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Impacted Mandibular Third Molars: Review of Literature and a Proposal of a Combined Clinical and Radiological Classification

Santosh P

Department of Oral Medicine and Radiology, College of Dentistry, Al Jouf University, Sakakah, Al Jouf, Kingdom of Saudi Arabia

Abstract

Tooth impaction is a pathological situation where a tooth fails to attain its normal functional position. Impacted third molars are commonly encountered in routine dental practice. The impaction rate is higher for third molars when compared with other teeth. The mandibular third molar impaction is said to be due to the inadequate space between the distal of the second mandibular molar and the anterior border of the ascending ramus of the mandible. Impacted teeth may remain asymptomatic or may be associated with various pathologies such as caries, pericoronitis, cysts, tumors, and also root resorption of the adjacent tooth. Even though various classifications exist in the literature, none of those address the combined clinical and radiologic assessment of the impacted third molar. Literature search using the advanced features of various databases such as PubMed, Scopus, Embase, Google Scholar, Directory of Open Access Journals and Cochrane electronic databases was carried out. Keywords like impaction, mandibular third molar, impacted mandibular third molar, complications, anatomy, inferior alveolar nerve injury, lingual nerve injury were used to search the databases. A total of 826 articles were screened, and 50 articles were included in the review which was obtained from 1980 to February 2015. In the present paper, the authors have proposed a classification based on clinical and radiological assessment of the impacted mandibular third molar.

Keywords: Classification, Complications, Impacted tooth, Management, Third molar

Introduction

Impacted tooth is a tooth which is completely or partially unerupted and is positioned against another tooth, bone or soft tissue so that its further eruption is unlikely, described according to its anatomic position.[1] The third molar impaction is occurring in about 73% of the young adults in Europe,[2] these teeth generally erupt between the ages of 17 and 21 years.[3] It has also been reported that the third molar eruption varies with races, such as in Nigeria[4] mandibular third molars may erupt as early as 14 years and in Europe,[5,6] it may erupt up to the age of 26 years. Factors such as the nature of the diet that may lead to attrition, reduced mesiodistal crown diameter, degree of use of the masticatory apparatus and genetic inheritance also affect the timing of third molar eruption.[7] Most of the researchers suggest that the females have a higher incidence of mandibular third molar impaction when compared to males.[8,9]

Methods of Literature Search

A web-based literature search using the advanced features of various databases such as PubMed, Scopus, Embase, Google Scholar, Directory of Open Access Journals (DOAJ), and Cochrane electronic databases was carried out. The major MeSH and other keywords like impaction, mandibular third molar, impacted mandibular third molar, complications, anatomy, inferior alveolar nerve injury, lingual nerve injury were used to search the databases. The search encompassed articles published from 1980 to February 2015, and the search was limited to articles published in English language. A total of 826 articles were screened, and 50 articles were included in the review.

Causes of Impacted Teeth

Various causes have been suggested in the literature for the impaction of the third molar. It has been suggested that the
gradual evolutionary reduction in the size of the human mandible/maxilla has resulted in too small mandible/maxilla that may accommodate the corresponding molars.\textsuperscript{[10]} It has also been found that the modern diet does not offer a decided effort in mastication, resulting in loss of growth stimulation of jaws, and thus the modern man has impacted and unerupted teeth. It has been suggested that the major basic cause of aberrant/impacted teeth in the adults of Western Europe, Great Britain and Ireland, U.S.A, and Canada is due to artificial feeding of babies, the habits developed during childhood, due to cross breeding, more consumption of sweet food by the children and youth which produces disproportion in the jaws and thus the teeth.\textsuperscript{[2,11]}

### Classification

#### The first classification for the impacted molar
- Vertical (38%)
- Mesioangular (43%)
- Horizontal (3%)
- Distoangular (6%)
- Buccoangular
- Linguoangular
- Inverted
- Unusual.\textsuperscript{[12]}

#### Pell and Gregory

**Relation of the tooth to ramus of mandible and second molar**
- Class I: Sufficient amount of space for accommodation of the mesiodistal diameter of the crown of the third molar
- Class II: The space between the ramus and distal side of second molar that is, less than the mesiodistal diameter of the third molar
- Class III: All/most of the third molars is located within the ramus.\textsuperscript{[13]}

**Relative depth of the third molar in the bone**
- Position A: The highest portion of the tooth is on a level with/above the occlusal line
- Position B: The highest portion of the tooth is below the occlusal plane, but above the cervical line of the second molar
- Position C: The highest portion of the tooth below the cervical line of the second molar teeth in relation to the long axis of impacted second molar.

#### According to nature of overlying tissue

This system is used by most dental insurance companies and one by which surgeons charge for his services.

- Soft tissue impaction
- Partial bony impaction
- Fully bony impaction.\textsuperscript{[14]}

### Pathological Changes Associated with Impacted Third Molars

The retained, unerupted mandibular third molars are often associated with varied pathologies which are listed in Table 1.

#### Pericoronitis

Many studies have looked at the association of pericoronitis and third molar impaction, and this is still the main cause for extraction of these teeth. However, one of the major flaws in these studies is the fact that there is no standard definition of pericoronitis. The eruption process is also likely to cause minor gingivitis, where the symptoms may be similar to pericoronitis, and the lack of a good definition for this disease may lead researchers and clinicians to misclassify it. Still pericoronitis is undoubtedly the main problem faced by dentists when it comes to lower impacted third molars.\textsuperscript{[10,12,15]}

#### Dental caries

The impacted lower third molars are extracted more commonly also due to dental caries, involving either the impacted third molar itself or the distal surface of the second molar. Majority of the researches in this context were carried out in patients who were referred for third molar removal and hence, the actual incidence of this disease in the general population.

### Table 1: Classification of pathologies associated with impacted mandibular third molars

<table>
<thead>
<tr>
<th>Clinical signs and symptoms</th>
<th>Noninflammatory radiological changes</th>
<th>Inflammatory radiological changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caries</td>
<td>Caries</td>
<td>Periapical radiolucent areas suggesting abcess</td>
</tr>
<tr>
<td>Pain</td>
<td>Root resorption (internal or external)</td>
<td>Pericoronal radiolucent areas suggesting pericoronitis</td>
</tr>
<tr>
<td>Swelling</td>
<td>Hyperplastic dental follicle</td>
<td>Severe inflammatory radiological changes</td>
</tr>
<tr>
<td>Paresthesia</td>
<td>Mild inflammatory radiological changes</td>
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<tr>
<td>Periodontal pocket</td>
<td>Pericoronitis</td>
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<td>Pericoronitis</td>
<td>Noninflammatory radiological changes</td>
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<td>Interdental bone loss</td>
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<td>Hyperplastic dental follicle</td>
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<td>Severe inflammatory radiological changes</td>
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<td></td>
<td>Osteomyelitis</td>
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<td></td>
<td>Radiological signs of cysts and benign tumors</td>
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<tr>
<td></td>
<td>Dentigerous cyst</td>
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<tr>
<td></td>
<td>Keratocystic odontogenic tumor</td>
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<tr>
<td></td>
<td>Odontomes</td>
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<td>Ameloblastoma</td>
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<td>Odontogenic fibroma</td>
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<td>Radiological signs of malignant tumors</td>
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<td>SCC</td>
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<td></td>
<td>Fibrosarcoma</td>
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<tr>
<td></td>
<td>Mucoepidermoid carcinoma</td>
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</table>

SCC: Squamous cell carcinoma
cannot be estimated. According to Nordenram et al., caries accounts for 15% of third molar extractions. Researchers in prospective studies of occlusal caries in patients with asymptomatic third molars reported an increased frequency of caries with an increase in age and erupted third molars.

Cysts and tumors associated with the tooth

Odontogenic cysts and tumors may be observed in some patients with impacted third molars, although they are relatively rare. The incidence of large cysts and tumors occurring around impacted third molars differs greatly in various studies, showing a wide range from 0.001% when a biopsy was indicated to 11% when the diagnosis was clinically established. This wide variation indicates that the presence of a cyst is a weak indication for prophylactic extraction of impacted third molars. Cystic changes may be encountered in the histopathological examination of the associated soft tissue of the asymptomatic impacted third molars, commonly in patients older than 20 years. The incidence, multiple presentation, and recurrence of aggressive cysts of the jaws and the malignant transformation of cysts have been discussed by Stoelinga and Bronkhorst.

Periodontitis

The incidence of periodontitis has been reported to vary from 1% to 5% on the distal surface of the second molar. The incidence and prevalence of periodontitis increases with age irrespective of the presence or absence of the third molars, and thus a higher incidence of periodontitis has been observed among the older patients in relation to the impacted wisdom teeth. There is a paucity of studies relating periodontitis associated with impacted third molars with oral hygiene, which may be a confounding factor.

Root resorption

It has been shown in some studies that a third molar left in situ may cause resorption of the distal root of the adjacent second molar. Some studies have also reported an association between root resorption at the apex and increasing age. However, these studies do not report the incidence of this problem in the general population since these are retrospective studies and are carried out in secondary care settings.

Late Crowding in Lower Incisors

One major controversy for indicating the prophylactic removal of lower third molars is the belief that their presence may result in late crowding of the lower incisors. However, it has been observed in a randomized controlled trial that the presence of impacted third molars had no significant clinical influence on the development of crowding in the lower incisors. Previous studies support these findings and suggest that crowding may be caused by other factors. A review of studies related to management of third molars by orthodontists suggested that the role of third molars may be controversial in the alignment of the anterior teeth and that no evidence exists in support of the fact that third molars may cause late incisor crowding.

Other related pathologies

One of the most commonly reported pathologies is an association of pain directly related to the presence of a third molar. The prevalence of this condition varies greatly from 5% to 53%. The incidence of cellulitis and osteomyelitis has been reported to be around 5%. Few other conditions which are also believed to be associated with impacted third molars include functional disorders such as occlusal interference, cheek biting, mastication disorders, trismus and temporomandibular joint problems. These pathologies and symptoms may result in distress and pain, but their correlation with third molars is not yet well-established due to lack of supporting evidence from the current literature.

Studies have shown that smoking causes pathological diversity, by augmenting expression of epidermal growth factor receptor and it has been suggested that this observation should be taken into account when deciding in case of removal of an asymptomatic impacted lower third molar. Ki67 and p53 are two markers which are commonly used to assess the pathologic proliferation and early-stage tumoral alterations in vital tissues. Results of recent studies shown that dental follicles of smokers have higher Ki67 and p53 protein expressions than nonsmokers’ follicles.

Assessment of Third Molar

Assessment of impacted tooth is done by physical and radiographic evaluation. The physical evaluation includes inspection and palpation of the temporomandibular joint and movement of the mandible, determination of mobility characteristics of lips and cheeks, size and contours of the tongue and appearance of soft tissue overlying the impacted teeth. Radiographic evaluation includes assessment of root morphology, size of follicular sac, density of the surrounding bone, contact with the second molar, nature of overlying tissues, inferior alveolar nerve and vessels, relationship to body and ramus of mandible, relation with adjacent teeth and buccal to lingual position of the third molar.

Haghanifar et al. have been carried out a study to find feasible radiographic criteria to help differentiate between normal and pathological dental follicles. The authors found that the average diameter of teeth associated with cystic follicular tissue was slightly more than the normal teeth; therefore, the average diameter of the follicles of these teeth was also slightly more than the normal follicles. Neither of the samples showed statistical significant differences but the probability of cystic epithelial changes thought to be increased when the dental follicles were noticed with the unusual wider surface. The results of the study concluded that the ratio of dental follicle diameter to the mesiodistal width of the teeth cannot be practicable as a diagnostic index to differentiate between normal and pathological dental follicle.
Management of Impacted Tooth

The treatment plans depend on the presenting complaint and the history of the patient, the physical evaluation, radiographic assessment, the diagnosis, and the prognosis. The management includes observation, exposure, transplantation or removal of the impacted tooth.

Observation

If the impacted mandibular third molar is embedded in bone with no perceptible to the follicle, as may be seen in an older individual and has no history, signs of associated pathology, long-term observation is appropriate. Most impacted teeth retain an erupting potential, and annual/biannual evaluation would be recommended if no indications for direct surgical management arise.

Exposure

This option is considered if there is probability that it may erupt into useful occlusion but is obstructed by follicle, sclerotic bone, hypertrophic soft tissue, odontoma, etc. If the second molar is absent, exposure of a blocked third molar may be considered.

Transplantation of mandibular third molar

The variety of crown and root shape on the impacted third molar makes them suitable for transplantation to other molar sites, bicuspid and even the cuspid locations depending on the anatomy of the coronal and radicular surface.

Removal

The primary reasons to remove impacted teeth are to correct associated pathology and to intercept reasonably expected pathological process.[22]

Indications for Mandibular Third Molar Extraction

As mentioned earlier, the third molar teeth are the last to erupt with a relatively high chance of becoming impacted. Hence, the surgical extraction of these impacted teeth has become the most common dentoalveolar surgeries.[14] In 1979, the National Institutes of Health Consensus Development Conference agreed on a number of indications for removal of impacted third molars, which included infection, nonrestorable carious lesions, cysts, tumors, and destruction of adjacent teeth and bone.[30] Some authors reported the absence of any associated problems over a period of several years due to the impacted third molars in edentulous patients. However, overemphasizing the development of dentigerous cysts due to impacted third molars have also been reported in the literature.[33]

The removal of impacted third molars is indicated for various therapeutic and prophylactic measures. However, no general indication has been agreed upon till date for the need of surgical removal of all asymptomatic impacted third molars.[18,34] The surgical extraction of many impacted mandibular third molars which have been asymptomatic for years are often carried out to prevent the development of any future complications and pathologic conditions.[35] Many investigators have questioned the necessity of removal for patients who are asymptomatic or have no associated pathologies, based on the view that retention of impacted teeth for a longer duration has less chances of pathological change in the tooth itself, or of deleterious effects on adjacent tooth and associated structures. Few authors have argued over the fact that all impacted third molars should be removed regardless of being asymptomatic; while others suggest that removing such impacted asymptomatic third molars is questionable in the light of the present lack of knowledge about the incidence of associated pathology.[19,22,36,37] Yet another group of authors considers that prophylactic surgical removal of impacted third molars is not necessary as the risk of development of pathological conditions in or around follicles of third molars is apparently low.[38]

Extraction of the impacted mandibular third molars significantly improved the periodontal status on the distal aspect of second molars, positively affecting the overall health of supporting periodontal tissues.[16] But it is also suggested that periodic exercising of arbitration to enhance the periodontal parameters on the distal surface of the second molar at the time of third molar extraction is not advisable for all subjects.[40-42]

The removal of asymptomatic impacted third molars that could not cause any complications for a known period of time thought to be an encumbrance from economic standpoint. The assessment of health risks and cost effectiveness regarding the prophylactic extraction of asymptomatic impacted third molars should be considered before tooth removal.[43] The dental practitioner, who scrutinize the healthy individual should monitor carefully regarding the pathologies which may incur an impacted third molar. He should procreate adult patients with asymptomatic third molars, fathom that there is no coercion or it is indispensable to remove the impacted third molars without any pathology. This aforesaid phenomenal proposition needs to be exercised for adolescents and their parents regarding the impact of the extraction of asymptomatic impacted third molar removal on lower incisor crowding at a later period.[44]

Complications and Risks Following Surgery

Complications associated with the removal of impacted teeth are relevant and is aided by local and general factors which include tooth position, age of the patient, health status, knowledge and experience of the dental surgeon, and surgical equipment used. Most common complications associated with the removal of the third molar include damage of the pain, sensory nerve leading to paresthesia, dry socket, infection, and hemorrhage. Severe
trismus, oro-antral fistula, buccal fat herniations, iatrogenic damage to the adjacent second molar, and intravenous mandibular fracture may also occur, though very rarely.[18,45] The rate of sensory nerve damage after third molar surgery ranges from 0.5% to 20%.[18,46] The overall rate of dry socket varies from 0% to 35% among studies.[18,47] The risk of dry socket increases with lack of surgical experience and tobacco use though this does not justify prophylactic removal. Many of these problems are not permanent; however, paresthesia may become permanent and lead to functional problems in some cases.[48,49] The pathological features associated with impacted third molars are summarized in Table 1, after a thorough review of the literature. According to these features, an attempt has been made to propose the first combined clinical and radiological classification of impacted mandibular third molars [Table 2]. This attempt of proposing the classification will assist the dental practitioners and researchers in accomplishing insight in terms of standardized assessment and categorization of impacted mandibular third molar which will further help in the management of this condition accordingly. This classification would expedite continued studies to be carried and analogizing to be made in a more categorical and propitious manner and allow an exceptional understanding of the pathophysiology underlying the impacted teeth. This proposed classification focus on diversified key characteristics that are believed to be of concernment to the dental practitioners and have also been hypothesized by others as important when it comes to various sequel of interest such as practice efficiency, operator satisfaction, and subject outcomes. It also provides a common lexicon and nomenclature for referring to group practices of different kinds and also serves common terminology to facilitate transmission among practitioners, researchers, academicians, and patients.

It is also implied that additional clinical research should be conducted for the substation and affirmation of the classification and also to know the authenticity of this proposed neoteric classification.

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Hygiene Practices among Workers in Local Eateries of Orolu Community in South Western Nigeria

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Introduction

The springing up of fast food shops and local eateries in nooks and crannies of South Western Nigeria has been recognized as one of the forces that boosted the economy of the region. Westernization, urbanization and the need to struggle for daily survival has made many Nigerians to abandon the culture of cooking at home. Foods at these eateries are available, accessible and sometimes affordable. These food premises also employ a significant number of staff who handles food items from the stage of processing to marketing.

As a matter of public health importance, food safety and hygienic practices employed in this food marketing sector, restaurants and hotels would play an important function in ensuring that safe food is available for consumption.[1] Safe foods ensure minimal risks and hazards to human health through protecting and preventing edible substances from becoming hazardous in the presence of chemical, physical and biological contaminants that deteriorate or spoil the food.[1] Contaminated food represents one of the greatest health risks in a population and a leading cause of disease outbreaks and transmission.

Food handlers in these food premises are responsible for food safety throughout the chain of producing, processing, storage and preparation.[2,3] Mishandling and disregard for hygiene measures on the part of these food handlers may result in food contamination and its attendant consequences[2] including food poisoning[4,5] and spread of diseases with resultant morbidity and occasional mortality.[2]

Many factors ranging from ignorance,[6] uncaring and poor attitude to personal hygiene,[3] lack of basic hygiene infrastructure and sanitary facilities such as water, soap and toilets and lack of food storage and preservation facilities, all

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Abstract

Background: Activities of local food premises and monitoring of food handlers are usually not regulated. Aim: The objective of this study was to determine food hygiene (FH) practices among food handlers in rural communities in South Western Nigeria. Subjects and Methods: Descriptive cross-sectional study was carried out among 235 food handlers; data collection was by interviewer administered questionnaires. Using the SPSS software, multivariate analysis in two separate models was done to explore the predictors of correct knowledge and good hygiene practices. The model fit was assessed as good using the Hosmer and Lemeshow test. Results: Mean age of respondents was 31.8 (10.8) years. Of the respondents (134) that had training, 17.2% (23/134) had formal training, and 82.8% (111/134) had apprenticeship; about 31.5% (74/235) of respondents maintained a good level of hygiene in their practices. Significant predictors of correct knowledge were found to be being trained (significant 0.01, odds ratio [OR] 2.4, 95% confidence interval [CI] 1.2–4.8) and receiving the training as an apprentice (significant 0.01, OR – referent group); or in a formal setting (significant 0.01, OR 3.3, 95% CI 1.6–7.0) and having no formal education (significant 0.04, OR – reference group). Conclusion: Good knowledge and attitude but low level of good practices toward FH characterized food handlers under study.

Keywords: Attitude and practice, Food handlers, Food hygiene, Knowledge, Local eateries

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contributed to poor attitude toward food hygiene (FH) practices among food handlers. In addition, lack of time and staff have been identified as some of the barriers to practice of FH. Another study carried out among food handlers in a Nigerian University campus showed a predominantly poor level of FH knowledge, very low frequency of hand washing practices and low level of personal hygiene generally.

Unfortunately, the agency of government saddled with the responsibility of regulating food sale and marketing National Agency for Food and Drug Administration and Control (NAFDAC) acts centrally, and little or no emphasis is placed on such regulation at state and local council levels. Past epidemics of food borne diseases outbreak in the region usually do not focus food handlers in local eateries despite common knowledge that they may be carriers of infectious diseases. Recently NAFDAC commenced nationwide crackdown on fast food outlets following an outbreak of food poisoning in Nigeria in 2008, and this led to the closure of some popular eateries.

Studying food handlers in food premises could determine hygiene knowledge and practice toward prevention of food borne and food related diseases. The study focused mainly on local eateries which are generally more accessible and affordable to the majority of the local populace. Data emanating from such studies could also guide the regulating agencies and other stakeholders into formulating policies that would reduce the occurrences of outbreak of communicable diarrheal diseases as well as making food premises liable to negative effects resulting from their hygiene practices. This study therefore assessed hygiene practices among workers in local eateries in Orolu community in South Western Nigeria.

Subjects and Methods

Study area

Orolu community is located in the heart of Orolu Local Government Area in Osun State in South Western Nigeria. The 2006 population census puts the population of the area at around 200,000 people. Majority are traders, artisans, farmers or civil servants. There are thirty (30) local eateries in the community. There are social amenities such as PHCs, hotels, banks, private clinics, primary and secondary schools, etc.

Study design and duration

It was a descriptive cross-sectional study carried out among food handlers at local eateries (bukaterias) in Orolu community of Osun State, South Western Nigeria from January to March, 2012.

Study population

The target populations in the community under study were food handlers in local eateries popularly referred to as “bukaterias.” Food handlers in hotels and fast food shops or joints were excluded from the study. Eligible respondents should have been directly involved with handling of food items for a minimum of 6 months.

Sample size estimation

Using the formula for calculating sample size for population <10,000, a sample size of 208 was calculated based on hygiene knowledge prevalence of 50%. Total sample size was taken as 240 after adding 10% for possible non response.

Sampling method

There were thirty local eateries in the community; twenty-four of which were selected through simple random sampling by balloting. In each bukateria, a list of staff involved with handling of raw and processed food was made, and each was found to have an average of 15–18 food handlers. Ten food handlers were selected using simple random sampling by balloting from each bukateria.

Data collection

All eligible respondents were interviewed using precoded, pretested questionnaires. The interview was conducted by trained research assistants who could also speak the local language. A vernacular version of the questionnaire was prepared for the uneducated respondents to reduce inter-observer variation in interpretation during the interview.

Study variables

Information collected were based on socioeconomic characteristics of the respondents, the operations of their eateries as regards food sanitation, their knowledge and attitude toward FH practices and its relation to public health.

Ethical consideration

Ethical approval to conduct this study was obtained from the Research Ethics Committee of Lautech Teaching Hospital Osogbo. Permission was also obtained from the Local Government Department of Health and Community Development, and operators of the eateries selected to take part in the study. Informed consent was obtained from each respondent verbally before being interviewed.

Data management

The SPSS version 17.0 statistical package (Chicago, IL, USA) was used for data entry and analysis. Validity of data collected was ensured by double entry and random checks for errors. Knowledge, attitude, and practices were scored based on responses of respondents to questions that were asked. Correct or favorable response was scored +1 while
negative or wrong responses were scored 0. Pertaining to knowledge and perception, the scale was from 0 to 12, and a score of 0–6 was taken as poor knowledge and poor perception, while a score of 7–12 was taken as good. Scoring for attitude was on a scale of 0–6, with 0–3 taken as negative attitude, and 4–6 taken as positive attitude. The scale for practice was based on 0–7; poor or low-level practice was for respondents with 0–3 while good or high-level practice was taken for scores of 4–7.

We conducted a multivariate analysis in two separate models to explore the predictors of correct knowledge and good hygiene practices. In a first model, we fit a forward stepwise logistic regression model to the data to explore factors that influenced knowledge of FH. The model fit was assessed as good using the Hosmer and Lemeshow test. In the second model, the outcome variable (practice) was coded 1 for respondents who had good FH practices and 0 for those who did not. The independent variables included in the model were age, sex, education, religion, tribe, marital status, role in the eatery, years of experience, type of training, certification, aware that FH is an important issue for caterers, has had training on FH, can correctly describe FH and believes FH is necessary in food preparation. The model fit was assessed as good using the Hosmer and Lemeshow test.

Relevant frequency distributions and summary measures were done. We used the Chi-square test to examine bivariate relationship and multivariate logistic regression to examine the predictors of correct knowledge and good hygiene practice. All tests were performed at the 5% significance level, and two independent sample t-test analyses was used to compare mean differences between quantitative variables.

## Results

A total of 235 respondents questionnaire were completely analyzed giving a response rate of 97.9% (235/240). Mean age of respondents was 31.8 (10.8) years. They had spent a medium of 4 years in the local eatery business (interquartile range: 2–10 years). Of the respondents, 57.0% (134/235) have had training on FH of which 17.2% (23/134) had formal training, and 82.8% (111/134) had apprenticeship. They all had varying roles in the eatery. These and other baseline characteristics are presented in Table 1.

In Table 2, about 94% (220/235) of respondents were aware that FH was an important issue for caterers but only 55.7% (131/235) could correctly describe FH. Respondents differed in their disposition to issues related to FH. Almost all felt that FH is necessary in food preparation (93.2% [219/235]) and should be practiced at all steps of food preparation and serving (97.9% [230/235]). Questions relating to their practices elicited that 31.5% (74/235) of respondents maintained a good level of hygiene in their practices. Other details of disposition and practices are as shown in Table 2.

### Table 1: Baseline characteristics of respondents

<table>
<thead>
<tr>
<th>Variables</th>
<th>Frequency (n)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Mean=31.8 years</td>
<td>SD=10.8 years</td>
</tr>
<tr>
<td>Sex (n=235)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>35</td>
<td>14.9</td>
</tr>
<tr>
<td>Female</td>
<td>200</td>
<td>85.1</td>
</tr>
<tr>
<td>Education level (n=235)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>30</td>
<td>12.8</td>
</tr>
<tr>
<td>Primary/Arabic school</td>
<td>94</td>
<td>40.0</td>
</tr>
<tr>
<td>Secondary school</td>
<td>97</td>
<td>41.3</td>
</tr>
<tr>
<td>Tertiary</td>
<td>14</td>
<td>6.0</td>
</tr>
<tr>
<td>Religion (n=235)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Christian</td>
<td>109</td>
<td>46.4</td>
</tr>
<tr>
<td>Islam</td>
<td>119</td>
<td>50.6</td>
</tr>
<tr>
<td>Traditionalist</td>
<td>7</td>
<td>3.0</td>
</tr>
<tr>
<td>Tribe (n=235)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yoruba</td>
<td>210</td>
<td>89.4</td>
</tr>
<tr>
<td>Igbo</td>
<td>22</td>
<td>9.4</td>
</tr>
<tr>
<td>Hausa/others</td>
<td>3</td>
<td>1.3</td>
</tr>
<tr>
<td>Marital status (n=235)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>89</td>
<td>37.9</td>
</tr>
<tr>
<td>Married</td>
<td>126</td>
<td>53.6</td>
</tr>
<tr>
<td>Divorced/widowed</td>
<td>20</td>
<td>8.5</td>
</tr>
<tr>
<td>Role in the eatery (n=235)</td>
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<td></td>
</tr>
<tr>
<td>Administrator</td>
<td>41</td>
<td>17.4</td>
</tr>
<tr>
<td>Cook</td>
<td>59</td>
<td>25.1</td>
</tr>
<tr>
<td>Vendor</td>
<td>14</td>
<td>6.0</td>
</tr>
<tr>
<td>Waiter/waitress</td>
<td>73</td>
<td>31.1</td>
</tr>
<tr>
<td>Cleaning duties</td>
<td>32</td>
<td>13.6</td>
</tr>
<tr>
<td>Multiple roles as assigned</td>
<td>16</td>
<td>6.8</td>
</tr>
<tr>
<td>Years of experience</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median=4 years</td>
<td>IQR=2-10 years</td>
<td></td>
</tr>
<tr>
<td>Ever undergone training on FH (n=235)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>134</td>
<td>57.0</td>
</tr>
<tr>
<td>No</td>
<td>101</td>
<td>43.0</td>
</tr>
<tr>
<td>Type of training (n=134)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formal</td>
<td>23</td>
<td>17.2</td>
</tr>
<tr>
<td>Apprenticeship</td>
<td>111</td>
<td>82.8</td>
</tr>
</tbody>
</table>

In a first multivariate analysis model, the descriptive information for these variables has been presented in Tables 1 and 2. The model fit was assessed as good using the Hosmer and Lemeshow test (significant of 0.5 at a df of 8). Significant predictors of correct knowledge were found to be being trained (significant 0.01, odds ratio [OR] 2.4, 95% confidence interval [CI] 1.2–4.8) and receiving the training as an apprentice (significant 0.01, OR = referent group); or in a formal setting (significant 0.01, OR 3.3, 95% CI 1.6–7.0).
In the second model, the descriptive information for these variables has been presented in Tables 1 and 2. The model fit was assessed as good using the Hosmer and Lemeshow test (significant of 0.44 at a df of 8). Significant predictors of good FH practice were found to be being a cook (OR 10.6, 95% CI 2.0–55.2); having no formal education (significant 0.04, OR – referent group); and having received training in an informal setting (significant 0.05, OR – referent group), as an apprentice (significant 0.03, OR 3.9, 95% CI 1.1–13.4), and in a formal setting (significant 0.04, OR 2.3, 95% CI 1.1–5.1).

### Discussion

Mean age in this study is close to that of a study carried out among food handlers in fast food restaurants in Benin. This constitutes the young adult age group that is expected to be
actively working and contributing their quota to economic growth by working in eateries. In this study, about one-tenth of total respondents (and one-third of those trained) had formal training on FH. This is higher when compared to a similar study in which none of the respondents under study acquired food handling skills through formal training. However, the figure is low when compared to another study in Benin that reveals that more than half of the food handlers had no training in FH and safety. In Nigeria, regulatory authorities such as NAFDAC and Standard Organization of Nigeria are regularly on top of regulating FH practices and registration of food premises including eateries.

Training of food handlers is a fundamental requirement for ideal FH practices, and this might have forced many eateries toward training of newly employed food handlers. This could explain the figures reported in this study. Training will assist workers to have adequate information about hand washing before and after touching food items, and adequate cooking of raw food and basic practices related to FH. According to Food and Agricultural Organization (FAO), food handlers should have the necessary knowledge and skills to enable them to handle food hygienically; they are expected to receive training at least to a level suitable for their roles and responsibilities. FAO recommends that every vendor/helper of food should undergo a basic training in FH before licensing. All this could reduce new infections and burden from diarrheal diseases and food borne infections.

Training in FH and safety, longer years of work and in some cases, the level of education as reflected by Hosmer and Lemeshow tests are predictive factors that have been shown to influence knowledge and practices of FH in this study. This agreed with other studies among food handler. However, lack of formal education was reported to be a predictor for good food hygienic practice in this study. This agreed with another study which revealed that the level of education of food handlers did not significantly influenced their practice of FH and safety.

In this study, most (above 90%) have good knowledge about FH, a finding similar to previous studies in Benin where good knowledge about FH was found among the respondents, and Mauritius where food vendors were found to be quite aware of hygienic conditions, and which have to be respected while handling and preparing foods. This study thus further reveals that respondents with good knowledge about FH may have more positive attitude and good practice than those without knowledge.

In this study, in spite most respondents having good knowledge and majority having good attitude or disposition about FH, only about one-third had a high level or good practice of FH. This finding has also been seen in previous studies where it was found that majority of food handlers were not implementing their knowledge into practice, that level of knowledge of food handlers does not impact on the hygiene standard of their food premises, and that more than two-thirds of respondents admitted to sometimes not carrying out food safety behaviors despite their knowledge about FH in Wales. In a study carried out about food-borne diseases outbreaks in schools, contamination of food by food handlers as one of the most common practices that contributed to these diarrheal disease outbreaks.

Another study carried out in Ilorin on food vendors observed that respondents who used soap and water for cleaning, vended food at locations that were relatively closer to water source, had good level of FH practices compared to other vendors who used unsanitary methods to clean their utensils as supported by findings from this study. This stresses the importance of good access to water and sanitary facilities as a possible panacea for the practice of good FH. This supports reported attitude of majority of food handlers in this study, that eateries should be compelled to procure materials and infrastructure necessary for FH and the claims of respondents that they usually wash their hands regularly before and after touching food and food items.

**Limitations of this study**

There could be a possible response bias on the part of some respondents that may try to portray their eateries as compliant with safety regulations out of fear of being sacked. This was appropriately handled by persuasive training and sensitivity of the research assistants and several reassurances of the respondents.

**Conclusion**

Good knowledge and attitude but low level of good practices toward FH characterized food handlers under study. Authors advocate formal pre-employment training on FH to prospective food handlers by their employers. Other recommendations include periodic medical examinations and on the job health education and promotion exercises for food handlers. Stakeholders involved in regulating the operations of food premises should also remove barriers to good FH practices toward ensuring food sanitation and prevention of food borne diseases in local eateries in Nigeria.

**Acknowledgment**

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Characterization of Escherichia coli Phylogenetic Groups Associated with Extraintestinal Infections in South Indian Population

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Abstract

Background: Escherichia coli strains mainly fall into four phylogenetic groups (A, B1, B2, and D) and that virulent extra‑intestinal strains mainly belong to groups B2 and D. Aim: The aim was to determine the association between phylogenetic groups of E. coli causing extraintestinal infections (ExPEC) regarding the site of infection, expression of virulence factors, antimicrobial resistance patterns, and clinical outcome. This descriptive study was carried out in a multi-speciality Tertiary Care Hospital. Materials and Methods: A total of 300 E. coli causing ExPEC were studied. Triplex polymerase chain reaction was used to classify the phylogenetic groups; hemolysin production was assessed on sheep blood agar and biofilm production in a microtiter plate assay. Production of extended spectrum of beta‑lactamase (ESBLs) was detected by combination disk method; AmpC was detected by AmpC disk test, Carbapenemase production was detected by modified Hodge test and metallo-β‑lactamase by metallo-beta-lactamasases (MBL) E-test. Results: Of 300 isolates, 61/300 (20%) belonged to phylogroup A, 27/300 (9%) to phylogroup B1, 104/300 (35%) were B2 and 108/300 (36%) belonged to group D, respectively. Phylogroups B2 and D were the most predominant groups in urinary tract infection and sepsis. Prognoses were better in infections with group A and B1 isolates, and relapses and death were common in infections with B2 and D. Expression of biofilm was greatest in B1 and hemolysin in group B2. Group A and B1 showed higher resistance to ciprofloxacin and were most frequent β‑lactamase (ESBL, AmpC, Carbapenemase and MBL) producers. Conclusions: Phylogenetic group B2 and D were predominant in ExPEC and exhibited least antimicrobial resistance among the groups. Resistance to multiple antibiotics was most prevalent in group A and B1. Regular monitoring of antimicrobial susceptibility in commensal strains is essential as they might transfer the property of antimicrobial resistance to pathogenic strains.

Keywords: Drug resistance, Escherichia coli; Extraintestinal infections, Polymerase chain reaction, Phylogenetic group, Virulence

Introduction

Escherichia coli strains are implicated in a large number of extraintestinal infections (ExPEC) in humans such as urinary tract infection (UTI), bacteremia, pneumonia, soft-tissue infection, and neonatal meningitis.\textsuperscript{[1]} Phylogenetic analysis has shown that E. coli strains fall into four main groups (A, B1, B2, and D). It has been found that pathogenic E. coli strains causing extraintestinal infections mainly belong to group B2 and a lesser extent to group D whereas commensal strains belong to group A and B1.\textsuperscript{[2]} Results of various studies have also indicated that the phylogroups A and B1 (commensal strains) exhibit an increased drug resistance pattern but possess few virulence genes whereas the phylogroups B2 and D (pathogenic strains) possess several pathogenicity associated islands and express multiple virulence factors such as adherence factors including biofilm production and high surface hydrophobicity, toxin (hemolysin and CNF)
and siderophore production but are more susceptible to antibiotics.\[3\]

In recent years, much attention has been given to the analysis of the phylogenetic affiliation of pathogenic E. coli strains, so as to understand the sources of such ExPEC and also to limit the spread of multidrug resistance among such strains.\[4\] Although several studies have been reported from outside India, not much information is available regarding the phylogenetic lineage of ExPEC causing extraintestinal infections from within our country with only very few studies being reported regarding phylogenetic grouping. The aim of the present study was to assess the association between phylogenetic groups of E. coli and site of infection, phenotypic characters, antibiotic resistance, and clinical outcome, respectively.

**Materials and Methods**

**Participants and clinical isolates**

The study was conducted during the period from August 2010 to January 2012, from hospitalized patients of two Tertiary Care Hospitals, after obtaining permission by way of informed consent from patients and ethical clearance from the Kasturba Medical College, Mangalore institutional ethical committee. Sample size was determined with 55% confidence and 90% power based on previous studies. Three hundred non-repeat strains of E. coli were isolated from specimen such as urine, blood, wound swab, pus, cerebrospinal fluid, ascitic fluid, and intravascular devices, collected using standard sterile procedures from the study population. The study population included hospitalized patients of all age groups whose extraintestinal clinical samples grew E. coli and excluded those subjects who had received antimicrobial drugs during the past 1 month, who had asymptomatic UTI, polymicrobial infections and those who were discharged without treatment with antimicrobial drugs. Clinical data from the patient’s records were collected in a proforma. All patients were followed up for the period of 1 year to monitor clinical outcome. The samples were processed immediately using standard procedures. The isolates were identified based on colony morphology on Blood agar, MacConkey’s agar, Gram staining and by standard biochemical tests.\[5\] ExPEC isolates from blood were identified using automated biochemical system Vitek 2 (BioMerieux, France).

**Biofilm production**

The capacity to form biofilms was assayed in microtiter plates with minor modification as described by O’Toole and Kolter.\[6\] Briefly, cells were initially grown for 18 h in Trypticase soy broth (TSB) at 37°C, subsequently cultures were diluted 1:100 with fresh TSB and 200 µl were inoculated into 96 well polystyrene microtiter plates and incubated for 24 h at 37°C. After incubation, content of each well was gently removed by tapping the plates. The wells were washed four times with 200 µl of phosphate buffer saline (PBS, pH 7.2) to remove free-floating planktonic bacteria. Biofilms formed by adherent organisms in plate were fixed with Bovin’s fixative and stained with crystal violet (0.1% w/v). Excess stain was rinsed off by thorough washing with deionized water and plates were kept for drying. Optical densities (OD) of stained adherent bacteria were determined with an ELISA reader at wavelength of 630 nm. These OD values were considered as an index of bacteria adhering to surface and forming biofilms; strains with mean OD values of <0.120 were considered as non-biofilm producers and those with mean OD values >0.120 were considered to be biofilm producers. Pseudomonas aeruginosa ATCC 27853 was used as the control organism.

**Hemolysin production**

Production of α-hemolysin was tested on 5% sheep blood agar. E. coli strains were inoculated onto blood agar plates, incubated overnight at 37°C and hemolysis was detected by the presence of a zone of complete lysis of the erythrocytes around the colony.\[7\]

**Antimicrobial susceptibility testing**

Antibiotic susceptibility testing was done by the modified Kirby-Bauer disk diffusion method in accordance with CLSI guidelines.\[8\] The antibiotic disks (HiMedia, Mumbai) used were ampicillin (10 µg), piperacillin (10 µg), piperacillin + tazobactam (100/10 µg), ceftriaxone (30 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), amikacin (30 µg), gentamicin (10 µg), co-trimoxazole (1.25/23.75 µg), cefoperazone + sulbactam (75/30 µg), imipenem (IPM; 10 µg), meropenem (MRP; 10 µg), and ertapenem (ETP; 10 µg).

**Screening for extended spectrum of beta-lactamase production**

Isolates which were resistant to one or more third-generation cephalosporins were tested for Extended spectrum of beta-lactamase (ESBL) production by the combination disk method using, ceftazidime (30 µg) and ceftazidime/clavulanic acid (10 µg). A ≥5 mm increase in diameter of the inhibition zone of the cephalosporin-plus-clavulanate disc, when compared to the cephalosporin disc alone was interpreted as phenotypic evidence of ESBL production.\[9\]

**Detection of AmpC production**

Isolates were tested for AmpC enzyme production by AmpC disk test.\[9\] Briefly, a suspension of ATCC E. coli 25922 standardized to 0.5 McFarland was inoculated on the surface of Mueller–Hinton Agar (MHA) plate. A 30 µg cefoxitin disk was placed on the inoculated surface of the agar. A sterile plain disc containing Tris-ethylenediaminetetraacetic acid (EDTA) inoculated with several colonies of the test isolate was placed beside the cefoxitin disc almost touching it, with the inoculate in contact with the agar surface. The plates were incubated overnight at 35°C, aerobically. A positive test was indicated as flattening or indentation of cefoxitin inhibitory zone in the vicinity of the test disc. A negative test had an undistorted zone.
Detection of carbapenemase production

Plates of MHA were inoculated with suspensions of test strains and adjusted to a turbidity equivalent to 0.5 McFarland standard. A set of discs (HiMedia) of IPM, MRP, and ETP (10 µg each) were applied to the surface of the agar, plates were incubated overnight at 35°C aerobically, and diameters of zone of inhibition (≥23 mm indicating sensitivity, 20–22 mm indicating intermediate resistance and ≤19 mm indicating resistance) were recorded. Carbapenemase production was further confirmed by modified Hodge test.[6]

Detection of metallo-β-lactamase producers

Identification of metallo-beta-lactamases (MBL) activity was performed by two methods: A carbapenem–EDTA combined disk method and MBL E-test (HiMedia).[10] A known MBL producing isolate (PCR positive with MBL genes) was used as a positive control for all tests.

Determination of Escherichia coli phylogenetic groups

Isolates were assigned to one of the four main phylogenetic groups of E. coli (A, B1, B2, and D) by using the triplex PCR as described by Clermont et al.[11] Briefly, template DNA was obtained by boiling-lysis method. The genes chuA, yjaA and TSPE4.C2 were amplified using appropriate oligonucleotide primers.[11] The PCR was performed in a final reaction volume of 50 µl containing 750 mM Tris–HCl, 200 mM (NH₄)₂SO₄, 2.5 mM MgCl₂, 0.2 mM each of dNTP’s, 0.4 µM of each primers, 1 U of Taq DNA polymerase and 5 µl template DNA. An Eppendorf thermocycler was used for amplification. The program for amplification included a step of initial denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 30 s and a final extension step at 72°C for 7 min. The PCR products were loaded in 2% w/v agarose gel prepared in Tris-borate–EDTA buffer at 120V for 1 h and detected by ethidium bromide staining after electrophoresis.

Statistical analysis

Chi-square test was used to find an association between the phylogroups, virulence factor genes and patient’s clinical outcome. Analysis was performed using statistical package SPSS version 17.0 (USA).

Results

A total of 300 E. coli isolates from extraintestinal sources were collected. One hundred forty-three isolates were from the medical unit, 44 from surgical, 43 from urology, 20 from oncology, 20 from gastroenterology, 13 from obstetrics and gynecology, 12 from orthopedics and 5 from pediatrics units. The primary site of infection included 159/300 (53%) cases of UTI, 77/300 (25.6%) with bacteremia, 40/300 (13.3%) with wound infection, 19/300 (6.3%) with pneumonia, 3/300 (1%) intravascular device infection, and 2/300 (0.6%) with meningitis.

Phylogenetic analysis of isolates carried out by triplex PCR [Figure 1] indicated that 61/300 (20%) isolates belonged to Group A, 27/300 (9%) were group B1, 104/300 (35%) were group B2, and 108/300 (36%) belonged to group D. The distribution for each phylogenetic group according to gender, age group, site of infection, and outcome is summarized in Table 1. The phylogroups B2 and D occurred with greater frequency (statistically significant, P < 0.05) than A and B1 among all isolates of ExPEC without any sex disparity. Furthermore, phylogroups B2 and D were seen to occur at a far greater frequency in all age groups, when compared to A and B1. The largest number of ExPEC infections were observed in the > 60 years age group (130/300 strains; 43.3% of all infections, statistically significant, P = 0.04) indicating that this group was more susceptible than others to infection with ExPEC. UTI was the clinical entity wherein highest number of ExPEC were isolated (159/300 strains; 53% statistically significant, P = 0.03), again with phylogroups B2 and D being isolated most frequently (38.4 and 33.9% respectively, P < 0.01). Regarding clinical outcome, prognosis of infections with strains belonging to A and B1 phylogroups was far better than with infections due to strains from B2 and D. Seventy-eight percent of cases due to ExPEC strains belonging to A and 62.9% of cases due to infection with strains belonging to B1 were successfully treated and such ExPEC were eradicated completely with appropriate antibiotic therapy. On the other hand, higher morbidity (relapses and recurrent infections) and mortality was observed in infections due to ExPEC strains belonging to the virulent phylogroups B2 and D (37% and 26%, respectively; Table 1).

The study of phenotypic characteristics such as expression of virulence factors and antimicrobial resistance of the isolates belonging to each phylogenetic group showed that expression of biofilm production was greatest with ExPEC strains belonging to A and B1 isolates were observed to occur at a far greater frequency in all age groups, when compared to A and B1. The largest number of ExPEC infections were observed in the > 60 years age group (130/300 strains; 43.3% of all infections, statistically significant, P = 0.04) indicating that this group was more susceptible than others to infection with ExPEC. UTI was the clinical entity wherein highest number of ExPEC were isolated (159/300 strains; 53% statistically significant, P = 0.03), again with phylogroups B2 and D being isolated most frequently (38.4 and 33.9% respectively, P < 0.01). Regarding clinical outcome, prognosis of infections with strains belonging to A and B1 phylogroups was far better than with infections due to strains from B2 and D. Seventy-eight percent of cases due to ExPEC strains belonging to A and 62.9% of cases due to infection with strains belonging to B1 were successfully treated and such ExPEC were eradicated completely with appropriate antibiotic therapy. On the other hand, higher morbidity (relapses and recurrent infections) and mortality was observed in infections due to ExPEC strains belonging to the virulent phylogroups B2 and D (37% and 26%, respectively; Table 1).

The study of phenotypic characteristics such as expression of virulence factors and antimicrobial resistance of the isolates belonging to each phylogenetic group showed that expression of biofilm production was greatest with groups B1 and B2; group B2 isolates were observed to be most frequently associated with hemolysin production (37.5%, statistically significant, P < 0.05) and 17.3%
were non-lactose fermenters [Table 2]. The expression of various enzymes associated with antimicrobial resistance and resistance patterns exhibited by isolates of the four phylogenetic groups against representative antimicrobials from different classes are shown in Table 2 and Figure 2. Results of our study showed that group B2 isolates produced the least amount of beta lactamases when compared with group A, B1, and D. Resistance to ciprofloxacin, norfloxacin, co-trimoxazole, nalidixic acid and cefoperazone/sulbactam was especially prevalent among group B1 isolates and group A isolates showed comparatively higher resistance to ciprofloxacin and norfloxacin when compared with groups B2 and D. However, in case of ampicillin and cefotaxime, resistance rates were higher with group B2 and D. The presence of multi-drug resistance (resistance to three or more antibiotic groups) strains were significantly higher in group B1 isolates and group A isolates showed comparatively higher resistance to ciprofloxacin and norfloxacin when compared with groups B2 and D. However, in case of ampicillin and cefotaxime, resistance rates were higher with group B2 and D. The presence of multi-drug resistance (resistance to three or more antibiotic groups) strains were significantly higher in group B1 isolates (statistically significant, P = 0.04). Among the four groups, A and B1 group isolates were most frequent β-lactamase (ESBL, AmpC, Carbapenemases, MBL) producers when compared to group B2 and D [Table 2].

**Discussion**

*Escherichia coli* is emerging as an important cause of extraintestinal infections in our hospitals. These strains are seen to exhibit several virulence properties as well as a high rate of antibiotic resistance which is of major concern for management of cases. Extraintestinal pathogenic *E. coli* which routinely cause infections have been shown to belong to phylogroups B2 and D. Results of our study indicated that approximately 70.6% of the *E. coli* isolates from our patients also belonged to phylogenetic group B2. However, in cases of UTI, B2, B1, and D, results of our study showed that group B2 isolates produced the least amount of beta lactamases when compared with group A, B1, and D. Resistance to ciprofloxacin, norfloxacin, co-trimoxazole, nalidixic acid and cefoperazone/sulbactam was especially prevalent among group B1 isolates and group A isolates showed comparatively higher resistance to ciprofloxacin and norfloxacin when compared with groups B2 and D. However, in case of ampicillin and cefotaxime, resistance rates were higher with group B2 and D. The presence of multi-drug resistance (resistance to three or more antibiotic groups) strains were significantly higher in group B1 isolates (statistically significant, P = 0.04). Among the four groups, A and B1 group isolates were most frequent β-lactamase (ESBL, AmpC, Carbapenemases, MBL) producers when compared to group B2 and D [Table 2].
and D phylogroups were more common. The most common phylogroup which was responsible for relapses and/or deaths was group B2, which probably indicates the capability of strains of this group to cause persistent and severe infections.

Previous studies have indicated that phylogroups B2 and D possess more virulence properties such as biofilm formation and hemolysin secretion when compared with phylogroups A and B1 isolates.\[15,14\] In our study, we found group B2 consisted of a greater number of non-lactose fermenting isolates, and also more strains produced hemolysin which is considered to be a potent virulence factor essential for survival in extraintestinal sites.\[3,13\] Interestingly, it was group B1 isolates which expressed a higher capacity to form biofilms, a virulence factor which has been shown to be responsible for the initial step of adherence to various cell/tissue surfaces, during the process of infection.\[14\]

Regarding antimicrobial resistance exhibited by isolates from different phylogroups, our study results indicated that phylogroup B2 isolates were the most susceptible (statistically significant) to antibiotics than isolates belonging to the other three groups. Similar results have been obtained by other investigators, indicating that although being more virulent, the isolates of phylogroup B2 were more susceptible to antibiotics.\[12\] However, in our study, we found gentamicin resistance was more common in group B2 isolates. Several studies have reported a significant association of ciprofloxacin-resistant extraintestinal \textit{E. coli} with phylogeny group A and ciprofloxacin susceptibility with group B2, which is similar to our study findings.\[15,16\]

Our study of the ability of isolates to produce antimicrobial degrading enzymes such as ESBLs, AmpC, carbapenemase, and MBLs indicated that beta lactamase producing isolates occurred with greater frequency in group A and B1 isolates when compared with B2 and D, a finding which is in accordance with other studies.\[15,17\] This finding indicates that strains belonging to phylogroups A and B1 were carrying more resistance properties than the strains belonging to phylogroups B2 and D. By this finding, it may be assumed that in our population a large number of people act as a reservoir for such resistant strains. This may be due to inappropriate antibiotic use in both animals and humans.

### Table 2: Expression of phenotypic properties associated with virulence and antimicrobial resistance among ExPEC strains

<table>
<thead>
<tr>
<th>Phenotypic properties</th>
<th>A (n=61)</th>
<th>B1 (n=27)</th>
<th>B2 (n=104)</th>
<th>D (n=108)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biofilm production</td>
<td>29 (47.5)</td>
<td>14 (51.8)</td>
<td>51 (49.1)</td>
<td>35 (32.4)</td>
</tr>
<tr>
<td>Hemolysis on sheep blood agar</td>
<td>12 (19.6)</td>
<td>4 (14.8)</td>
<td>39 (37.5)</td>
<td>21 (19.4)</td>
</tr>
<tr>
<td>Lactose fermentation</td>
<td>58 (95.1)</td>
<td>26 (96.3)</td>
<td>86 (82.7)</td>
<td>91 (84.3)</td>
</tr>
<tr>
<td>ESBL production</td>
<td>42 (68.8)</td>
<td>20 (74.1)</td>
<td>72 (69.3)</td>
<td>78 (72.2)</td>
</tr>
<tr>
<td>AmpC producers</td>
<td>20 (32.7)</td>
<td>12 (44.4)</td>
<td>23 (22.1)</td>
<td>40 (37.1)</td>
</tr>
<tr>
<td>Carbapenemase producers</td>
<td>5 (8.2)</td>
<td>10 (37.1)</td>
<td>4 (3.8)</td>
<td>10 (9.3)</td>
</tr>
<tr>
<td>MBL producers</td>
<td>3 (4.9)</td>
<td>7 (25.9)</td>
<td>3 (2.8)</td>
<td>4 (3.70)</td>
</tr>
</tbody>
</table>

*Percentage of ExPEC strains exhibiting a particular phenotypic character. ESBL: Extended spectrum of beta-lactamase, MBL: Metallo-beta-lactamases.

### Conclusion

Results of our study indicate that pathogenic strains belonging to phylogenetic groups B2 and D express more virulent properties but were seen to be more susceptible to antibiotics than phylogroups A and B1 isolates. This finding indicates a need for regular monitoring of antimicrobial susceptibility in commensal strains as these strains might transfer the property of antimicrobial resistance to pathogenic strains. There is also an urgent need to undertake measures which would prevent the development of multidrug resistance among commensal strains such as reducing inappropriate antibiotic treatment, the overuse of antimicrobials in the agricultural industry which might contribute to increased numbers of multidrug resistant strains. Clearly, antimicrobial resistance may lead to decreased therapeutic outcomes in the near future.

### Acknowledgment

We would like to thank API Karnataka for their financial support and Manipal University for permitting us to conduct the study.

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6. O’Toole GA, Kolter R. Initiation of biofilm formation in \textit{Pseudomonas fluorescens} WCS365 proceeds via multiple,


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Perception of Simulation-based Learning among Medical Students in South India

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Abstract

Background: Traditional methods of educating medical students are no longer sufficient in the current era largely influenced by multimedia. Simulation-based techniques may play a pivotal role in bridging this educational gap. Aim: This study was conducted to explore the perception of medical students towards simulation-based learning (SBL). Subjects and Methods: This cross-sectional study was conducted in May 2013 in a private medical college in Mangalore, Karnataka, India. A total of 247 participants from fourth, sixth, eighth semester and internship were chosen by convenience sampling method. Attitudinal data on perception towards SBL were collected using a self-administered questionnaire with responses in a 5-point Likert’s scale. Results: The mean age of students was 21.3 (standard deviation 1.9) years, and males constituted 55.5% (137/247). Most participants 72.5% (179/247) had favorable perceptions of SBL, with scores of 92–118 out of a possible 118 points. Favorable perception towards SBL was seen significantly more among female students (P = 0.04) and senior MBBS students of sixth and eighth semesters (P = 0.05). Nearly, all students (90.7%; 224/247) agreed that simulation supports the development of clinical skills. As many as 29.6% (73/247) agreed that real patients might be replaced with simulated patients in practical examinations. Conclusion: SBL was perceived as favorable by a large number of participants in this study indicating a bright prospect for its implementation in the medical curriculum.

Keywords: Interns, Medical students, Perception, Simulation-based learning

Introduction

The current medical education training system regarding clinical care of patients in terms of history taking, physical examination, diagnosis and management in medical schools has been reported to be inadequate by students even after graduation.[¹] Not seeing a number of important diagnosis during training periods could pose a significant knowledge gap which eventually gets carried forward to internship and would eventually affect patient care.[¹][²]

Use of simulation in teaching medicine could be a solution to bridge this knowledge gap.[¹] Simulation is an instructional process that substitutes real patient encounters with artificial models created by screen-based computer simulations, partial-task simulators and high-fidelity whole body mannequins. Simulators replicate patient care scenarios in a realistic environment and also have the benefit of enabling repetition of the same scenario in a controlled environment. This allows practice without risk to the patient thereby minimizing chances of medical error. Furthermore, the recording and feedback options in modern simulators make them a useful tool for student assessment.[⁴][⁵] Simulation-based learning (SBL) can also provide an ideal background for learning and improving teamwork skills and behavioral skills. These skills are essential for trainees as health care delivery has become increasingly a multidisciplinary activity.[⁶][⁷] It is therefore not surprising that prior research has favored integration of SBL into the formal medical curriculum.[⁸][⁹]
Health services and academic institutions around the world are now recognizing the importance of SBL and are establishing more of skills labs in simulated clinical settings. Professional and regulatory organizations have also begun to accept teaching hours spent on simulation-based practice as a proxy for some clinical placements or operative skills.\(^{11-14}\)

In spite of advancements in the field of SBL elsewhere, it has so far not been formally introduced in medical colleges in our settings. With this background, this study was conducted to explore the perception of medical students in South India towards SBL.

**Subjects and Methods**

This cross-sectional study was done in a private medical college in Mangalore city in May 2013. The ethical approval for conducting this study was obtained from Institutional Ethics committee. Permission to conduct the study was subsequently obtained from the head of the institution. A sample size of 178 was determined using a confidence level of 95% with 15% degree of precision of the expected proportion and an estimated minimum prevalence of favorable perception toward SBL to be 50%. Adding a nonresponse rate of 10%, the final minimum sample size was calculated as 196. Students were chosen from the fourth, sixth, eighth semester and housemanship through convenience sampling method so that the sample will have a representation of second, third, final phase medical students and junior doctors of this institution. The students were briefed about the objective of the study and written informed consent was taken for their participation. Only students who have heard about SBL were invited to participate in this study.

A pretested self-administered semi-structured questionnaire was used for data collection which contained questions on demographic information and perception towards SBL.

The questionnaire was subjected to a pilot trial on 10 students before it was distributed in its final form. Each response for the question meant to assess perceptions towards SBL was given responses “strongly disagree,” “disagree,” “neutral,” “agree,” and “strongly agree” on a Likert’s scale with scores ranging from 1 to 5 points respectively. Scoring was done in the reverse order for negative questions on perception. Cumulative scores from 38 to 64 was considered poor, 65–91 as neutral and 92–118 as favorable level of perception.

Incompletely filled questionnaires were excluded from the analysis. The data entry and analysis were done using Statistical Package for Social Sciences software package (SPSS Inc., Chicago, IL, USA) version 16. Chi-square test was used to find out the association of demographic variables with the perception level among participants. \(P \leq 0.05\) was taken as a statistically significant association.

**Results**

Of the total 265 participants who took part in this study completely filled in questionnaires were returned by 247 participants. Among these 247 participants, about half were males 55.5% (137/247). Mean age of students was 21.3 (standard deviation 1.9) years (95% confidence interval being 21.1 years to 21.5 years). Most participants had a favorable perception about SBL 72.5% (179/247) and the rest had a neutral perception 27.5% (68/247). None of the students had poor perception towards SBL. Age of students was not influencing their perception towards SBL \((P = 0.82)\). Favorable perception towards SBL was seen significantly higher among female students \((P = 0.04)\) and senior MBBS students of sixth and eighth semesters \((P = 0.05)\) [Table 1].

Nearly, all students (90.7%, 224/247) felt that simulation can support the development of clinical skills. But most students felt that repeated use of SBL in medical training would adversely affect communication skills, team behavior, ethical values and feeling of empathy to real patients. Although most participants believed that SBL can create a highly realistic, safe and reproducible learning environment, majority were not in favor of replacing real patients in clinical examinations with simulators. Most participants were also concerned about the cost associated with simulation equipments. However, majority did not feel that use of SBL will any way minimize role of teachers in training process nor did they think that teachers would minimize their efforts in training students by use of simulators [Table 2].

**Table 1: Association between sociodemographic variables with perception towards SBL among participants**

<table>
<thead>
<tr>
<th>Socio-demographic characteristics</th>
<th>Neutral perception (%)</th>
<th>Good perception (%)</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>≤ 19</td>
<td>11 (24.4)</td>
<td>34 (75.6)</td>
<td>45</td>
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<tr>
<td>20-21</td>
<td>30 (29.7)</td>
<td>71 (70.3)</td>
<td>101</td>
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<tr>
<td>22-23</td>
<td>18 (29)</td>
<td>44 (71)</td>
<td>62</td>
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<tr>
<td>≥ 24</td>
<td>9 (23.1)</td>
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<td>39</td>
</tr>
<tr>
<td>(\chi^2=0.912), (P=0.82)</td>
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<tr>
<td><strong>Gender</strong></td>
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<tr>
<td>Males</td>
<td>45 (32.8)</td>
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<tr>
<td>(\chi^2=4.36), (P=0.04)</td>
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<tr>
<td><strong>Nationality</strong></td>
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<tr>
<td>Indians</td>
<td>61 (27.2)</td>
<td>163 (72.8)</td>
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<tr>
<td>Foreigners</td>
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<tr>
<td><strong>Term of study</strong></td>
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<tr>
<td>4th semester</td>
<td>46 (34.8)</td>
<td>86 (65.2)</td>
<td>132</td>
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<tr>
<td>6th semester</td>
<td>7 (17.1)</td>
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<td>8th semester</td>
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</tr>
<tr>
<td>Internship</td>
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</tr>
<tr>
<td>(\chi^2=7.9), (P=0.05)</td>
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<tr>
<td>Total</td>
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SBL: Simulation-based learning

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SBL: Simulation-based learning
Discussion

Medical training in the current era is multimodal and SBL may play a pivotal role in improving training standards in medical schools. In this study, nearly three-fourth of students had a favorable perception about SBL indicating a bright prospect for its acceptance if implemented in future as a part of training.

Favorable perception towards SBL was seen significantly more among senior MBBS students of sixth and eighth semesters. This could be because of increase in course requirements felt among senior MBBS students of sixth and eighth semesters.

Of the 247 participants, 20 had undergone formal training in at least one simulation-based procedure. The most common procedure in which training was received was in life support—65% (13/20) [Table 3]. Most of these students underwent training at private hospitals—70% (14/20) and most were trained by resource person for training—65% (13/20) [Table 3].

Table 2: Distribution of perception scores on various aspects of SBL among medical students (n=247)

<table>
<thead>
<tr>
<th>Questions on various aspects of SBL</th>
<th>Number of students who strongly agreed/agreed (%)</th>
<th>Number of students with neutral response (%)</th>
<th>Number of students who disagreed/strongly disagreed (%)</th>
<th>Mean score (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Can simulation support the development of clinical skills?</td>
<td>224 (90.7)</td>
<td>12 (4.9)</td>
<td>11 (4.4)</td>
<td>4.25 (0.74)</td>
</tr>
<tr>
<td>Can simulation help to see and manage even the rarest of cases in medicine?</td>
<td>167 (67.6)</td>
<td>53 (21.5)</td>
<td>27 (10.9)</td>
<td>3.78 (0.92)</td>
</tr>
<tr>
<td>Do you think SBL can help to address the following problems faced by students during clinical postings?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimize standing hours</td>
<td>187 (75.7)</td>
<td>41 (16.6)</td>
<td>19 (7.7)</td>
<td>3.95 (0.89)</td>
</tr>
<tr>
<td>Reduce overcrowding</td>
<td>201 (81.4)</td>
<td>29 (11.7)</td>
<td>17 (6.9)</td>
<td>3.99 (0.83)</td>
</tr>
<tr>
<td>Reduce learners’ fatigue</td>
<td>188 (76.1)</td>
<td>40 (16.2)</td>
<td>19 (7.7)</td>
<td>3.91 (0.89)</td>
</tr>
<tr>
<td>Overcome the problem of uncooperative patients</td>
<td>195 (78.9)</td>
<td>36 (14.6)</td>
<td>16 (6.5)</td>
<td>4.04 (0.89)</td>
</tr>
<tr>
<td>Solve the problem of getting patients during exams</td>
<td>161 (65.2)</td>
<td>52 (21.1)</td>
<td>34 (13.7)</td>
<td>3.74 (1.04)</td>
</tr>
<tr>
<td>Minimize the stressful learning environment usually seen in wards</td>
<td>162 (65.6)</td>
<td>49 (19.8)</td>
<td>36 (14.6)</td>
<td>3.73 (1.04)</td>
</tr>
<tr>
<td>Overcome language barrier</td>
<td>175 (70.9)</td>
<td>39 (15.8)</td>
<td>33 (13.3)</td>
<td>3.89 (1.06)</td>
</tr>
<tr>
<td>Will constant usage of SBL lead to deterioration in communication skills with the patients?</td>
<td>187 (75.7)</td>
<td>40 (16.2)</td>
<td>20 (8.1)</td>
<td>3.95 (0.93)</td>
</tr>
<tr>
<td>Do you feel that repeated practice of the procedure in SBL will improve the performance of the user?</td>
<td>164 (66.4)</td>
<td>66 (26.7)</td>
<td>17 (6.9)</td>
<td>3.83 (0.9)</td>
</tr>
<tr>
<td>Do you feel that SBL might improve patient safety?</td>
<td>133 (53.8)</td>
<td>80 (32.4)</td>
<td>34 (13.8)</td>
<td>3.59 (0.6)</td>
</tr>
<tr>
<td>Do you feel that SBL can replace live patients in practical examination?</td>
<td>73 (29.6)</td>
<td>57 (23.1)</td>
<td>117 (47.3)</td>
<td>2.71 (1.21)</td>
</tr>
<tr>
<td>Do you think that SBL will hamper the role of team efforts by minimizing role identity in an emergency situation?</td>
<td>166 (67.2)</td>
<td>56 (22.7)</td>
<td>25 (10.1)</td>
<td>3.69 (0.91)</td>
</tr>
<tr>
<td>Do you believe that the feedback provided by SBL at the end is better than that of bedside teaching?</td>
<td>88 (35.6)</td>
<td>91 (36.8)</td>
<td>68 (27.6)</td>
<td>3.1 (1.1)</td>
</tr>
<tr>
<td>Do you feel that SBL should be integrated into the medical educational curriculum?</td>
<td>170 (68.8)</td>
<td>57 (23.1)</td>
<td>20 (8.1)</td>
<td>3.81 (0.88)</td>
</tr>
<tr>
<td>Will SBL help to increase the confidence levels of students while dealing with real patients?</td>
<td>161 (65.2)</td>
<td>48 (19.4)</td>
<td>38 (15.4)</td>
<td>3.67 (1.48)</td>
</tr>
<tr>
<td>Do you feel that SBL can be used as an adjuvant for clinical practice and not as a replacement to it?</td>
<td>195 (78.9)</td>
<td>38 (15.4)</td>
<td>14 (5.7)</td>
<td>4.09 (0.92)</td>
</tr>
<tr>
<td>Do you feel that SBL makes learning medicine easier?</td>
<td>180 (72.9)</td>
<td>55 (22.3)</td>
<td>12 (4.8)</td>
<td>3.86 (0.78)</td>
</tr>
<tr>
<td>Do you feel that SBL can create a highly realistic, safe and reproducible learning environment?</td>
<td>137 (55.5)</td>
<td>54 (21.9)</td>
<td>56 (22.6)</td>
<td>3.46 (1.07)</td>
</tr>
<tr>
<td>Do you feel that SBL will minimize the role of the teacher?</td>
<td>86 (34.8)</td>
<td>60 (24.3)</td>
<td>101 (40.9)</td>
<td>2.88 (1.2)</td>
</tr>
<tr>
<td>Do you feel that SBL will be relatively costly than employing a trained resource person for training?</td>
<td>143 (57.9)</td>
<td>75 (30.4)</td>
<td>29 (11.7)</td>
<td>3.64 (0.97)</td>
</tr>
<tr>
<td>Do you feel that importance of ethical issues will be reduced by repeated usage of SBL?</td>
<td>157 (63.6)</td>
<td>67 (27.1)</td>
<td>23 (9.3)</td>
<td>3.91 (3.32)</td>
</tr>
<tr>
<td>Do you feel that the teacher will minimize his or her efforts in clinical teaching if SBL becomes a part of the medical curriculum?</td>
<td>134 (54.3)</td>
<td>66 (26.7)</td>
<td>47 (19.0)</td>
<td>3.48 (1.06)</td>
</tr>
<tr>
<td>Do you feel that SBL should replace the use of animals in medical experiments?</td>
<td>150 (60.7)</td>
<td>59 (23.9)</td>
<td>38 (15.4)</td>
<td>3.67 (1.12)</td>
</tr>
<tr>
<td>Do you feel that more of SBL will minimize the empathy among doctors towards patients?</td>
<td>130 (52.6)</td>
<td>68 (27.5)</td>
<td>49 (19.9)</td>
<td>3.49 (1.1)</td>
</tr>
</tbody>
</table>

SBL: Simulation-based learning, SD: Standard deviation

Of the 247 participants, 20 had undergone formal training in at least one simulation-based procedure. The most common procedure in which training was received was in life support—60% (12/20). Most of these students underwent training at private hospitals—70% (14/20) and most were trained by resource person for training—65% (13/20) [Table 3].
Table 3: Distribution of training details of students in various simulation-based procedures (n=20)

<table>
<thead>
<tr>
<th>Training characteristics</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of procedure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basic life support</td>
<td>6</td>
<td>30</td>
</tr>
<tr>
<td>Advanced life support</td>
<td>6</td>
<td>30</td>
</tr>
<tr>
<td>Management of normal labor</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Animal experiments</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Surgical procedures</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>Place of training</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Government hospital</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Private hospital</td>
<td>14</td>
<td>70</td>
</tr>
<tr>
<td>Private clinics</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Trainer particulars</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medical officers</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Specialist</td>
<td>13</td>
<td>65</td>
</tr>
<tr>
<td>Parents who were practitioners</td>
<td>4</td>
<td>20</td>
</tr>
</tbody>
</table>

Limitations

Few studies have been done with regards to the role of simulators in training medical professionals and what students perceived of such type of teaching methods. Such innovative teaching methods will help to improve the quality medical care that meets the health needs of individuals, families and communities.

Strengths

Very few studies have been done with regards to the role of simulators in training medical professionals and what students perceived of such type of teaching methods. Such innovative teaching methods will help to improve the quality medical care that meets the health needs of individuals, families and communities.

Conclusion

Implementation of SBL in medical colleges has been perceived favorably by a large number of participants, particularly female students and senior medical students. While the use of SBL is likely to expand with the modernization of medicine and the advent of new technologies and methods, policy development is needed to ensure its coordinated and cost-effective implementation.
be generalizable to other settings. Even though SBL was perceived positively by students, it remains unclear whether the skills acquired with this teaching methodology transfer to the real-world settings such as improvement in patient care. Further research is needed to evaluate these aspects.

**Acknowledgments**

The authors would like to thank all MBBS students and interns of Kasturba Medical College, Mangalore, who took part in this study.

**References**

31. Issenberg SB, McGaghie WC, Gordon DL, Symes S, Petrusa ER,


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Introduction

Perinatal transmission of HIV can occur in utero, during delivery and postpartum through breastfeeding. The risk of perinatal HIV transmission depends on factors such as maternal viral load, infant mode of delivery, the presence of other sexually transmitted infections, and duration of ruptured membranes.\(^1,3\)

The risk of HIV transmission from an infected mother to a child varies from 15% to 30%.\(^4\) This risk can be reduced to <2% with appropriate intervention using combination antiretroviral (ARV) therapy during pregnancy and labor, delivery through cesarean section for HIV-infected mothers with unknown viral load or viral load >1000 copies/ml and avoidance of breastfeeding among HIV-infected women.\(^9\) This has resulted in a dramatic decline in the number of children with perinatal HIV infection from an estimated annual peak of 1650 infected infants in mid-1990s to 142 infants in 2005 in the United States.\(^5-8\) The framework for the elimination of...
perinatal transmission of HIV starts with the early identification of pregnant women who are HIV-infected. Initially, the recommendation was targeted HIV screening of pregnant women, which was eventually changed to routine screening for all pregnant women in the United States.\(^9,10\)

Nevada documented its highest cases of perinatally acquired HIV infection with eight confirmed cases in 1998 and was identified by the Centers for Disease Control and Prevention (CDC) as the only state west of the Mississippi River with an increasing number of women of childbearing age living with HIV infection in 2004.\(^11,12\) Although Nevada had the nation’s 35th largest population at the time, it ranked 14th in the nation for the rate of adolescents/adults living with AIDS.\(^13\) Six new cases of perinatally acquired HIV infections were documented in Clark County over a 9 months period from September 2005 to June 2006.\(^14\) Clark County comprises the southern tip of Nevada and includes the cities of Las Vegas, Henderson, North Las Vegas and Boulder City.

The Department of Pediatrics at the University of Nevada-School of Medicine received a Community Access to Child Health (CATCH) grant from the American Academy of Pediatrics (AAP) to determine why children were still being infected with HIV in Clark County. The medical records of HIV-infected pregnant women who delivered an infant from July 2005 to June 2006 were reviewed, and common systemic barriers to prevention were identified.\(^15\) Subsequently, funding from the Sawyer Foundation was utilized to develop and implement an intervention program based on the six-step process developed by the Quality Enhancement Research Initiative (QUERI) of the Department of Veteran Affairs (VA) was received.\(^16\) The program integrated public health services provided by the county health department (Southern Nevada Health District [SNHD]) with clinical care for HIV-infected pregnant women and HIV-exposed infant provided by a county hospital and the medical school, with a focus to reduce or eliminate mother-to-child transmission (MTCT) of HIV. This report summarizes the process and outcome of the integrated program. The institutional review boards of University of Nevada, Reno and the University Medical Center of Southern Nevada (UMC) approved this project.

**Subjects and Methods**

**Subjects**

Approximately 72% of the population in Nevada and 87% of people infected with HIV live in Clark County.\(^17\) The Wellness Center at the UMC provides medical care to the majority of adults with HIV including women in Clark County. The Nevada Care Program at the University of Nevada School of Medicine (UNSOM) provides care to pregnant women and HIV-exposed and infected children in Clark County. The SNHD provides surveillance and case management for HIV-infected persons and Aid for AIDS of Nevada provides social services for individuals infected with HIV in Clark County.

**Methods**

The method was based on the principles of implementation science developed in 1998 by the VA QUERI and community-based participatory research.\(^16\) The six-step approach includes: (a) identify a high burden clinical issue (b) identify evidence-based clinical practice guidelines (c) define existing practices and identify performance gaps (d) develop and implement an intervention (e) evaluate system improvement (f) evaluate health outcomes and disseminate findings.

**Identify a high burden clinical issue**

The confidential name-based HIV reporting system that was established in 1992 and is maintained by the SNHD was reviewed to identify HIV-infected women of childbearing age. Data were extracted on women who delivered an infant between July 2005 and June 2006, and their prenatal and hospital delivery records were reviewed. The medical records of HIV-exposed infants maintained by the UNSOM Department of Pediatrics were also reviewed. Data and medical record reviews were performed by a pediatric infectious disease specialist with expertise in the care of patients with HIV, who was assisted by a trained program coordinator.

**Identify evidence-based clinical practice guidelines**

The medical literature was reviewed to identify current evidence-based guidelines and recommendation in 2006, to reduce MTCT including those from the CDC, American College of Gynecologist, AAP; United States Preventive Services Task Force (USPSTF) and the Institute of Medicine (IOM).\(^18-21\) All of the above agencies identified routine prenatal HIV testing as a rate-limiting step toward prevention of mother-to-child HIV transmission (PMTCT) and lack of coordination of care as creating missed opportunities for preventing MTCT. HIV-infected pregnant women identified early in pregnancy who are able to be enrolled in prenatal care and have access to available ARV therapy are shown to reduce significantly the risk of HIV transmission. HIV-infected pregnant women identified during labor are still able to reduce the risk of transmission to <10% when they receive ARV therapy during labor, and prophylactic ARV therapy is administered to their HIV-exposed infant.

**Define existing practices and identify performance gaps**

Detailed review of cases where MTCT occurred was performed to identify performance gaps based on six main variables: (1) adequacy of prenatal care (2) HIV diagnoses of expectant mothers prior to delivery (3) appropriate use of ARV therapy before delivery (4) appropriate use of cesarean section for delivery (5) adequacy of zidovudine prophylaxis to newborn (6) HIV transmission rate.

In 2005–2006, pregnant women in Nevada were only screened for HIV if they were identified as “high risk” by their obstetricians. Only one hospital in Clark County had
a well-defined protocol for screening pregnant women who presented in labor with unknown HIV status. Only one center provided comprehensive care for HIV-infected pregnant women in Clark County with a population of 1.9 million in 2006. SNHD maintains data on individuals with HIV/AIDS, but there were no systematic methods for the hospitals, obstetricians, and pediatricians to confirm the HIV status of pregnant women to allow for early intervention. No pediatric program was dedicated to the follow-up of infants who had been perinatally exposed to HIV, and there was no defined communication between the SNHD, the obstetricians and pediatrician providing surveillance, or prenatal and pediatric care.

**Design an intervention and define outcome measures**

Information obtained from our review of performance gaps was used to design a program to address identified barriers. Five community organizations were invited to partner with the UNSOM in developing an intervention program that would integrate services provided by SNHD and community organizations with clinical services provided by the UNSOM Departments of Pediatrics and Obstetrics. Each agency was assigned specific roles [Figure 1]. A 7-step integrated intervention for the care of HIV-infected pregnant women and HIV-exposed infants was developed [Table 1]. For the purpose of this study and in order for consistency in evaluating pre- and post-intervention outcomes, certain terms were defined as follows: (a) adequate prenatal care was defined as cases where a pregnant woman with HIV had at least two prenatal visits prior to delivery; (b) HIV-infected pregnant women were considered to have received appropriate ARV therapy during pregnancy if the combination ARV therapy prescribed was “preferred” in USPSTF’s recommendations for HIV treatment were instituted prior to labor; (c) appropriate use of cesarean section for delivery was defined as cesarean section delivery for a HIV-infected pregnant woman with the most recent viral load prior to labor above 1000 copies/ml or for other medical reasons including previous cesarean section; (d) adequacy of zidovudine prophylaxis to newborn was defined as initiation of zidovudine within 12 h of birth and completion of prophylaxis for a 6 weeks following birth; (e) a child was defined to be HIV positive if he/she was <18 months with detectable HIV

![Figure 1](image-url): (a) Southern Nevada Health District, (b) University Medical Center of Southern Nevada Wellness Center, (c) Area Health Education Center, (e) Aid for AIDS of Nevada, (f) University of Nevada School of Medicine


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pro-viral particle by qualitative HIV DNA polymerase chain reaction on two separate specimens and HIV negative if he/she had two negative HIV test obtained at or after 1-month.

Evaluate system improvement
Six predefined outcome measure were used to evaluate system improvement after 6 years (2007–2012).

- adequacy of prenatal care
- HIV diagnoses of expectant mothers prior to delivery
- appropriate use of ARV therapy before delivery
- appropriate use of cesarean section for delivery
- adequacy of zidovudine prophylaxis to newborn
- HIV transmission rate.

Evaluate health outcomes and disseminate findings
Final HIV sero-status of the infant using appropriate diagnostic test for exposed infants was used to evaluate and define health outcomes and health-related quality of life. All descriptive and inferential statistics were calculated using IBM SPSS version 15.0.

Results

Overall
A review of the database for HIV infection maintained by the SNHD revealed that the final HIV status of all infants born between 2000 and 2004 were indeterminate due to “loss to follow-up” that did not allow a final HIV status to be established. In 2005, there were 5406 persons living with HIV/AIDS in Clark County, 495 (9%) were women of childbearing age. Twenty-six infants were born to women with HIV between September 2005 and June 2006. One hundred and five infants were born to women with HIV from 2007 to 2012. Demographic information is provided in Table 2. Of the HIV-positive women, 69% had Medicaid, 5% were uninsured and 27% had private health insurance.

Prior to 2007, only one hospital implemented a rapid HIV testing during labor for women presenting in labor with unknown HIV sero-status. At the end of 2012, all ten local hospitals in Clark County with a labor and delivery unit had a protocol for rapid HIV testing for women presenting in labor with known HIV sero-status.

Evaluate system improvement
We evaluated improvements in the six predefined outcome measure after 6 years.

Among the 26 HIV-infected women who delivered prior to the implementation of the integrated program, 58% (15/26) had adequate prenatal care. Among women who delivered

<table>
<thead>
<tr>
<th>Table 1: Seven-step approach to care of HIV-infected pregnant women and HIV-exposed infants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive HIV tests are reported to the Clark County Health Department from all laboratories and physician practices as mandated by state law.</td>
</tr>
<tr>
<td>Health Department Surveillance team reaches out to contact patient, and if confirmed pregnant, brings it to the attention of the maternal-child HIV team.</td>
</tr>
<tr>
<td>Pregnant woman is assisted in enrolling into care at the wellness center under the care of an adult HIV specialist and an obstetrician. Patients are also assisted in identifying other care providers and obstetricians not within the county hospital system if they so desire.</td>
</tr>
<tr>
<td>Pregnant women are scheduled to meet with pediatrician to discuss expected plan of care for their infant once he/she is delivered. Education is provided with regards to risk of transmission and available interventions to reduce risk of transmission.</td>
</tr>
<tr>
<td>Social support is provided through the health department and AFAN including but not limited to transportation and housing as needed.</td>
</tr>
<tr>
<td>All pregnant women presenting in labor at all hospitals in Las Vegas were assessed for documented HIV sero-status. Rapid HIV testing is performed on all pregnant women with unknown HIV sero-status unless patient declines.</td>
</tr>
<tr>
<td>Patients are discussed during a monthly clinical meeting where barriers to care are assessed, identified and a specific plan was developed to eliminate or reduce barrier.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2: Demographic characteristics of mothers: Pre- and post intervention</th>
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</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Pre-intervention</td>
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<tr>
<td></td>
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<tr>
<td>Post-intervention</td>
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</table>

*Pre-intervention data collection began September 2005, **Pre-intervention ended June 2006
following the implementation of the integrated intervention, 85% (89/105) had adequate prenatal care. Preintervention, 46% (12/26) of HIV-positive mothers were diagnosed prior to pregnancy, 35% (9/26) during pregnancy, 4% (1/26) during labor and 15% after delivery. Postintervention, 63% (66/105) of HIV-positive mothers were diagnosed prior to pregnancy, 31% (33/105) during pregnancy, 5% (5/105) during labor and 1% (1/105) after delivery.

Lack of appropriate screening during pregnancy, labor and delivery led to missed opportunities to initiate ARV therapy to pregnant women. Of the 26 HIV-positive women who delivered from September 2005 to June 2006, 62% (16/26) received appropriate ARV therapy during pregnancy, and 73% (19/26) received ARV therapy during labor. Postintervention, 81% (85/105) of the HIV-positive women, received ARV therapy during pregnancy, and 86% (90/105) received ARV therapy during labor.

Preintervention, 62% (16/26) of HIV-infected pregnant received appropriate delivery by cesarean section. Postintervention, cesarean sections were performed on 74% (78/105) of the HIV-infected mother and all (100%) were judged to be medical appropriate. We made this determination by reviewing our cesarean section cases and found that the majority of cases were due to the history of previous cesarean and request by the pregnant women for repeat cesarean section. Cesarean sections were not related to persistent lack of viral suppression.

Only 69% (18/26) of the 26 infants born from September 2005 to June 2006 received adequate zidovudine prophylaxis defined as initiation of zidovudine within 12 h of birth and 54% (14/26) had documented completion of 6 weeks therapy with zidovudine. Postintervention, 87% (91/105) of HIV-exposed infants, received zidovudine within 12 h of birth, and 87% (91/105) had documented completion of 6 weeks therapy with zidovudine.

Six infants were documented to have been infected with HIV among the 26 infants delivered during the preintervention period September 2005 through June 2006. No MTCT was documented among the 105 infants born postintervention from 2007 to 2012.

**Discussion**

The World Health Organization in its recent publication of PMTCT strategic vision 2010–2015 outlined that “priority will be given to strengthening linkages between PMTCT and HIV care and treatment services for women, their children, and other family members in order to support an effective continuum of care.”[22] Such an integrated approach between public health departments, primary care practices and hospitals, provides support to women who are known to be HIV-infected or test positive for HIV during pregnancy and is also in line with the World Health Organization and the IOM’s recommendations.[22,23]

The result from our program validates the point that MTCT of HIV can be eliminated when an integrated approach to the care of HIV-infected women and exposed newborns is implemented.

Although women accounted for an estimated 8% of HIV/AIDS infected adults in 1985, that proportion had increased steadily to an estimated 25% of new infections in 2006, with a majority of infections occurring among women of childbearing age.[24] Studies evaluating missed opportunities to prevent perinatal HIV transmission in the United States conducted between 1996–2000 and 2005–2008 noted that the majority of HIV-infected pregnant women had one or more missed opportunities to prevent perinatal HIV transmission.[25]

In response to the six cases of perinatal HIV-infection in newborns in 2005–2006, we began the process of implementing the comprehensive HIV program in Clark County. To obtain buy in from the community hospitals, we formed the community advisory board for the elimination of pediatric HIV. The advisory board was comprised of staff representatives from pediatric and adult HIV clinics, nursing case managers from the county Health Department and hospital representatives from the 10 local hospitals that had a delivery unit. This committee met once a month and reviewed the national HIV perinatal guideline to produce a local version that detailed the management of women who present in labor with known and unknown HIV status and their infants. These hospital representatives, who were in most cases nurses and directors of their hospital delivery units, were critical to the success of our program. They acted as liaisons between the hospitals and the SNHD. They were the contact person to verify the HIV status of women presenting in labor through the county name-based database. The privacy and confidentiality requirement was maintained through existing memorandum between the hospitals and the health department.

Once the local guidelines were approved by the hospitals, we embarked on a 6 months training session where in-service trainings were conducted in all the hospitals. Each hospital had four training sessions organized in such a manner that it occurred during the morning and evening hand-over session and included the nursing and laboratory staff. This allowed the team the opportunity to reach most of the nursing and laboratory staff including those on day and night shifts. The training curriculum included appropriate test requisitions, rapid HIV testing by the laboratory team, a protocol for the management of the HIV-exposed infant including the appropriate blood specimen for testing.

The program team met once a month with the nursing case management team from the health department. Under an existing memorandum of understanding, we discussed each new HIV case documented in a pregnant woman. We also reviewed the care of existing pregnant women who were in care to identify barriers to care especially social issues that included but were not limited to transportation and housing during pregnancy. We
discussed postpartum follow-up and discharge of infants from the program to their regular pediatricians once HIV infection had been ruled out. A result of the work of the program team was mandatory HIV screening for all pregnant women, which was implemented in Nevada following the passage of Nevada Senate Bill 266. This bill was passed in 2007 and was instrumental for enhanced identification of HIV-infected women who became pregnant.\(^{[26]}\) The comprehensive nature of this intervention and the taskforce approach was instrumental to reducing the missed opportunities for PMTCT in our community.

After the integrated intervention, women who were identified early in their pregnancy had multiple opportunities to be brought into care. The integrated approach provided coordination and collaboration where prenatal care was provided by an obstetrician. In addition, they meet at least once with the pediatric infectious diseases physician during pregnancy to discuss factors that affected the risk of perinatal transmission and to develop a plan of care once the infant was delivered. Our report shows that such initial contact with a pediatrician led to increased compliance with follow-up of the infant and compliance with postnatal zidovudine prophylaxis. Approximately 69% of our patient had some form of public sponsored insurance which presented an initial challenge to enrollment into prenatal care, but ultimately removed cost as a barrier to prenatal care attendance.

Our report shows that lack of prenatal screening for HIV during pregnancy and labor increased the risk that HIV-infected women were not identified in time and opportunities for initiating zidovudine prophylaxis were missed. The lack of a coordinated program led to poor follow-up of HIV-exposed infants and adherence to zidovudine prophylaxis during the preintervention period which improved dramatically to 87% postintervention. We believe that early involvement of a pediatrician can tremendously improve follow-up after delivery as relationship are established early, and a clear follow-up plan is developed. This analysis showed that all cases of perinatal transmission in the preimplementation period of the integrated program were among minority women (25% Hispanics and 75% African American) who had limited or no prenatal care. This is consistent with recent results in the US that showed that during 2007–2009, 85% of diagnoses of perinatal HIV-infection were in Blacks (63%) or Hispanics (22%).\(^{[27]}\) Overall, while the proportion of women with HIV infection has risen in the US, the number of reported cases of perinatal transmission has declined from an estimated annual peak of 1650 infected infants in mid-1990s to 162 infants in 2010.\(^{[27]}\) Our finding of no cases of perinatal transmission diagnosed postimplementation is consistent with other studies that showed reductions in perinatal transmission with successful implementation of strategies aimed at identifying HIV-infected pregnant women before or early in pregnancy, treating HIV-infection in pregnant women with highly active ARV therapy, zidovudine prophylaxis during labor and delivery, and zidovudine prophylaxis to HIV-exposed infants.\(^{[10]}\)

**Limitations**

Our analysis has a number of limitations. First, it is a single site experience, and even though it included all deliveries in the county, our experience may be different from other part of the country. Second, care for HIV-infected adults and children were concentrated in two major medical centers in our community, and it was easier to implement an integrated program. It may be more difficult to implement in communities without consolidated HIV care centers. Thirdly, successful passage of the Nevada Senate Bill mandating HIV screening of all pregnant women in our community could have made our educational and outreach interventions more successful by allowing for increased communication between obstetricians and pediatricians and greater collaboration among HIV service organization, hospitals and the health district.

**Conclusion**

Our study indicated that an integrated approach to the care of pregnant HIV-infected women and their newborns can led to a reduction in missed opportunities for prevention and eventual eradication of perinatal HIV transmission by increasing the odds that the women and their newborns receive recommended interventions.

**Ethical approvals**

This project received ethical approval from the University of Nevada Institutional Review Board and the Institutional Review Board of the University Medical Center of Southern Nevada.

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7. Centers for Disease Control and Prevention (CDC).

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Conflict of Interest: None declared.
Influence of Intensity and Duration of Yoga on Anxiety and Depression Scores Associated with Chronic Illness

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Abstract
Background: Chronic illness is commonly associated with anxiety and depression. Both anxiety and depression respond to yoga. However, there is no report on the association between the intensity and duration of yoga practice with the benefits seen. Aim: The present study was intended to determine whether the daily duration of yoga practice and the duration of experience in months would predict anxiety and depression, associated with chronic illness. Subjects and Methods: Seven hundred and sixty-three volunteers with ages between 14 and 86 years (group mean age standard deviation, 50.2 [14.2]) who attended a 7 day residential yoga camp in the north of India were included in this cross-sectional study. All participants had chronic illnesses, which were under control with treatment, and which were categorized and are detailed. Participants were assessed for state anxiety scores using State-Trait Anxiety Inventory and for anxiety with hospital anxiety and depression scale (HADS-A), and depression was assessed using HADS-D scores of the HADS. Linear multiple regression analyses were performed using PASW SPSS version 18.0 (Armonk, New York, U.S.) to determine how the daily and monthly duration of yoga practice could influence state anxiety, hospital anxiety and depression of the participants. Results: Yoga practice in months and the time spent practicing yoga each day significantly predict the level of state anxiety (P < 0.001, P = 0.03) and HAD-A (P < 0.01, P < 0.01). The duration of yoga practice in months alone was a significant predictor of the HAD-D (P < 0.01). Conclusions: The results suggest that the duration of yoga practice in months and daily practice in minutes predict anxiety associated with chronic illness. In contrast the duration of yoga practice in months alone, predicted depression scores.

Keywords: Anxiety, Chronic illness, Depression, Linear multiple regression, Yoga practice

Introduction
Physicians often focus on the somatic component of illness while emotional aspects are overlooked.[1] The reasons for this are usually valid. All the same emotional disorders require adequate consideration, as an emotional disorder (i) may frequently occur concomitantly with a somatic illness, or (ii) may present as a somatic disorder.[2-4] Chronic diseases are often associated with symptoms of an emotional disorder. Chronic diseases are being considered seriously the world over, as conditions such as heart disease, stroke, and diabetes are leading causes of death in the U.S.[5] and other countries.[6] The association between chronic disease and unhealthy lifestyle choices is becoming increasingly apparent.[7] As a result, nonpharmacological therapies that change the lifestyle are being considered in the management of many chronic diseases.[8] Among them, yoga has been shown to have therapeutic benefits and is useful in stress reduction.[9]

A review, conducted by searching three electronic databases (Ovid Medline, PsycINFO, CINAHL), with yoga as the keyword, yielded 2349 articles published between 1980 and 2007.[10] Among the articles, 861 considered yoga and chronic disease. The number of articles post-2007 is likely to
have increased. However, this article is referred to here, as it examined the importance of noting the frequency and duration of yoga sessions. The author, Yang (2007) stated that ‘the total dose of yoga training, which depends on both the duration and frequency of yoga sessions, also needs to be considered in evaluating and comparing yoga studies’.

As described earlier, chronic disease is often associated with an emotional component. Two aspects of emotional disorders that clinically have the most relevance are anxiety and depression. Both anxiety and depression have benefited through yoga practice. The present study was conducted on participants who had already been practicing yoga but had self-elected to enroll for a yoga program as they had a somatic disorder.

The aim of the study was to determine whether there would be associations between duration or intensity of yoga practice and the anxiety and/or depression experienced by these participants who already had a chronic somatic disease.

**Subjects and Methods**

Seven hundred and sixty-three participants self-elected to enroll for 7 day yoga based stress management program. Statistical calculation of the sample size was not done prior to the experiment. However, post-hoc analyses were carried out for the present study, with the sample size as 763 in each group. The power was calculated for the multiple linear regression of total time and time per day as a predictor of State-Trait Anxiety Inventory (STAI-S) using G* Power Software version 2.0 (University of Dusseldorf, Dusseldorf, Germany). The adjusted $R^2$ value was used, and an effect size of 0.028 (small) was found; however with a sample size of 763 (large) the power was 0.989. For HAD-A, the adjusted $R^2$ value was used and an effect size of 0.25 (large) was found; however with a sample size of 763 (large) the power was 0.6446. The $\alpha$-level was set as 0.05.

The program was held in a Residential Yoga Center in North India. Their ages ranged between 14 and 86 years (group mean standard deviation, 50.2 [14.2] years), 95% confidence interval (CI) ±1.01 (49.2, 51.2) and there were 260 females in the group. Recruitment was by advertisements in a television channel and a yoga magazine. To be included in the trial participants had to meet the following criteria (i) their chronic illness had to be under control through the use of conventional medicine or other remedies; verified by the appropriate tests, (ii) they had to be sufficiently physically and mentally healthy to perform the yoga techniques, and (iii) literate, to complete the questionnaires. Participants were excluded from the trial if they could not complete the questionnaires ($n=331$, 30.3%). Participants were excluded due to incomplete or inadequately completed questionnaires. The details of the participants are provided in Table 1. The signed consent of all participants was obtained. The project was approved by the Patanjali Research Foundation Ethics Committee, in February 2014. The study was completed between February 2014 and April 2014.

**Design**

The study was a single time, cross-sectional assessment.

**Assessments**

All participants were given with three questionnaires to fill in. These were (i) STAI-the subsection for state anxiety (II) the hospital anxiety and depression scale (HADS) and (iii) a set of questions to determine the participants’ experience and daily duration of yoga practice.

**State-trait anxiety inventory**

State anxiety was measured using a sub-scale of Spielberger’s STAI, which contains 20 items used to describe the intensity

<table>
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<tr>
<th>Table 1: Baseline characteristics of the 763 participants</th>
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<tr>
<td><strong>Characteristics</strong></td>
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<td>Diseases (blocks,[^3] number) %</td>
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[^1] Values are group mean (SD); SD: Standard deviation

[^2] **Table 1:** Baseline characteristics of the 763 participants

[^3] **Table 1:** Baseline characteristics of the 763 participants
of feelings at the moment of testing. The participants had to choose one out of the four options provided for each item that is, not at all = 1, somewhat = 2, moderately = 3, and very much so = 4. The STAI scores range from 20 to 80, and they increase in response to stress and decrease under relaxing conditions. The reliability and validity have been established for use in an Indian population.[16]

Hospital anxiety and depression scale
The HADS was used to assess anxiety and depression in the participants. This scale was designed to identify cases of anxiety disorder (HADS-A) and depression (HADS-D) in a nonpsychiatric hospital and clinic patients.[11] The HADS has 14 items out of which item numbers 1, 3, 5, 7, 9, 11, 13 measure anxiety and the remaining items that is, 2, 4, 6, 8, 10, 12, 14 measure depression. Each item is on a 4-point scale, and the scores are added to give a total ranging from 0 to 21 for anxiety and 0–21 for depression. The reliability and validity have been established for use in an Indian population.[17]

Yoga practice was ascertained by three questions
For these questions, it was mentioned that yoga practice meant the practice of postures (asanas), regulated breathing (pranayamas), and meditation, either in combination or as a single technique selected out of the three.

Of the three questions, one was a close-ended dichotomous question (question 1, below), the second question (question 2, below), and the third question were both open ended.

The three questions were as follows. Question 1: ‘Are you a regular practitioner of yoga?’ Here regular was specified as a minimum of 4 days in a week. The options were two, yes or no. Question 2 was ‘For how long have you been practicing yoga?‘ (With the option to fill in the number of days, months or years).

The third question was 3. For how many minutes/hours do you practice yoga daily?

Since this was not a regular questionnaire, but three questions with straightforward responses, there was no attempt to establish the reliability and validity for these questions.

Results
Linear multiple regression analysis using PASW SPSS version 18.0 (Armonk, New York, U.S.) was performed with STAI scores (State Trait Anxiety Inventory), HADS-A, and HADS-D scores of the HADS as the dependent variables and with the duration of yoga practice in months and time spent practicing yoga each day as independent variables. Linear multiple regression showed that yoga practice in months and time spent practicing yoga each day both predicted STAI scores (State Trait Anxiety Inventory). The resulting model had an adjusted $R^2 = 0.03$ (standard error of the estimate = 11.4), $df (2,760)$, $F = 11.4$, and was significant for yoga practice in months at $P < 0.001$ with 95% CI = (−0.02, −0.05) and time spent practicing yoga each day at $P = 0.03$ with 95% CI = (−0.01, −0.04) and for HADS-A, adjusted $R^2 = 0.02$ (standard error of the estimate = 4.0), $df (2,760)$, $F = 8.8$, and was significant for yoga practice in months at $P < 0.01$ with 95% CI = (−0.01, −0.01) and time spent practicing yoga each day at $P < 0.01$ with 95% CI = (−0.01, −0.02). For depression scores, the results were different; the duration of yoga practice in months alone predicted HAD-D scores and the resulting model had an adjusted $R^2 = 0.01$ (standard error of the estimate = 3.7), $df (2,760)$, $F = 4.6$, and was significant at $P < 0.01$ with 95% CI = [−0.01, −0.01], while time spent practicing yoga each day did not predict depression scores.

The group mean values (SD) for STAI-state subscale scores, HAD-A anxiety scores, and HAD-D depression scores and details of the linear multiple regression are given in Table 2.

Discussion
In 763 persons who had a chronic illness, the duration of yoga practice in months was associated with lower anxiety and depression scores, whereas the time spent practicing yoga each day, for at least 4 days in a week was associated with lower anxiety scores, but was not associated with the depression scores.

Most studies on the therapeutic benefits of yoga for chronic diseases have examined the effects on the quality of life, anxiety and depression, in addition to the clinical outcomes.[18] In this study, an attempt has been made to understand which factor is, duration or daily intensity of yoga practice has a greater effect on the two most common emotional responses to chronic illness, anxiety and depression.

| Table 2: Anxiety and depression scores predicted by duration and intensity of yoga practice for 763 participants |
|---------------------------------|------------------|------------------|------------------|------------------|
| Measures                        | Mean value (SD)  | Duration of practice in months as predictor | Intensity of practice in minutes/day for at least 4 days/week as predictor |
|---------------------------------|------------------|------------------|------------------|------------------|
|                                |                  | $F$   | $df$ | Adjusted $R^2$ | $\beta$ | Tolerance values | $F$   | $df$ | Adjusted $R^2$ | $\beta$ | Tolerance values |
| STAI-state subscale             | 35.5 (11.5)      | 11.4*** | 2760 | 0.03           | −0.2 | 1.0 | 11.4* | 2760 | 0.03           | −0.1 | 1.0 |
| HAD-A                           | 5.3 (4.0)        | 8.8**  | 2760 | 0.02           | −0.1 | 1.0 | 8.8** | 2760 | 0.02           | −0.1 | 1.0 |
| HAD-D                           | 5.3 (3.7)        | 4.6**  | 2760 | 0.01           | −0.1 | 1.0 | 4.6   | 2760 | 0.01           | 0.0  | 1.0 |

*P<0.05, **P<0.01, ***P<0.001, linear multiple regression. SD: Standard deviation, STAI: State-Trait Anxiety Inventory, HAD-A: Hospital Anxiety and Depression Anxiety, HAD-D: Hospital anxiety and depression-depression
It is known that anxiety is associated with high rates of medically unexplained symptoms and increased utilization of healthcare resources.[19-24] In fact, the disability and related poor physical and economic outcomes associated with anxiety disorders may be as great as with depression.

The neural pathways that process visceral pain also regulate the stress response and anxiety.[25] Prolonged anxiety can lead to dysregulation of the hypothalamic pituitary axis, as well as altered autonomic control with reduced heart rate variability.[26]

The practice of yoga has been found to help in stress reduction[9] and in correcting imbalances of the autonomic nervous system associated with sympathetic hyperactivity.[27] This may explain why yoga practice helps to reduce anxiety associated with chronic illness. The amount of time spent in practicing yoga each day as well as the duration of yoga practice were associated with a reduction in anxiety (HADS) and state anxiety.

Depression increases symptom burden and functional impairment and also worsens the prognosis for heart disease, stroke, diabetes mellitus, HIV/AIDS, cancer and other chronic illnesses.[19,28,29] A survey of over 130,000 Canadian adults indicated that depression independently increased role impairment by 21% compared to healthy persons.[29] When depression occurred along with chronic lung disease, diabetes mellitus or heart disease, the rate of disability increased by over 50%.[29] It is apparent that depression has adverse effects on biological mechanisms and self-care in persons who are chronically ill.[30-38] The latter includes adherence to diet, addictive behavior, taking medication, and exercise, among other factors.

Depression is associated with several changes at the synaptic level including aminergic reuptake inhibition, presynaptic autoregulatory desensitization, up- and down-regulation of post synaptor receptor sites and receptor-mediated second messenger and neurotrophic intracellular signaling effects.[39-43] Neuroimaging studies have identified areas of over- and under-activity.[44]

The present results show that a long duration of yoga practice was associated with lower depression scores in the present group of chronically ill persons. The amount of time spent practicing each day did not make the same difference.

The inverse relation between physical activity and depression is known.[45,46] The therapeutic benefits are particularly found if exercise is continued over time.[47] Yoga has a physical activity component, in addition to its psychological, philosophical (and depending on the program, Spiritual) components. Yoga practice has been found beneficial in depression.[12] The mechanisms underlying the benefits have not been worked out. However, it is possible that among other effects, yoga practice acts on neurotransmitters,[48] hence acting mainly at the synaptic level. The fact that the changes required may involve synaptic receptors[49] in addition to hyper/hyposecretion of certain neurotransmitters may explain why the duration of yoga practice in months, rather than the intensity (as minutes of practice each day) may be associated with lower levels of depression associated with chronic illness.

While the findings are of interest as several chronically ill people practice yoga, it has the following limitations: (i) The study may not prove a causative effect; rather it shows strong association between intensity and duration of yoga on anxiety and depression associated with chronic illness (ii) there was no attempt to assess the impact of the frequency of yoga practice; instead persons were considered regular practitioners if they practiced yoga for at least 4 days in a week. (iii) Other factors such as the severity of illness, social support, and personality traits could influence the level of anxiety and depression experienced by a person with chronic illness. Also, the same factors could influence the intensity and duration of yoga practice. However looking at these factors was not the aim of the present study. (iv) The participants varied widely in their age, severity of disease and other social factors. (v) Yoga practice included yoga postures (asanas), breathing techniques (pranayamas) and meditation. There was no attempt to differentiate between different schools of yoga or amount of time spent practicing the different techniques. Also, there was no attempt to ask the participants about whether they practiced yoga cleansing practices (yoga kriyas), yoga physiological ‘locks’ (bandhas) and followed a yoga lifestyle. (vi) There was no attempt to look at other aspects of emotional distress, other than anxiety and depression. (vii) Approximately 30% of the participants returned one of the questionnaires incorrectly filled in and hence had to be excluded.

Despite these limitations, some of which suggest directions for future study, the present results suggest that the duration and intensity of yoga practice are associated with a decrease in anxiety and depression associated with chronic illness.

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Introduction

Diabetes mellitus (DM) is a chronic disorder characterized by elevated blood sugar levels that occur when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. The first one is called as type 1 diabetes mellitus (T1DM) and later one is called as type 2 diabetes mellitus (T2DM).[1] The prevalence of diabetes is more heavily due to T2DM, and its adverse health effects have risen more rapidly in South Asian region than in any other region of the world.[2] According to the International Diabetes Federation, currently 39.5 million people in India have prediabetes, and of them seven million will develop diabetes every year. The number of people with diabetes in India is expected to increase from 51 million in 2010 to 87 million in 2030.[3] Studies have documented 2.6% and 1.5% prevalence of diabetes among men and women in the urban areas while in rural areas had a lower prevalence: 1.8% and 1.3% respectively.[4] By 2010, the average age-adjusted prevalence of diabetes in India was 8%, higher than that in most European countries.[3] DM is associated with a large variety of complications and a greater risk of all manifestations of atherosclerosis.[5]
Diabetes is a silent disease—many sufferers became aware that they have diabetes only when they develop one of its life-threatening complications. Once diabetes develops, it is a costly disease to manage because of its chronic nature and severity of complications.[7] Over 70% of diabetes-related cost is attributed to its complications, particularly for macro-vascular diseases that most commonly occur in type 2 diabetics.[8]

It is known that adequate control of diabetes is essential if complications are to be reduced.[9] There is evidence available that knowledge about DM and its complications has a positive association with attitude and practices for self-care and glycemic control among diabetics.[10,11]

If patients are to contribute to the effective control of their diabetes, their awareness and practices can assist in reducing the incidence of its complications. The interaction between demography and awareness about DM has already been emphasized by some authors in other countries. Similarly, the rural-urban difference could be present.[12]

This article discusses the results of a study conducted among people with T2DM in rural and urban areas of Delhi with an objective to assess their knowledge about DM and its complications, practices, treatment seeking behavior and average expenditure incurred on its management among those who are suffering.

### Subjects and Methods

#### Study design, setting and participants

In this cross-sectional study, 98 adult patients with T2DM were included. These patients were detected from the screening survey conducted 4 months back on 200 adults in urban area and 1005 adults in rural area selected by systematic random sampling[13] from approximately 22,000 population of following areas: urban areas: An urban slum[14] Balmiki Basti and a resettlement colony Vikram Nagar and rural area: Barwala and adjoining Pooth Khurd village. This sample was calculated on the basis of previous recorded prevalence of diabetes in a rural population in multicentric study as 3.1% and for urban 7.3%.[15] The two areas were selected by convenience sampling. The study was conducted over a period of 3 months from July 2012 to September 2012 and four months after the screening survey.

#### Methodology

All subjects were diagnosed during a screening survey by fasting and postprandial blood glucose tests in both rural and urban areas described elsewhere.[13] The patients were traced after 4 months in those two areas. Seven patients in the urban area could not be traced even after three visits. Finally, 31 DM patients in the urban area and 67 in the rural area were interviewed after taking informed consent and analyzed.

### Study tool

A pretested, predesigned, semi-structured questionnaire schedule in local language consisting of items on the demographic profile including age, sex, religion, marital status, education, occupation, etc., was used. Questionnaire consisted of items to assess their knowledge (cause, types, symptoms and complications), practices for management and prevention of complications (exercise and dietary modifications) and health seeking behavior about DM and its complications (drugs, compliance to treatment, reasons for not taking treatment, complications of management etc.) Questionnaire was pilot tested in a different setting among adult DM patients for assessing its feasibility and reliability. Suitable modifications were done afterward. Cronbach’s alpha that is a coefficient of internal consistency was calculated which came out to be 0.82. Opinion of experts on each questionnaire item was obtained, and all graded excellent in its construct and meaning. Data were also collected about the average out of pocket expenditure incurred on DM treatment. Average time duration of each interview was approximately 10-15 minutes.

### Inclusion and exclusion criteria

All adult patients who is, aged equal to or more than 18 years with DM diagnosed through screening survey were included. No patient refused to participate, and none was seriously ill who could not complete the interview.

### Statistical analysis

Data were analyzed using SPSS software, version 17 (Chicago II, USA). Results are presented in averages and proportions. Difference in proportions between groups was assessed using Chi-square or fisher test and means by Mann–Whitney test for nonnormal distribution. It was accepted for statistical significance when error was <5%.

### Ethical issues

All patients were explained the purpose of the study and confidentiality was assured. A written informed consent was taken from each patient before collecting data. The study was approved by the University’s Institutional Ethical Committee.

### Results

#### Demographic profile of participants

Of 98 participants, 31.6% (31/98) were from the urban area, and 68.4% (67/98) were from the rural area. In urban area, there were 38.7% (12/31) males and 61.3% (19/31) females while in rural area there were 41.8% (28/67) males and 58.2% (39/67) females who participated in the study. In both urban and rural areas, majority were Hindu (74.2%; 23/31 and 97%; 65/67), married (83.9%; 26/31 and 92.5%; 62/67), literate (77.4%; 24/31 and 80.6%; 54/67) and unemployed (71%; 22/31 and 61.2%; 41/67). Mean age (standard division) in the urban area was 49.58 (12.07) years and in a rural area was 51.18 (11.47) years.
Average monthly family income and per capita income were significantly higher in urban areas than in rural areas. Details of socio demographic profile are given in Table 1.

Knowledge of type 2 diabetes mellitus and its complications

When participants were asked about what happens to blood glucose levels in DM, 74.2% (23/31) participants in urban area and 49.3% (33/67) in rural area responded correctly that glucose levels increases in DM that was statistically significant \( (P = 0.02, \text{OR} = 0.3, 95\% \text{CI} = 0.1–0.9). \) When they were asked if they know about types of DM, 22.6% (7/31) in urban slum area and 32.8% (22/67) in rural area answered positively but it was not statistically significant \( (P = 0.3, \text{OR} = 1.7, 95\% \text{CI} = 0.6–4.5). \) Participants were also asked about the cause of DM in which only 19.4% (6/31) in urban slum area and 11.9% (8/67) in rural area knew that it is because of decreased availability of insulin in the body but this difference was not significant \( (P = 0.32, \text{OR} = 0.6, 95\% \text{CI} = 0.2–1.8). \) 61.3% (19/31) patients in urban area and only 1.5% (1/67) in rural area reported that they knew about the genetic inheritance of DM, but 38.7% (12/31) in urban slum area and 98.5% (66/67) in rural area had no knowledge about the same. This difference in knowledge was statistically significant \( (P < 0.01, \text{OR} = 0.01, 95\% \text{CI} = 0.01–0.08 ). \)

When asked about their awareness on symptoms of DM, 87.1% (27/31) patients in urban slum area and 91.0% (61/67) in rural area were able to name at least one symptom of DM but this difference was not significant \( (P = 0.54, \text{OR} = 1.5, 95\% \text{CI} = 0.4–5.8). \) The responses that were given by participants are shown in Figure 1.

But when asked about their awareness on complications of DM, then 61.3% (19/31) in urban area and 85.1% (57/67) in rural area could name at least one complications of DM and this difference was statistically significant \( (P < 0.01, \text{OR} = 3.6, 95\% \text{CI} = 1.3–9.6). \)

Figure 2 shows responses given by respondents on complications of DM.

The majority of participants in both urban slum 83.9% (26/31) and rural area 97.0% (65/67) were reported to have knowledge about at least one component of DM management which was statistically significant \( (P = 0.01, \text{OR} = 6.2, 95\% \text{CI} = 1.1–34.2) \) as shown in Figure 3.

67.7% (21/31) in urban slum area and 70.1% (47/67) in rural area knew that lifestyle modifications are important for a patient with DM but the difference was not significant \( (P = 0.81, \text{OR} = 0.3, 95\% \text{CI} = 0.1–0.8). \) Surprisingly, the rural population showed more health literacy about T2DM.

About 51.6% (16/31) in urban slum area and 71.6% (48/67) in rural area knew that a DM patient should get his/her eye checked by doctor which was not significant \( (P = 0.05, \text{OR} = 2.7, 95\% \text{CI} = 1.0–5.7). \) When asked about awareness on symptoms of hypoglycemia when a patient is on treatment of DM, then patients in urban slum area have significantly higher knowledge where 58.1% (18/31) patients as compared to 35.8% (24/67) in the rural area could tell at least one symptom of hypoglycemia \( (P = 0.03, \text{OR} = 0.4, 95\% \text{CI} = 0.2–0.9). \)

Only 35.5% (11/31) in urban and 20.4% (14/67) in the rural area knew that symptoms of hypoglycemia can be corrected by taking items like sugar candies. 64.5% (20/31) patients in urban area and 50.7% (34/67) in rural area had no awareness of insulin in the body but this difference was not significant \( (P = 0.54, \text{OR} = 1.5, 95\% \text{CI} = 0.4–5.8). \) The responses that were given by participants are shown in Figure 1.

Table 1: Demographic characteristics of study subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Urban n=31 (%)</th>
<th>Rural n=67 (%)</th>
<th>Total n=98 (%)</th>
<th>OR, 95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Male</td>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 (38.7)</td>
<td>19 (61.3)</td>
<td>31 (31.9)</td>
<td>1.1, 0.5-2.2</td>
<td>0.77</td>
</tr>
<tr>
<td>Religion</td>
<td>Hindu</td>
<td>Muslim</td>
<td>Sikh</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>23 (74.2)</td>
<td>1 (3.2)</td>
<td>7 (22.6)</td>
<td>13 (19.4)</td>
<td>20 (20.4)</td>
</tr>
<tr>
<td></td>
<td>65 (97)</td>
<td>1 (1.5)</td>
<td>1 (1.5)</td>
<td>54 (80.6)</td>
<td>78 (79.6)</td>
</tr>
<tr>
<td>Education</td>
<td>Illiterate</td>
<td>Literate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7 (22.6)</td>
<td>24 (77.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>13 (19.4)</td>
<td>54 (80.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occupation</td>
<td>Unemployed</td>
<td>Employed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>22 (71.0)</td>
<td>9 (29.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>41 (61.2)</td>
<td>26 (38.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marital status</td>
<td>Married</td>
<td>Unmarried</td>
<td>Widow/separated</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>26 (83.9)</td>
<td>3 (9.7)</td>
<td>2 (6.4)</td>
<td>62 (92.5)</td>
<td>6 (2)</td>
</tr>
<tr>
<td></td>
<td>62 (92.5)</td>
<td>0 (0.0)</td>
<td>5 (7.5)</td>
<td>88 (98.8)</td>
<td>7 (7.1)</td>
</tr>
<tr>
<td>Income (monthly in rupee)*</td>
<td>Total family, mean (SD)</td>
<td>22,419.35 (19,621.03)</td>
<td>16,295.52 (12,431.25)</td>
<td>18,232.65 (15,244.98)</td>
<td>0.07-0.08</td>
</tr>
<tr>
<td></td>
<td>Per capita, mean (SD)</td>
<td>4412.22 (3474.23)</td>
<td>2642.29 (2081.55)</td>
<td>3202.16 (2713.97)</td>
<td>0.03-0.04</td>
</tr>
</tbody>
</table>

* Rupee is official currency of Republic of India. SD: Standard deviation, OR: Odds ratio, CI: Confidence interval
knowledge about the practices a DM patient should adopt like care for their feet, carrying candies when they go out, regular eye check-up and blood sugar monitoring etc., \( \text{OR} = 0.6, 95\% \text{ CI} = 0.2–1.4 \). Similarly, 54.8\% (17/31) and 80.6\% (54/67) patients in urban and rural areas respectively had no knowledge about forbidden practices that a diabetic patient should not do like wearing tight shoes, skipping meals, taking alcohol etc., which was statistically significant \( \text{OR} = 3.4, 95\% \text{ CI} = 1.3–8.7 \), 61.3\% (19/31) and 79.1\% (53/67) in urban slum and rural area, respectively, had no knowledge about recommended diabetes foot care practices like selecting proper footwear, washing and inspecting feet daily for cuts and abrasions, not walking barefoot etc., but difference was not significant \( \text{OR} = 2.4, 95\% \text{ CI} = 1.0–6.1 \).

**Practices**

About 64.5\% (20/31) in urban slum and 61.2\% (41/67) in rural area said that they do exercise \( \text{OR} = 0.9, 95\% \text{ CI} = 0.3–2.1 \). Out of those who answered positively, 75.0\% (15/20) in urban slum and 68.3\% (28/41) in rural area use to do exercise daily. 5.0\% (1/20) in urban slum and 2.4\% (1/41) in rural area reported once weekly exercise, while 20.0\% (4/20) and 29.3\% (12/41) used to exercise occasionally in urban slum and rural area, respectively. When asked about their dietary practices, 67.7\% (21/31) in urban slum and 83.6\% (56/67) in rural area replied positively \( \text{OR} = 2.4, 95\% \text{ CI} = 0.9–6.5 \) about following diabetic diet. Out of those replied positively, more than one-third (8/21; 38.1\% urban) and about half (29/56; 51.7\% rural) used to follow DM diet always, 61.9\% (13/21) in urban and 35.7\% (20/56) in rural used to follow it sometimes while 0.0\% (0/21) in urban and 12.5\% (7/56) in rural followed it occasionally, which was statistically significant \( \text{OR} = 0.02 \).

**Treatment seeking behavior**

About 29.0\% (9/31) in urban area and 7.5\% (5/67) patients in rural area reported that they are not taking any treatment for DM which was statistically significant \( \text{OR} = 0.2, 95\% \text{ CI} = 0.1–0.6 \). The majority of patients reported to be taking metformin. One patient each in rural and urban area reported using herbal remedies for DM while one patient in the urban area reported use of homeopathic medicines. None of DM patient reported self-monitoring of glucose at home. When asked about the reasons for not taking any treatment, lack of money, distance...
of the health facility from residence, dissatisfied with long queues and waiting time and no need of taking treatment were some of the reasons given by the patients as given in Table 2. Out of those who were taking treatment, slightly more than one-third, 36.4% (8/22) in urban slum area and 11.3% (7/62) in rural area said that they used to miss medicines, this difference was significant \((P < 0.01, OR = 0.2, 95\% CI = 0.1–0.7)\). Out of those eight patients in urban slum area, 62.5% (5/8) reported that they used to forget taking medicines once a week, and 37.5% (3/8) reported that they forgot occasionally. Similarly out of seven patients in a rural area, 14.3% (1/7) reported that they used to forget taking medicines once a week, and 85.7% (6/7) reported that they do only occasionally.

About 9.7% (3/31) and 13.4% (9/67) in urban and rural area respectively reported to have suffered from complications related to DM \((P = 0.59, OR = 1.4, 95\% CI = 0.4–5.8)\). 7.5% (5/67) patients in the rural area reported that they had suffered from complications of management of DM. All five patients reported histories of hospital admissions after symptoms of hypoglycemia. When asked about the health facility where they go for treatment, it was found that those who were taking treatment used to follow multiple systems of medicines like allopathic, ayurvedic, homeopathic and home remedies and used to avail both government and private health care facilities [Figure 4].

**Expenditure on diabetes management**

Patients were asked about out of pocket expenditure on management of DM. The details are shown in Table 3.

From the table data, rough estimate for out of pocket expenditure for 1000 diabetic patients/year for drugs, insulin, travel, consultation, investigations, hospitalization would be Indian National Rupee (INR) 112,008.97 (1789.85$), 9058.77 (144.76$), 4052.65 (64.76$), 9808.57 (156.74$), 14,656.32 (234.2$), 17,367.75 (277.09$) respectively. However, not all patients were taking insulin or hospitalized. Weighted mean came out to be Rs. 433.84 (6.92)$/person in last 3 months or Rs. 1735.36 (27.23$) annually.

Questions were also asked to assess the effect of DM management on the patients and their families. In urban area, 32.2% (10/31) patients told that it is a burden on their family while in rural area 44.8% (30/67) of the patients told that they have to squeeze money from the family expenditure to afford drugs as given by Table 4.

**Discussion**

**Knowledge about diabetes mellitus and its complications**

The study shows that there is a difference in knowledge among patients in urban slum and rural areas. Study conducted by Pardhan et al. in Badford, UK to compare Caucasian and Asian diabetic patient’s awareness about DM showed that Asians reported a significantly lower perceived knowledge of diabetes, its complications and of the dietary practices required for optimal diabetes management.[16] When the questions were asked to assess their knowledge on pathophysiology of DM like types of DM, causes of DM, it was found that not even half of patients in both urban slum and rural areas knew about these. A

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### Table 2: Reasons given by patients for not taking treatment

<table>
<thead>
<tr>
<th>Reason for not taking treatment**</th>
<th>Urban (%)</th>
<th>Rural (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lack of money</td>
<td>4 (44.4%)</td>
<td>2 (40.0%)</td>
<td>6 (42.85%)</td>
</tr>
<tr>
<td>Distance of health facility from residence*</td>
<td>0 (0.0%)</td>
<td>2 (40.0%)</td>
<td>2 (14.28%)</td>
</tr>
<tr>
<td>Dissatisfied with long queues and waiting hours</td>
<td>3 (33.33%)</td>
<td>1 (20.0%)</td>
<td>4 (28.5%)</td>
</tr>
<tr>
<td>No need of treatment</td>
<td>3 (33.33%)</td>
<td>2 (40.0%)</td>
<td>5 (35.7%)</td>
</tr>
</tbody>
</table>

*Response statistically significant \((P<0.01)\), **Responses are not mutually exclusive

### Table 3: Out of pocket expenditure (in Indian rupee) by the patients on management of DM

<table>
<thead>
<tr>
<th>Expenses</th>
<th>Urban area</th>
<th>Rural area</th>
<th>Total</th>
<th>P, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n)</td>
<td>Mean (SD)</td>
<td>(n)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Drugs (monthly)</td>
<td>16</td>
<td>972.58 (1354.98)</td>
<td>53</td>
<td>887.99 (1603.20)</td>
</tr>
<tr>
<td>Insulin (monthly)</td>
<td>5</td>
<td>209 (535.63)</td>
<td>2</td>
<td>11.19 (67.88)</td>
</tr>
<tr>
<td>Travel*</td>
<td>8</td>
<td>73.87 (150.63)</td>
<td>42</td>
<td>111.04 (160.09)</td>
</tr>
<tr>
<td>Consultation*</td>
<td>9</td>
<td>254.84 (537.17)</td>
<td>40</td>
<td>233.58 (224.84)</td>
</tr>
<tr>
<td>Investigations*</td>
<td>11</td>
<td>829.03 (2743.01)</td>
<td>48</td>
<td>141.64 (138.64)</td>
</tr>
<tr>
<td>Hospitalization related to DM*</td>
<td>4</td>
<td>1338.71 (4459.79)</td>
<td>1</td>
<td>2.99 (24.43)</td>
</tr>
</tbody>
</table>

*In last 3 months. DM: Diabetes mellitus, SD: Standard deviation
similar finding was reported by Shah et al. (2009) among DM patients attending three health centers in Saurashtra, Gujarat where about 46% of patients knew the pathophysiology of diabetes.\cite{17} When asked about the awareness on symptoms of DM, it was found that good percentage that is, 87.1% in urban slum area and 91.0% in rural area were able to name at least one symptom of DM. Most common symptom known to patients in both urban slum and rural areas was increased frequency of urination, and least known symptoms were DM being asymptomatic in the urban area and recurrent infections in the rural area. This corresponds to the findings of a study conducted by Mukhopadhyay et al. (2010) in Kolkata in which frequent urination was most common symptom known to the patients (42.2%) and being asymptomatic was least commonly known (to only 3.1%).\cite{18} The possible reason for higher knowledge about DM among rural patients than urban patients in some aspects may be actual suffering from the symptom or complications by rural patients that leads to their diagnosis. Patients in the urban area might be screened by opportunistic screening for DM while contacting some health facility for some other morbidity. Similarly, when asked about the complications of DM, eye and kidney related complications were most common complications known to patients, again corresponding to the finding of previous study.\cite{19} The majority of patients knew one of the components of management needed for a DM patient. Most commonly known were dietary modifications and drugs as found in the previous study.\cite{18} Another study has found lacunae in knowledge prevailed in drug therapy of diabetes.\cite{17} More than 50% of patients in both areas knew that they should get their eye examination done. In another study conducted by Khandekar et al. (2010) in Oman, knowledge of eye complications of diabetes was excellent in 72.9% of patients.\cite{19} Similar findings were given by Rani et al. in rural districts of Tamil Nadu in which 65.9% patients had the right knowledge of getting an eye examination done despite no knowledge about diabetic retinopathy.\cite{10} For some questions like knowledge about complications and management of DM, knowledge was higher in a rural area as compared to urban slum area. This could be because of higher percentage of literates in the rural area of Delhi as compared to urban slum and resettlement colony. Although 58% of patients in urban slum area could tell at least one symptom of hypoglycemia, but patients in both areas reported low knowledge about how to manage hypoglycemic symptoms. In a study carried out by Upadhyay et al. (2012) in Nepal, only 10.49% of patients knew symptoms, and only 17.28% patients knew how to manage hypoglycemic symptoms in their study.\cite{20} The majority of patients in both areas had no knowledge about overall do’s and don’ts for a diabetic patient. Similarly awareness about foot care was also found to be low. The same findings were reported by Matwa et al. (2003)\cite{21} in Eastern Cape Province concerning poor foot care knowledge and practices and Hasnain and Sheikh (2009)\cite{22} in Lahore where one-third of diabetic patients had poor knowledge about foot care.

### Practices

It is a well-established fact that healthy planned eating and regular exercise can delay diabetes and its complications.\cite{23} Although more than 50% patients in both areas said that they used to exercise (30 minutes of brisk walk for at-least 5 days in a week), not all of them used to exercise daily. This is in line with previous studies; one by Raj and Angadi (2010) in Karnataka in which only 40.68% of the respondents reported to exercising regularly.\cite{18,24} Dietary adherence findings are also in line with a previous study in which only 1.85% of the respondents used to follow a diet plan “frequently” at home.\cite{20}

### Treatment seeking behavior

The study revealed that 29% of patients in urban and 7.5% of patients in a rural area were not taking any treatment for diabetes. The reason could be higher out of pocket expenditure and higher percentage of patients being unemployed in the urban area. In a study done in rural areas of Tanzania by Baskin (2012) reported 14.9% of the diabetic patients were not taking any treatment at the time of interview. Most common reasons for not taking treatment were lack of money and long waiting hours and queues apart from a distance of health facility from the residence. In the previous study also, cost burden was prime barrier to medications.\cite{23} Poor availability of transport, physical distance to the health facility and the time taken to reach such facilities have been found to influence health-seeking behavior and health service utilization.\cite{26}

In the present study, patients were using different systems of medicine apart from allopathic. This is similar to findings of a study carried out by Mehrotra et al. in Allahabad, India, which showed that 67.8% of patients were using the alternative system of medicine apart from allopathic system of medicine.\cite{27}

### Economic impact

The International Diabetes Federation, Diabetes Atlas (2006) reported that public mechanisms for financing health care are nonexistent in most developing countries, hence, health costs typically represent out-of-pocket expenditure.\cite{28} Studies in India, for example, have shown that a low-income family with one adult with diabetes may spend as much as 25% of family income on the care of the patient.\cite{29} Mean direct annual cost for outpatient care for all patients with diabetes was INR 4724/-, those without complication had 18% lower

| Table 4: Impact of expenditure on DM management on family |
|-----------------|-----------------|-----------------|
| **Effect of DM** | **Urban (%)**   | **Rural (%)**   | **Total (%)**   |
| **management on family** |               |               |               |
| Squeeze family expenditure to buy drugs* | 8 (25.8) | 30 (44.7) | 38 (38.8) |
| Burden on family budget* | 10 (32.2) | 5 (7.4) | 15 (15.3) |
| Sacrifice family needs | 6 (19.3) | 6 (8.9) | 12 (12.2) |
| Not a burden | 7 (22.5) | 26 (38.8) | 33 (33.6) |

*Responses statistically significant (P < 0.01). DM: Diabetes mellitus
According to Ramachandran (2007), annual expenditure on inpatient care on investigations, physicians fees and medicine were Rs. 6725 (107.29$), on hospitalization was Rs. 5000 (79.77$) and transport was Rs. 300 (4.79$) for diabetes.\(^{[31]}\)

The present study also found that patients have to bear a significant out of pocket expenditure on management of diabetes. Expenditure on drugs and hospitalization was higher than travel. For 66.4% of the patients, the cost of DM management was a burden that is consistent with a previous study where almost all patients considered treatment of DM as a cost burden on their families.\(^{[23]}\)

**Conclusion**

Although patients have some knowledge about diabetes symptoms and complications, awareness about their management was lacking. Patients need to be made aware of long-term complications of diabetes on eye, heart, kidney, etc., and precautions that should be taken and that they can be prevented. At the same time, efforts should be made to sensitize them about the importance of taking regular treatment. Public health care facilities should be utilized for easy and affordable availability of drugs so that burden of disease on patient family can be reduced.

**Strengths and Limitations**

The present study focused upon an important emerging disease DM in India. Strengths of the study are its defined objectives, large sample size, use of validated tool and interpretation of results. Treatment compliance, health seeking behavior and expenditure incurred on management were mainstay of results. Possible limitations are rural study area chosen may not be representative of rural areas in other states in India due to the difference in pace of urbanization and health care facilities available in Delhi and other states.

Multi centric studies should be conducted in future so as to get the results with better external validity. Policy level changes can be undertaken to plan interventions to raise awareness, compliance, better availability of cheaper drugs and comprehensive health education services at the primary health centers.

**Acknowledgment**

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Vulvovaginal Candidiasis in Aminu Kano Teaching Hospital, North-West Nigeria: Hospital-Based Epidemiological Study

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Abstract
Background: Vulvovaginal candidiasis (VVC) remains a common problem worldwide and the role of douching as a predisposing factor is unclear. Aim: This study was undertaken to highlight the prevalence and predisposing factors of VVC in North-west Nigeria. Subjects and Methods: This was a prospective study done at Aminu Kano Teaching Hospital (AKTH), North-west. AKTH is a 500-bed tertiary hospital located in Kano, the most populous state in Nigeria. Ethical clearance was obtained. Three hundred patients with VVC were recruited from the gynecologic and general outpatients’ clinics of AKTH. Research structured questionnaires were used to obtain sociodemographic and clinical information. The data obtained were analyzed using SPSS version 16.0 statistical software (SPSS Inc., Chicago IL, USA). Frequency, mean and simple percentages were used to analyze data. Result: Candida albicans was the most frequent cause of the positive high vaginal swabs constituting 84.5% (316/374) while Proteus vulgaris was the least frequent cause constituting 0.53% (2/374). Fifty-three percent (143/270) of those with VVC were aged 26–35 years; the married were 80% (216/270) and those who were unmarried were 20% (54/270). Douching was the commonest predisposing factor occurring in 42.5% (115/270) of cases. Conclusion: VVC was the most prevalent cause of vaginosis in North-west Nigeria, and douching was the commonest predisposing factor.

Keywords: Epidemiology, Hospital, Nigeria, North-west, Vulvovaginal candidiasis

Introduction
Vulvovaginal candidiasis (VVC) remains a common problem worldwide, affecting all strata of society. The absence of rapid, simple, and inexpensive diagnostic tests continues to result in both overdiagnosis and underdiagnosis of VVC. Although commonly caused by Candida albicans, non-albican species and immunosuppression have led to the development of recurrent diseases some of which are nonresponsive to conventional antifungal regimes.

Nwadioha and colleagues reported that C. albicans were responsible for 60% of high vaginal swab (HVS) specimens. Ibrahim and colleagues in a study done in Maiduguri, north-east Nigeria, reported a prevalence of 41% among pregnant women attending antenatal care. Another study involving a cohort of apparently healthy women reported that about 30% had yeast isolated, confirming the diagnosis of VVC.

Studies have reported the prevalence of VVC as 25%, 24% and 18.5%. Parveen et al. Maccato and Kaufman reported a high rate among pregnant women and Okonofua et al., reported a high carriage of C. albicans in Nigeria infertile women compared with controls. Okonkwo reported no significant difference in prevalence of VVC among women of various socioeconomic status in Nnewi.

A higher prevalence of vaginal colonization and symptomatic vaginitis is more often seen in pregnant women than in those who are not pregnant and this is due to high concentrations of reproductive hormones that increase the glycogen content in the vaginal tissue thereby providing a carbon source for candida organisms.

Increased vaginal colonization with candida has been shown after the use of oral contraceptives with high estrogen
content.[12] Increased carriage of yeast is reported in users of intrauterine contraceptive devices, contraceptive sponges, diaphragms, and condoms, with or without spermicides.[13]

However, an extensive study of college students did not show an increase in the risk of symptomatic VVC in users of oral contraceptives, diaphragms, condoms, or spermicides.[14] Women with type 2 diabetes are more prone to colonization with *Candida glabrata*.[15] Symptomatic VVC frequently follows use of vaginal or systemic antibiotics.[16] Douching has been shown to be a risk factor for candida vulvovaginitis for some women, and for others, there was no relationship found. Ekpenyong and Davis, in Uyo, found a paradoxical relation between vaginal douching and adverse reproductive tract disorders.[17] Heng, *et al.*, [18] found a high prevalence of VVC among Cambodian women who douchedit, while no similar association was reported among Indian women by Sujit, *et al.*[19]

With much emphasis on drug use and misuse by the female folks with respect to reproductive tract infections, little interest is shown in some cultural practices such as douching which can also predispose women to certain reproductive tract infections including VVC.

The aim of this study is to highlight the prevalence and predisposing factors of VVC in North-west Nigeria and make a recommendation on how to reduce the prevalence.

**Subjects and Methods**

This was a prospective study done at Aminu Kano Teaching Hospital (AKTH), North-west. AKTH is a 500-bed Tertiary Hospital Located in Kano, the most populous state in Nigeria. Ethical approval was obtained from AKTH Research and Ethics Committee with reference number AKTH/MAC/SUB/12A/P3/796 and dated January 21, 2010. Informed consent was also obtained from the respondents. The study period was between November 1, 2011 and February 28, 2012 and involved 300 women who had clinical and laboratory evidence of VVC. Selection was by simple random sampling.

Sample size was calculated based on prevalence of VVC from previous studies.[6–9]

\[ n = Z^2 \frac{P \times (1-P)}{d^2} \]

\( n = \text{Sample size}, \ Z = \text{Standard normal deviation} = 1.96 \)

at 95% confidence limit, \( P = \text{Prevalence rate} = 22.5\% \),
\( q = 1-P = 1-22.5\% = 0.775, d = \text{Error margin} = 5\% ) \).

The minimum sample size was 268. Because of the possibility of drop-out the sample size was increased to 300 after applying the exclusion criteria. Exclusion criteria were females less than 16 years of age, those who were menstruating, those who had any immunosuppressive illness, those with history of diabetes mellitus, those with recurrent VVC, those with premalignant or malignant cervical lesions and those who were pregnant.

Postmenarchal females with a diagnosis of VVC were included in the study. The patients had a clinical diagnosis of VVC based on history and physical examination (including vaginal examination).

Signs and symptoms that were evaluated include: Itching, burning, irritation, edema, erythema and/or excoriation of the vagina/vulva. Each evaluated sign and/or symptom was given a numerical rating based on severity (absent = 0; mild = 1; moderate = 2; severe = 3). Patients with VVC may have a vaginal discharge, which is usually described as white, creamy, and cottage cheese-like in appearance and adherent to the epithelium.

Research structured questionnaires were administered to all the 300 subjects. These gave various sociodemographic and clinical informations. To ensure content validity of the research instrument, the draft questionnaire was submitted to a senior colleague for scrutiny regarding the relevance of each item. Pretesting of the questionnaire was also done where 20 self-administered questionnaires were distributed to volunteers with vulvovaginal symptoms to comment on the clarity of the questions. All the recruited patients were examined by the researcher. They were put in lithotomy position, and the vulva was inspected. Under good light, a sterile Cusco’s bivalve speculum was used to expose the vagina after swabbing the vulva with sterile water. Specimens were taken from the posterior fornix with a sterile cotton swab that was immediately put into a sterile tube containing about 3 ml of saline. A screening 10% potassium hydroxide (KOH) preparation from the inflamed vaginal mucosa or vaginal discharge was done to identify yeast forms (hyphae/pseudohyphae) or budding yeasts. A drop of 10% KOH on the pool of the secretion on the speculum when it produced a fishy smell denoted a positive test for *Gardinerella vaginalis*. The tube was labeled with the patient’s initials and case form number and taken to the laboratory for further investigations that included wet microscopy and preparation of a dry Gram stain slide for microscopy. A Gram stain slide can reveal candida (pseudohyphae) or *Bacteria vaginosis* (clue cells and proportions of lactobacilli and other organisms). Wet microscopy was prepared in the laboratory by dipping a small amount of discharge from a HVS into saline on a microscope slide. This was useful in identifying pseudohyphae in candida. Culture in Sabouraud’s medium was used to detect the candida when microscopy was inconclusive. Whenever the HVS was not transported immediately to the laboratory, it was stored at 4°C for no longer than 48 h. Those HVS specimens in which *C. albicans* was isolated were included in the study.

The data obtained were analyzed using SPSS version 16.0 statistical software (SPSS Inc., Chicago IL, USA). Absolute numbers and simple percentages were used to describe categorical variables. Similarly, quantitative variables were described using measures of central tendency (mean, median) and measures of dispersion (range, standard deviation) as appropriate.
Results

Of the 300 women included in the study, 30 were drop-outs and 90% (270/300) completed the study. These 30 droppers did not continue with the study because their husbands did not give consent for them to participate in the study. The culture in many parts of Northern Nigeria is that the husbands consent is required about aspects of the woman’s reproductive and sexual health. VVC constituted 84.5% of all HVS specimens. The results are shown in Tables 1-3.

Discussions

This study reports a very high prevalence (84.5%) of VVC compared to 25%,[4] 24%,[5] 18.5%[6] and 61%[1] from previous studies. The report of this study is similar to that of Onifade and Olorunfemi in Ondo State who reported a prevalence of 81.5%.[20] This present study has shown that marital factor affects the prevalence of VVC. Although Enweani et al.,[24] reported that marital factor had no effect on the prevalence of VVC, Okungbowa et al.,[22] are of the view that marital factor was important. This study showed higher incidence among the married (69.6%) compared to the unmarried (30.4%). The report of this study agrees with that of Okungbowa, et al.,[22] that VVC is commoner among the married women.

Vulvovaginal candidiasis occurs among women in the age group 16–45 years as shown in this study. This was similarly reported.[4,21] The reason is the high estrogen levels in this group of women resulting in a favorable pH for candida colonization. The mean age of women with VVC in this study was 27.7 (7.8) years. The condition was more prevalent in women aged 26–35 years (53%) and lowest in those aged 36–45 years (13.7%). This report is a replication of that of another study[22] that showed that the occurrence of VVC peaks in the third decade of life, declining in women older than 40 years. It is, however, contrary to study by Ako-Nai et al.,[23] where highest incidence was among women 20–25 years of age.

The result of this study has shown that cheesy vaginal discharge was the commonest presentation in patients with VVC occurring in 47.4% of cases. This was followed by vulval itching or pruritus that occurred in 30.4% of cases. Vulval redness occurred in 12.2% and vulval burning sensation in 10% of cases. This is compatible with the reported symptoms for

Table 1: Microbiological pattern in 374 positive high vaginal swabs specimens in AKTH

<table>
<thead>
<tr>
<th>Organism</th>
<th>Frequency percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida albicans</td>
<td>316 (84.5)</td>
</tr>
<tr>
<td>Streptococcus species</td>
<td>20 (5.35)</td>
</tr>
<tr>
<td>Staphylococcus species</td>
<td>14 (3.74)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>14 (3.74)</td>
</tr>
<tr>
<td>Bacterial vaginosis</td>
<td>8 (2.14)</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>2 (0.53)</td>
</tr>
</tbody>
</table>

AKTH: Aminu Kano Teaching Hospital

Table 2: Distribution of sociodemographic characteristics of study population

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Frequency (%)</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td></td>
<td>27.7 (7.8)</td>
</tr>
<tr>
<td>16-25</td>
<td>90 (33.3)</td>
<td></td>
</tr>
<tr>
<td>26-35</td>
<td>143 (53)</td>
<td></td>
</tr>
<tr>
<td>36-45</td>
<td>37 (13.70)</td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td>2 (2)</td>
</tr>
<tr>
<td>0</td>
<td>60 (22.2)</td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td>113 (41.9)</td>
<td></td>
</tr>
<tr>
<td>3-4</td>
<td>62 (23.00)</td>
<td></td>
</tr>
<tr>
<td>≥5</td>
<td>35 (13.0)</td>
<td></td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>216 (80)</td>
<td></td>
</tr>
<tr>
<td>Unmarried</td>
<td>54 (20)</td>
<td></td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Housewife</td>
<td>119 (44.1)</td>
<td></td>
</tr>
<tr>
<td>Employee</td>
<td>61 (22.6)</td>
<td></td>
</tr>
<tr>
<td>Trader</td>
<td>29 (10.7)</td>
<td></td>
</tr>
<tr>
<td>Students</td>
<td>61 (22.6)</td>
<td></td>
</tr>
<tr>
<td>Educational status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>64 (23.7)</td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>34 (12.6)</td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>72 (26.7)</td>
<td></td>
</tr>
<tr>
<td>Tertiary</td>
<td>100 (37.0)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Distribution of clinical presentations, perceptions and predisposing factors of vulvovaginal candidiasis in the study population

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Frequency (%)</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presenting clinical features</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheesy vaginal discharge</td>
<td>128 (47.4)</td>
<td></td>
</tr>
<tr>
<td>Itching</td>
<td>82 (30.4)</td>
<td></td>
</tr>
<tr>
<td>Burning</td>
<td>27 (10)</td>
<td></td>
</tr>
<tr>
<td>Redness</td>
<td>33 (12.2)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>270 (100)</td>
<td>13.3 (7.4)</td>
</tr>
<tr>
<td>Duration of symptoms (days)</td>
<td></td>
<td>7 (26.3)</td>
</tr>
<tr>
<td>0-7</td>
<td>71 (26.3)</td>
<td></td>
</tr>
<tr>
<td>8-14</td>
<td>105 (38.9)</td>
<td></td>
</tr>
<tr>
<td>15-21</td>
<td>57 (21.1)</td>
<td></td>
</tr>
<tr>
<td>22-29</td>
<td>37 (13.7)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>270 (100)</td>
<td></td>
</tr>
<tr>
<td>Patient’s perception of the source of infection</td>
<td>73 (27)</td>
<td></td>
</tr>
<tr>
<td>Sexual</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toilet</td>
<td>113 (41.9)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>84 (31.1)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>270 (100)</td>
<td></td>
</tr>
<tr>
<td>Risk factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contraceptive</td>
<td>78 (28.9)</td>
<td></td>
</tr>
<tr>
<td>Antibiotics</td>
<td>52 (19.3)</td>
<td></td>
</tr>
<tr>
<td>Douching</td>
<td>115 (42.8)</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>25 (9.3)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>270 (100)</td>
<td></td>
</tr>
</tbody>
</table>
VVC including vaginal discharge, burning and pruritus.\textsuperscript{25,26} Vaginal discharge is a common presentation in gynecological patients.\textsuperscript{27,28}

Of 41.9% of the patients believed that they contracted VVC from the toilet, 31.1% did not know the source of the infection and 27% believed that it was sexually transmitted. Rabiu \textit{et al.},\textsuperscript{29} from Lagos, Nigeria, also reported that 44.6% of women perceived that they contracted reproductive tract infection from the toilet, followed by sexual intercourse and poor hygiene. Although VVC is not a sexually transmitted infection from the toilet, followed by sexual intercourse and antibiotics use in 19.3% of cases. Douching has been shown to be a risk factor for candida vulvovaginitis for some women, and for others, there was no relationship found.\textsuperscript{18,19,30}

This study has also shown that VVC is associated with usage of the oral contraceptive pills and intrauterine contraceptive devices. While this finding is similar to some studies,\textsuperscript{14} it is not replicated by others.\textsuperscript{101}

Vulvovaginal candidiasis is the most prevalent cause of vaginosis in North-west Nigeria and douching is the commonest predisposing factor. It will be a good practice to discourage women from douching.

The limitation of this study is that the use of the questionnaire means that individual opinion was assessed which may not be very objective. Also, future research using case-control design may help make better deductions.

\section*{Acknowledgment}
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\section*{References}


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Prevalence and Predictors of Erectile Dysfunctions among Men on Antiretroviral Therapy in South-western Nigeria

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Abstract

Background: Erectile dysfunctions (EDs) are common presentations among men on anti-retroviral therapy, many of who had a course to discontinue anti-retroviral drugs or search for alternative treatments. Aim: This study assessed the prevalence and predictors of ED among men on anti-retro viral therapies (ART) in a Nigerian population. Subjects and Methods: It was a descriptive cross-sectional survey among 234 HIV-positive men on anti-retroviral therapy selected using stratified sampling method after excluding for co-morbidities. Research instrument was semi-structured interviewer administered questionnaire, and data were analyzed using the SPSS software version 17.0 (Chicago IL, USA), while binary logistic regression and Chi-square test were used to demonstrate association between selected categorical variable. Results: Mean age of respondents was 37.1 (1.6) years, 85.6% have not missed their medications, self-reported adherence was reported as good among 213 [(90.8%) 213/234], though calculated adherence was 90% among as many as 201 [(85.6%) 201/234]. Pattern of EDs revealed weak erection among 42 [(37.8%) 42/111], 15 [(13.5%) 15/111] said they could no longer achieve erection, 33 [(29.7%) 33/111] said they could not maintain erections, while 27 [(24.3%) 27/111] presented with loss of libido. Delayed and premature ejaculations were reported among 24 [(21.6%) 24/111] and 8 [(7.2%) 8/111] respectively. About 14% (33/234) of respondents said that anti-retroviral drugs could have caused their ED while 78% (183/234) said it should not. A statistically significant association exists between having weak erections and age above 65 years and calculated the adherence < 95%, while none exists between having weak erections and missing pills. Conclusion: Anti-retroviral drugs are common causes of EDs. Concerns of clients should always be addressed most especially issues that may compromise adherence.

Keywords: Adherence, Anti-retroviral therapy, Erectile dysfunction

Introduction

Studies have shown an association between anti-retroviral therapies (ART) and different degrees of sexual dysfunction in men.[1–3] The highest rates of dysfunction are associated with Indinavir and the lowest with Nevirapine.[3] Among people living with HIV/AIDS (PLHWA) on ART, sexual dysfunctions have been reported in form of erectile dysfunction (ED) (9–74%), ejaculatory disturbances (36–42%), and low sexual desire (24–73%).[3] Ejaculatory dysfunction has been shown to be associated with the use of didanosine, as well as other protease inhibitors.[3,4]

These sexual dysfunctions could be of psychogenic or organic etiology.

There is also a subdivision into mental, hormonal, pharmacological, and other morbid conditions. Hypogonadism was one of the most frequent causes of sexual dysfunction before the advent of highly active anti-retroviral therapy (HAART), and it still remains the most common endocrine disorder of HIV-infected men.[5] After receiving the diagnosis of HIV
infection, it is common for people to experience negative moods, show less interest in sex and decrease the frequency of sexual activity. Depression is one of the important mental factors associated with sexual dysfunctions.\[1,6\]

In addition, HIV-infected individuals use many other medications that could also be associated with decreased sexual responses. Medications such as ketoconazole, fluconazole, ganciclovir, megestrol, methadone, and antipsychotics including antidepressants may decrease testosterone levels and cause sexual dysfunctions.\[3\] Sexual dysfunction has an impact on the quality of life and very often leads to negative attitudes on the part of the individual, including poor adherence to antiretroviral (ARV) regimens and to safer sex strategies.\[8-10\]

Huge data gap exists on pattern of ED in Nigeria most especially among PLWHA. Even among few studies done outside Nigeria, most emphasis were laid on 2nd line ART and clients on salvage therapy, with little or no emphasis on clients on first line ART which constitutes majority of HIV-positive Nigerians on ART addressed by this study. Despite few prevalence figures reported, the pathophysiology of sexual dysfunction in the HAART era is still not completely understood. We investigated the prevalence of ED in a cohort of HIV-infected people on ART, to identify risk factors for developing ED among HIV-infected men especially in developing countries where many socio-economic factors influence clients’ management and outcomes.

**Subjects and Methods**

**Study design**

The study was a descriptive cross-sectional survey carried out in Osogbo, Osun State.

**Study area**

Osogbo is the capital of Osun State in South-western Nigeria. HIV treatment, care and support in the capital city, takes place in the secondary (State Government Hospital) and tertiary (State Government University Teaching Hospital) health care levels. Primary health care centers were mainly for HIV counseling and testing services, and were excluded from this study. HIV prevalence in the city was 2.5%, a bit lower than the national average put at 4.1%.

**Study population**

Target population constitutes HIV positive men accessing treatment in some selected health facilities. Eligible men would have been on ART for at least 1-year. In addition, men on medications that could influence erectile functions (such as ketoconazole, fluconazole, ganciclovir, megestrol, methadone, and antipsychotics including antidepressants) were totally excluded from this study. Co-morbidities such as hypertension and diabetes that could also serve as confounders were also excluded among study participants through past medical history. Using Leslie Fischer’s formula for calculation of sample size for the population <10,000, a sample size of 221 was estimated, and this was increased to 240 to account for non-response. A total of 250 questionnaires were taken to the field.

There were two eligible facilities in Osogbo, Asubiaro General Hospital, which is secondary, and LAUTECH Teaching Hospital which is tertiary in nature, and both have about 2000 registered clients on ART. Questionnaires were equally shared among the 2 facilities. On a bi-weekly clinic day per facility, a list or sampling frame of all eligible men was obtained from the triage nurse. A systematic sampling of one in three eligible men on the list was done, and this continued until the questionnaires allocated for that day got exhausted. Apart from pre-testing and training of data collectors, data validity were further ensured by translating and back-translating the questionnaire between English and the native Yoruba languages to assist further understanding among the illiterate and semi-literate respondents. Study period was from January 2013 to June 2013.

Data were collected by trained research assistants using pre-tested interviewer administered semi-structured questionnaires. Interviews were conducted under strict confidentiality and privacy in the post-test counseling rooms of the clinics. Details of the study and its objectives were explained to all respondents and participation voluntary, informed consent was obtained from each participant. Ethical clearance was obtained from Osun State University, Osogbo ethical review committee. Permission was also obtained from the Project Coordinators of the respective HIV/ART program as well as the Medical Director of the health facilities used.

Questionnaires were manually sorted out and data obtained were entered into the computer. Statistical Package for social Sciences (SPSS) version 17 (Chicago IL, USA) was used to analyze the double-entered data that were also checked for outlier values to ensure its validity. Frequency tables were generated, and relevant summary measures calculated. The Chi-square test was used to demonstrate an association between categorical variables while level of significance for the statistical tests was considered at \( P < 0.05 \).

**Results**

Mean age of respondents was 37.1 (1.6) years, 88.2% (207/234) were married, and 74.8% (175/234) had up to secondary school level education as seen in Table 1. Table 2 showed that about 85.6% (201/234) have not missed their medications, self-reported adherence was reported as good among 90.8% (213/234), though calculated adherence was 90% among as many as 85.6% (201/234).

Pattern of EDs as shown in Table 3 revealed weak erection among 37.8% (42/111), 13.5% (15/111) said they could no
longer achieve erection, 29.7% (33/111) said they could not maintain erections, while 24.3% (27/111) presented with loss of libido.

Table 1: Sociodemographic characteristics of respondents

<table>
<thead>
<tr>
<th>Variable</th>
<th>Frequency (n=234)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-39</td>
<td>78</td>
<td>33.3</td>
</tr>
<tr>
<td>40-59</td>
<td>135</td>
<td>57.7</td>
</tr>
<tr>
<td>60-79</td>
<td>21</td>
<td>9.0</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>12</td>
<td>5.3</td>
</tr>
<tr>
<td>Married</td>
<td>207</td>
<td>88.2</td>
</tr>
<tr>
<td>Divorced/separated</td>
<td>15</td>
<td>6.5</td>
</tr>
<tr>
<td>Education level</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>25</td>
<td>10.7</td>
</tr>
<tr>
<td>Primary</td>
<td>74</td>
<td>31.6</td>
</tr>
<tr>
<td>Secondary</td>
<td>76</td>
<td>32.5</td>
</tr>
<tr>
<td>Tertiary</td>
<td>59</td>
<td>25.2</td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Student</td>
<td>6</td>
<td>2.6</td>
</tr>
<tr>
<td>Trader</td>
<td>40</td>
<td>17.1</td>
</tr>
<tr>
<td>Farmer</td>
<td>19</td>
<td>7.9</td>
</tr>
<tr>
<td>Artisan</td>
<td>80</td>
<td>34.2</td>
</tr>
<tr>
<td>Professionals</td>
<td>15</td>
<td>6.6</td>
</tr>
<tr>
<td>Civil servants</td>
<td>55</td>
<td>23.7</td>
</tr>
<tr>
<td>Unemployed</td>
<td>19</td>
<td>7.9</td>
</tr>
<tr>
<td>Religion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Christian</td>
<td>132</td>
<td>56.6</td>
</tr>
<tr>
<td>Islam</td>
<td>96</td>
<td>40.8</td>
</tr>
<tr>
<td>Traditional</td>
<td>3</td>
<td>1.3</td>
</tr>
<tr>
<td>Others</td>
<td>3</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Table 2: Pattern of adherence to ART

<table>
<thead>
<tr>
<th>Variable</th>
<th>Frequency (n=234)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Missed medications</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In the last 6 months</td>
<td>15</td>
<td>6.6</td>
</tr>
<tr>
<td>In the last 1-month</td>
<td>9</td>
<td>3.9</td>
</tr>
<tr>
<td>In the last 1-week</td>
<td>9</td>
<td>3.9</td>
</tr>
<tr>
<td>None</td>
<td>201</td>
<td>85.6</td>
</tr>
<tr>
<td>Self-reported adherence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>213</td>
<td>90.8</td>
</tr>
<tr>
<td>Bad</td>
<td>9</td>
<td>3.9</td>
</tr>
<tr>
<td>Can’t say</td>
<td>12</td>
<td>5.3</td>
</tr>
<tr>
<td>Calculated adherence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;90</td>
<td>43</td>
<td>18.4</td>
</tr>
<tr>
<td>90-95</td>
<td>188</td>
<td>80.3</td>
</tr>
<tr>
<td>&gt;95</td>
<td>3</td>
<td>1.3</td>
</tr>
<tr>
<td>Duration of commencement of ART</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-5 years</td>
<td>194</td>
<td>82.9</td>
</tr>
<tr>
<td>&gt;5 years</td>
<td>40</td>
<td>17.1</td>
</tr>
<tr>
<td>ART regimen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st line</td>
<td>234</td>
<td>100.0</td>
</tr>
<tr>
<td>2nd line</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Discussion

Mean age of respondents was 37.1 (1.6) years, 207 (88.2%) were married, 175 (74.8%) had up to secondary school level education. About 80% of respondents never missed their delayed and premature ejaculations were reported among 21.6% (24/111) and 7.2% (8/111), respectively. Figure 1 showed that about 14% (33/234) of respondents said that ARVs could have caused their ED while 78% (183/234) said it should not. Table 4 showed statistically significant association (P < 0.01) between having weak erections and age above 65 years, and calculated adherence <95%, while none exists between having weak erections and missing pills (P = 0.17).

Respondents with age >40 years are two and a half times more likely to develop ED compared to respondents with age <40 years (P = 0.35; odds ratio [OR] = 2.5; 95% confidence interval [CI] = 0.56–2.35). There is no observed difference between the different education levels of respondents coming up with weak erections (P = 0.08; OR = 1.1; 95% CI = 0.66–1.94). Respondents who have also missed their pills were about four and a half times more likely to have weak erections compared to those who have not missed their medication in the last 6 months (P = 0.01; OR = 44.7; 95% CI = 1.14–19.39). However, respondents with longer duration of being on ART (>5 years) are three times more likely to develop EDs compared to those who had been on ART for <5 years (P < 0.01; OR = 0.3; 95% CI = 0.01–0.11). Respondents with calculated adherence level above 90% were five times more likely to have developed weak erections compared to those with poor adherence (<90%), in which case (P < 0.01; OR = 5.1; 95% CI = 2.01–13.09). Thus, poor adherence to ART, longer duration of being on ART, missing of ART medications and older age are predictors of having EDs or weak erection on binary logistic regression analysis.

Figure 1: Respondents’ perception about anti-retroviral therapy being the cause of their sexual dysfunction

ART: Anti-retroviral therapy
Table 3: Pattern of sexual dysfunction

<table>
<thead>
<tr>
<th>Variable (n=111 with multiple responses)</th>
<th>Frequency (n=111)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>My erection is now weaker than before</td>
<td>42</td>
<td>37.8</td>
</tr>
<tr>
<td>Cannot achieve erection again</td>
<td>15</td>
<td>13.5</td>
</tr>
<tr>
<td>Could not sustain erection for long during sexual intercourse</td>
<td>33</td>
<td>29.7</td>
</tr>
<tr>
<td>Loss of libido</td>
<td>27</td>
<td>24.3</td>
</tr>
<tr>
<td>Getting more difficult to achieve orgasm during sexual intercourse</td>
<td>12</td>
<td>10.8</td>
</tr>
<tr>
<td>Delayed ejaculation</td>
<td>24</td>
<td>21.6</td>
</tr>
<tr>
<td>Premature ejaculation</td>
<td>8</td>
<td>7.2</td>
</tr>
</tbody>
</table>

Table 4: Association between weak erections and some selected socio-demographic and treatment variables on bivariate and binary logistics regression

<table>
<thead>
<tr>
<th>Variables</th>
<th>Weak erection</th>
<th>χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &gt;65 years</td>
<td>4 (18.4)</td>
<td>129.1</td>
<td>0.001</td>
</tr>
<tr>
<td>Missed pills in the last 6 months</td>
<td>1 (4.0)</td>
<td>3.503</td>
<td>0.06</td>
</tr>
<tr>
<td>Calculated adherence &lt;95%</td>
<td>15 (6.6)</td>
<td>17.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Duration of ART &gt;1-year</td>
<td>34 (17.4)</td>
<td>2.974</td>
<td>0.15</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variables</th>
<th>Had weak erections</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (constant = &lt;40)</td>
<td>2.5</td>
<td>0.558</td>
<td>2.353</td>
<td>0.35</td>
</tr>
<tr>
<td>Education level (constant = secondary and above)</td>
<td>1.1</td>
<td>0.658</td>
<td>7.941</td>
<td>0.06</td>
</tr>
<tr>
<td>Missed medications (constant = not in the last 6 months)</td>
<td>4.7</td>
<td>1.140</td>
<td>1.939</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Self-reported adherence (constant = good)</td>
<td>0.2</td>
<td>0.031</td>
<td>1.058</td>
<td>0.05</td>
</tr>
<tr>
<td>Calculated adherence (constant = &lt;90%)</td>
<td>5.1</td>
<td>2.009</td>
<td>13.949</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Duration of commencement of ART (constant &gt;15 years)</td>
<td>0.3</td>
<td>0.007</td>
<td>0.109</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

OR: Odds ratio, CI: Confidence interval, ART: Anti-retroviral therapy

Numerous medications are also known to cause sexual dysfunction and studies have suggested that a decrease in sexual interest and ED were found in individuals who are on HAART, especially if their regimens contain Protease Inhibitors. This study also showed that respondents who are on ART could have weak erection in spite of their adherence. Almost 20% of respondents >65 years of age were found to significantly have weak erection. Older age is one of the risk factors for ED.

Findings from binary logistic regression in this study proved wrong the notion that there is no age difference among those HIV positive clients having ED and those not having it. Atrophy of old age could also explain this phenomenon. Similarly, statistical difference exists to prove any alternative hypothesis supporting that there is a difference between adherence pattern and duration of taking ART among those who developed ED compared to those not having ED. Thus, clinicians working in ART clinics should be wary of all possible implications of weak erections among clients on ART most especially those in the middle age or older age groups. It is important that clinicians take priority in ensuring that medications of those who had been on ART for long are reviewed from time to time to prevent this menace of ED among HIV-positive men.

This study has demonstrated sexual dysfunctions among PLWHA. Further research may be needed to improve the description of the development of sexual dysfunctions in such individuals so as to identify pathophysiological mechanisms and to study the management/treatment of this disorder. The recovery of the sexual function, associated with a good adherence to safe sex practices, will improve the quality of life of the PLWHAs.

Though this study evidently excluded drugs that may serve as cofounders, and employed oral (and some common laboratory tests) methods to exclude possible common organic causes of ED as stated, a need for further sophisticated methods of excluding remote causes may be necessary in future studies.

Conclusion

Antiretroviral are common causes of EDs among HIV-positive men on ART. Many of such men may have contemplated missing their ARV drugs or seek alternative care elsewhere when these menstrual abnormalities are getting unbearable. Stakeholders in Art care should always strive to address concerns of clients, most especially issues that may compromise ARV adherence.

References

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Lipid and Some Other Cardiovascular Risk Factors Assessment in a Rural Community in Eastern Nigeria

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Abstract

Background: Continuous re-evaluation of modifiable cardiovascular risk factors (cardiovascular diseases [CVDs]) in developing nations is imperative as it lays foundation for early preventive/intervention measures at grass root level to improve/prevent CVD morbidity and mortality in those nations where health indices still score below the standard. Aim: The aim was to assess CVD risk factors as a continuous re-evaluation of these may underscore the need for early intervention measures at grass root level. Subjects and Methods: A total of 257 apparently healthy inhabitants aged 18–85 years were recruited in a rural community in South Eastern Nigeria by convenient sampling. Blood pressure, waist circumference and blood lipid analysis were done procedurally and data analyzed using SPSS 16.0 statistical software. Results: The males were older (59.41 [5.22]) than the females (53.31 [16.90]). 69.2% (133/192) were low level farmers, retirees and dependents. Total cholesterol (TC), low density lipoprotein (LDL), and risk predictive index were higher in females while triglyceride (TG), high density lipoprotein and very LDL (VLDL) were higher in males. The middle aged and elderly respectively had higher TG and VLDL compared to the young. Aside hypertriglyceridemia, all lipid abnormalities were higher in females than males both singly (high TC: 28.9% [35/121] vs. 16.9% [12/71]; high LDL cholesterol: 52.0% [63/121] vs. 31.0% [22/71]) and in combination hypercholesterolemia (42.9% [32/121] vs. 36.6% [26/71]). “Multiple risk factors” also occurred more in females with seeming further increase in older age. Conclusion: The chances of a female having CVD after menopause seemed to outweigh that of the male. CVD preventive measures should be focused at the primary/community level as a means to curtail the increasing morbidity and eventual mortality from CVDs.

Keywords: Blood pressure, Homogenous community, Lipids, Waist circumference

Introduction

The concept of predicting future morbidity and mortality from cardiovascular diseases (CVDs) by measuring such factors as blood pressure (BP), body weight, or index of obesity and blood lipid originated in the life insurance industry during the 1940’s and 1950’s\textsuperscript{[1]} for individuals who sought and obtained life insurance. Hyperlipidemia, hypertension, and possibly obesity (all of which have long time recognized association with one another) appear to be the most important treatable factors that predispose patients to coronary heart disease. Coexistence of these factors is known to have multiplier effect with other CVD risk factors and has continued to translate to increasing CVD morbidity and mortality.

These noncommunicable diseases (NCDs) were some decades ago described as rare or low in blacks but more recent researches in Nigeria and other countries\textsuperscript{[2–17]} indicate that their incidence in developing countries is gradually taking a prime position.

Bearing this changing trend in mind, continuous re-evaluation of these CVD risk factors cannot be over emphasized; more so, in different communities in developing nations where health indices still score below the standard. This regular assessment will re-evaluate data for these variables and emphasize the need for better education and provision of other appropriate early intervention measures at the grass root level. The positive
The study was a cross-sectional community-based prevalence study carried out in August, 2011 in a homogenous (rural community) in Udi Local Government Area of Enugu State, South East Nigeria with a population of about 12,990 (projected at 15% increase every 5 years from 1991 census).

Before commencing the study, approval was obtained from the research and Ethics Committee of the Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi. Letters were written respectively to the traditional ruler and the town union of the community as well as to the local government authority and their written approval obtained. Informed consent was also obtained from each participant before being included in the study.

The sample size was calculated to be approximately 246 using the prevalence of multiple risk factors for coronary vascular disease as found in Ibadan, Nigeria which was 20%[18] using the standard formula. However, a total of 257 inhabitants of the community that participated in this study had their data analyzed.

All consenting apparently healthy subjects 18 years and above residing in the community were recruited into the study. All those with a history of current use of steroids, clinical evidence of fluid retention and all pregnant females were excluded from the study. Six medical officers were trained to help in this study along with two laboratory scientists. General physical examination was carried out on each participant who then had his/her waist circumference (WC) measured with a nonstretchable tape. The umbilicus was the landmark and where the abdomen was pendulous, the point in the abdomen with highest circumference was taken. WC ≥ 102 cm for males and ≥ 88 cm for females was regarded as abdominal obesity. Afterward, the participant got seated and a questionnaire detailing the individuals’ demographic and relevant family and social history was administered to each participant by a trained interviewer. This also afforded him/her opportunity to relax before the BP was checked.

Each participant’s BP was measured using the standard procedure. Three readings were taken at about 5–10 min interval and the mean of the last two was regarded as the subject’s BP. Hypertension was defined as BP ≥ 140/90 mmHg.

Ten milliliters of venous blood was withdrawn from willing participants who had not eaten into a container containing dry sodium ethylenediaminetetraacetic acid (1 mg/ml) mixed gently and separated and stored at −20°C until analysis. The fasting period before sample collection was a minimum of 10 h since their last meal was at night and blood samples were collected before breakfast. Those who came after they had breakfast were asked to come the next day for collection of their fasting blood sample. Plasma total cholesterol (TC), high-density lipoprotein cholesterol (HDLC) and triglycerides (TGs) were done by colorimeter (enzymatic methods) in the chemical pathology laboratory of NAUTH, Nnewi using diagnostic sera kits by Randox Laboratories UK while low-density lipoprotein cholesterol (LDLC), and very LDLC (VLDLC) were calculated using the Friedewald Formula;[19] thus:

\[
LDLC = \frac{TC - (HDLC + TG)}{5} \times 2.2 \text{ if units were expressed in mmol/L}
\]

or

\[
VLDLC = \frac{(Plasma \ TGs) \text{ in mmol/L}}{2.2}
\]

The coronary heart disease risk predictive index was also calculated for each participant as LDLC/HDLC with value <2.1 as desirable. For each batch of the assay, a commercial control serum of known value was always included and all the parameters were assayed within the same period in order to minimize inter and intra batch errors.

The NAUTH reference ranges were used in interpreting the lipid parameters. Hyperlipidemia was defined as raised plasma TC and/or raised plasma TG that is, TC > 5.17 mmol/L and or TG > 1.71 mmol/L (NAUTH reference ranges). Combined dyslipidemia was defined as TC > 5.17 mmol/L and or TG > 1.71 mmol/L plus low HDL. The lipid values for samples that were not properly labeled were all not included in the analysis. Lipid profile results were later sent to all participants who desired to have their cholesterol results sent to them.

Data analysis

The Microsoft Excel 2003 worksheet and SPSS (16.0) statistical software (manufacturer: SPSS Inc., 233 South Wacker Drive, 11th Floor, Chicago, IL 60606-6412. Patent No. 7,023,453) were used for data entry, validation, and analysis. Frequency distribution tables were formed from which percentages, mean values and standard deviations of the parameters studied were determined appropriately. Analysis of variance and Student’s t-test were used to look for gross differences in the parameters among groups of the subjects. \(P < 0.05\) was taken as significant.
Results

The occupations of the participants were farming (56.0% [144/257]), retirees/dependents (13.2% [34/257]), petty trading (7.8% [20/257]), Teaching/other paid jobs (9.3% [24/257]), artisans (7.4% [19/257]), and students (6.2% [16/257]). 27.6% (71/257) of the participants (27.5% [50/181]) of females and (14.5% [1176]) of males; \( P = 0.046 \) were overweight while 12.8% (33/257) (16.0% [29/181]) of females and 9.9% [776] of males; \( P = 0.26 \) were globally obese (body mass index \( \geq 30 \) kg/m²).

Table 1 shows that the males were significantly older than the females \( (P < 0.01) \). The TC and LDL as well as the risk predictive index (RPI) were significantly higher in the females than in the males \( (P < 0.01, P = 0.02 \) and \( P = 0.03 \), respectively). TG, HDL, and VLDL were higher in the males than the females though not significantly \( (P = 0.54, 0.26 \) and 0.92, respectively).

As shown in Table 2, apart from HDL which was either the same or slightly lower than the values of the other age groups, all the other lipid parameters and RPI were highest in the middle-aged subjects whereas HDL was highest in the elderly subjects. Except for TC, LDL, and RPI that were insignificantly higher in the young than in the elderly subjects, all the parameters were lowest in the young subjects. Age differed significantly among the three age groups and within pairs of the different age groups \( (P < 0.001) \). Between the young and the elderly, TG, VLDL, waist-to-hip ratio, and systolic BP (SBP) differed significantly \( (P = 0.02, P = 0.02, P < 0.001 \) and \( P < 0.001 \), respectively). Between the young and the middle-aged, TG as well as SBP and VLDL differed significantly \( (P = 0.03, P < 0.001, \) and \( P = 0.02, \) respectively). However, between the middle-aged and the elderly, LDL and RPI were significantly different \( (respectively \( P = 0.05 \) and 0.04).

As Table 3 shows, the percentage of males who had hypertension (47.4% [36/76]) was higher than that of the females who had hypertension (41.4% [75/181]) though the difference was not statistically significant \( (P = 0.67) \). Abdominal obesity was significantly higher \( (P < 0.001) \) among the females (38.1% [69/181]) than among the males (13.2% [10/76]). Compared to the males, females had higher prevalence of both hypercholesterolemia (high TC \( [P = 0.04] \) and high LDL \( [P = 0.02] \) and hypertriglyceridemia \( [P = 0.41] \)) as single entities (high TC: 52/181 28.7% vs. 13/7617.1%; \( P = 0.04 \), High LDL: 51.9% [94/181] vs. 31.6% [24/76]; \( P = 0.02 \) as well as in combination (hypercholesterolemia \( \pm \) hypertriglyceridemia: 43.1% [78/181] vs. 36.8% [28/76]; \( P = 0.19 \)). Combined dyslipidemia (hypercholesterolemia \( \pm \) hypertriglyceridemia and low HDL) was also more prevalent among the females (42.0% [76/181] vs. 34.2% [26/76]; \( P = 0.37 \)).

Among the subjects that had only one risk factor, there were more males (39.5% [30/76]) than females (34.3% [62/181]). Conversely, for those who had two or more risk factors, there were more females (30.9% [56/181]) than males (21.1% [16/76]).

Table 1: Distribution and comparison of age, BP, lipid, and obesity parameters according to gender

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Male (n=181)</th>
<th>Female (n=181)</th>
<th>All subjects (n=362)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>59.4(15.22)</td>
<td>53.3(16.90)</td>
<td>55.14(16.63)</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.22(0.89)</td>
<td>1.18(0.9)</td>
<td>1.20(0.89)</td>
<td>0.54</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>3.17(1.59)</td>
<td>3.75(1.45)</td>
<td>3.58(1.51)</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>0.39(0.27)</td>
<td>0.36(0.22)</td>
<td>0.37(0.23)</td>
<td>0.26</td>
</tr>
<tr>
<td>VLDL (mmol/L)</td>
<td>0.56(0.38)</td>
<td>0.54(0.42)</td>
<td>0.54(0.41)</td>
<td>0.92</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>2.23(1.52)</td>
<td>2.85(1.39)</td>
<td>2.67(1.45)</td>
<td>0.02*</td>
</tr>
<tr>
<td>RPI</td>
<td>8.00(7.43)</td>
<td>10.41(7.31)</td>
<td>9.72(7.41)</td>
<td>0.03*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.6(3.9)</td>
<td>24.9(5.1)</td>
<td>24.8(4.8)</td>
<td>0.21</td>
</tr>
<tr>
<td>WHR</td>
<td>0.99(0.07)</td>
<td>0.95(0.10)</td>
<td>0.96(0.96)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>140.29(31.15)</td>
<td>137.28(25.96)</td>
<td>137.80(27.26)</td>
<td>0.84</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>77.46(16.32)</td>
<td>79.23(14.02)</td>
<td>78.68(16.62)</td>
<td>0.64</td>
</tr>
</tbody>
</table>

Values expressed as means (SD). *Number of subjects. **Level of statistical significance at <0.001. SD: Standard deviation, BP: Blood pressure, TG: Triglyceride, HDL: High-density lipoprotein, LDL: Low density lipoprotein, VLDL: Very low density lipoprotein, TC: Total cholesterol, RPI: Rice protein isolate, BMI: Body mass index, WHR: Waist-to-hip ratio, SBP: Systolic blood pressure, DBP: Diastolic blood pressure

Table 2: Distribution and comparison of the parameters among the different age groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>&lt;45 (n=108)</th>
<th>45-64 (middle-aged) (n=181)</th>
<th>65+ (elderly) (n=73)</th>
<th>ANOVA (P)</th>
<th>Student’s t-test (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>30.25(8.71)</td>
<td>54.4(5.72)</td>
<td>72.28(5.85)</td>
<td>&lt;0.001**</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>0.93(0.82)</td>
<td>1.26(0.84)</td>
<td>1.20(0.89)</td>
<td>0.04</td>
<td>0.02*</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>3.41(1.50)</td>
<td>3.83(1.56)</td>
<td>3.37(1.41)</td>
<td>0.17</td>
<td>0.12</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>0.35(0.23)</td>
<td>0.36(0.25)</td>
<td>0.38(0.22)</td>
<td>0.75</td>
<td>0.45</td>
</tr>
<tr>
<td>VLDL (mmol/L)</td>
<td>0.42(0.37)</td>
<td>0.57(0.38)</td>
<td>0.54(0.45)</td>
<td>0.04</td>
<td>0.02*</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>2.64(1.47)</td>
<td>2.89(1.52)</td>
<td>2.40(1.32)</td>
<td>0.13</td>
<td>0.07</td>
</tr>
<tr>
<td>RPI</td>
<td>9.86(7.08)</td>
<td>10.80(8.29)</td>
<td>8.19(6.08)</td>
<td>0.06</td>
<td>0.03*</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>126.22(20.99)</td>
<td>139.81(29.08)</td>
<td>145.21(27.21)</td>
<td>0.001*</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>91.33(18.23)</td>
<td>81.46(15.67)</td>
<td>78.99(14.03)</td>
<td>0.48</td>
<td>0.37*</td>
</tr>
<tr>
<td>WHR</td>
<td>0.66(0.34)</td>
<td>0.77(0.31)</td>
<td>0.79(0.24)</td>
<td>0.3*</td>
<td>&lt;0.01*</td>
</tr>
</tbody>
</table>

Values expressed as means (SD). *Number of subjects. **Level of statistical significance at <0.001. SD: Standard deviation, BP: Blood pressure, TG: Triglyceride, HDL: High-density lipoprotein, LDL: Low density lipoprotein, VLDL: Very low density lipoprotein, TC: Total cholesterol, RPI: Rice protein isolate, WHR: Waist-to-hip ratio, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, ANOVA: Analysis of variance
The differences were, however, not significant ($P = 0.20$) as shown in Table 4a.

Those 55 years and above had higher prevalence of “two or more” risk factors (30.7% [42/137]) compared to those <55 years (25.0% [32/120]; $P = 0.37$). For those <55 years, the prevalence of “only one” risk factor was higher in males than the females but “two or more risk factors” was more prevalent in females than the males. However, in those 55 years and above, females had a higher prevalence of both “only one” and “two or more” risk factors [Table 4b].

Table 5 shows the number of risk factors in the young, middle aged and elderly subjects. Among the three age groups, the elderly subject had highest prevalence of “only one” risk factor followed by the young and then the middle aged subjects. The variations in the prevalence showed no statistical significance ($P = 0.53$ and 0.33, respectively). However, among the subjects that had “two or more” risk factors, the prevalence was highest in the middle aged subjects compared to the other age groups and this difference was significant between the middle aged and the young subjects ($P = 0.05$).

As Table 6 shows, the hypertensive subjects were significantly older than the nonhypertensive subjects ($P < 0.01$). All the lipid parameters with the exception of HDL were higher in the hypertensive subjects than in nonhypertensive subjects.

As shown in Table 7, the percentages of those with abdominal obesity who had high BP (HBP) (58.2% [46/79]) differed significantly ($P = 0.04$) when compared with 36.5% (65/178) who had HBP among those with normal WC. The prevalence of hypercholesterolemia among those with abdominal obesity was significantly higher than among those with normal WC (41.8% [33/79] vs. 18.0% [32/178]; $P = 0.03$).

Subjects with HBP had higher prevalence of both hypercholesterolemia (27.9% [31/111]) and combined dyslipidemia (41.4% [46/111]) compared to those with normal BP who had high TC (23.3% [34/146]) and combined dyslipidemia (38.4% [56/146]); the difference between these prevalence values was, however, not statistically significant ($P = 0.27$ and 0.14, respectively). Those with combined dyslipidemia had higher prevalence of hypertension (45.1% [46/102] vs. 41.3% [65/155]) and abdominal obesity (36.3% [37/102] vs. 27.1% [42/15]) compared to those without dyslipidemia.

**Discussion**

The mean values of the atherogenic lipids were similar to the finding in many other recent studies in being lower than the upper limits just as the mean HDL value was lower than the desirable minimum. When compared with those previous local studies, the values obtained in this study were generally much lower. This may be because even when the participants had similar age brackets, most of those previous studies were done in settings and socioeconomic classes that were mixed or different from this one. The participants in this study were mostly low-level farmers, petty trades, and elderly dependents. Thus, as serum lipids are known to be influenced by nutrition, the lower mean lipid values obtained in this study may suggest that the inhabitants of this community had poor/poorer nutrition.

Some previous researchers observed higher values of TG in males than females just as found in this study. However, contrary to some of these studies and in agreement with others, the difference in mean TG value in both sexes was
not statistically significant. Again, contrary to the finding of a recent study of elderly subjects in south-east Nigeria,[14] in which males had higher values of all lipids parameters measured, the females in this study had significantly higher TC and LDL values ($P < 0.01$ and $P = 0.02$, respectively) than their male counterparts. The observed sex variation in respect of lipid in this and the other study may be accounted for by the differences in the diets of the two different study populations. The findings in this study, however, agrees with the finding of the Nigeria National NCD survey report on cholesterol values in adult males and females.[20]

Whereas older studies observed that serum lipid concentration did not alter with age in underprivileged Africans after adulthood,[21] more recent studies[14] found an age-related trend in serum lipid concentrations. This study, however, showed no consistent trend. This inconsistent trend supports the finding by Miller[22] in which LDL values showed no consistent trend with age and just like in this study, the 45–64 years age group in their study had the highest values of the parameters measured. The finding of lowest concentration of TC in the elderly (above 65 years) subjects of this study may not necessarily be a contradiction or concordance of earlier studies[23]
Hypertriglyceridemia was found to be more prevalent in the study participants. All the atherogenic risk factors as well as RPI (i.e., LDL/HDL) were more favorable in those who were normotensive and/or “not obese” than in those who had either of these and this is in keeping with other studies\(^{[11,25]}\) that reported direct association between lipid levels and LDL/HDL ratio.

The general prevalence of HBP documented in this study was 43.2% (males: 47.4%, females: 41.4%) while abdominal obesity prevalence was 30.7% (males: 13.2%, females: 38.1%). Those with abdominal obesity had significantly higher prevalence of HBP (58.2% vs. 36.5%; \(P = 0.04\)) and high TC (41.8% vs. 18.0%; \(P = 0.03\)) than those without abdominal obesity; an association that has long been established.\(^{[12-26,27]}\) Those with combined dyslipidemia also had a higher prevalence of both HBP (45.1% vs. 41.3%) and abdominal obesity (36.3% vs. 27.1%) than those with normolipidemia, as documented in other studies.\(^{[8,9,26,28]}\) In agreement with previous studies which demonstrated high dyslipidemia prevalence in hypertensive patients in Nigeria, mean values of all atherogenic lipids were higher in those found to have hypertension in this study compared to normotensive subjects. Mean HDL value in this study was similar in those with HBP (0.36 [0.23] mmol/L) and in nonhypertensive subjects (0.37 [0.24] mmol/L) thus, collaborating the finding by some researchers that aside HDL every other cardiovascular co-morbidity was higher in hypertensives.\(^{[26]}\)

In this study, 35.8% of the participants (males: 39.5%, females: 34.3%); \(P = 0.17\) had “only one” of the three cardiovascular risk factors assessed while 28.0% (males: 21.1%, females: 30.9%) had “two or more” risk factors. This finding is higher than the prevalence of multiple risk factors for coronary vascular disease as found over a decade ago in a rural community in Ibadan, Western Nigeria\(^{[18]}\) which was 20% and even a more recent study which found prevalence of at least one CVD risk factor to 12.9%. Despite some sociocultural differences between that rural community and this one, our findings suggest that coronary vascular risk factors are not just increasing in Nigerians as single entities but in groups/multiples.

The prevalence of elevated serum TC was 25.3% (males: 17.1%, females: 28.7%), elevated LDLC was 45.9% (males: 31.6%, females: 51.9%) while that of combined hyperlipidemia (high TC and/or high TG level) was 39.7% (males: 34.2%, females: 42.0%). These prevalence values for high TC and high LDL varied with findings by other researchers in Nigeria\(^{[8,9,14,16,28]}\) and some other developing nations\(^{[12,13]}\) being lower in some and higher in others. This difference may be accounted for by the fact that those other studies were conducted in settings different from that in which this study was conducted in that those ones were either hospital-based or involved people of different or mixed socioeconomic class unlike the case in our study.

Low HDL prevalence in this study was as high as 96.5% while combined dyslipidemia was 39.7%; both being higher than previous research findings in Nigerians\(^{[8,9,14,16,28]}\) and some Asian countries.\(^{[29]}\) On the same note, hypertriglyceridemia prevalence was higher in this study (24.5%) compared to previous research findings in different parts of Nigeria\(^{[8,9,15,26,28,30]}\) and South Asia\(^{[29]}\) but lower than the finding in one study in Iran.\(^{[12]}\) It is, however, similar to the finding in a Lagos; commercial city in Nigeria\(^{[46]}\) and in a South African study.\(^{[13]}\) Contrary to high LDL which was said to be the most common lipid abnormality followed by LDL, this study found the reverse with low HDL being the most common lipid abnormality before LDL. This opposite finding may be because that was a review study\(^{[4]}\) which include studies done in healthy people as well as those done on patients. The finding of low HDL as the most prevalent lipid abnormality has been demonstrated in other community-based studies.\(^{[9,15,28,30]}\) Hypertriglyceridemia was also the least occurring lipid abnormality as found in some other study, though hospital based.\(^{[51]}\) In relation to gender, the females had higher prevalence of high TC (28.7% vs. 17.1%; \(P < 0.05\)) and low HDL (97.8% vs. 93.3%) as found in other studies\(^{[9,12,15]}\) but contrary to some studies\(^{[31]}\) and in agreement with some,\(^{[29]}\) the higher low HDL prevalence in females showed no statistical significance compared to the males; \(P > 0.05\). Unlike in one of those studies,\(^{[9]}\) this study found that the prevalence of high HDL was still higher in females (51.9% vs. 31.6%; \(P < 0.05\)) like in some others.\(^{[15]}\)

In the general population, the prevalence of “only one risk factor” in this study was the same both above and below 55 years, whereas “two or more risk factors” was more prevalent above 55 years. Furthermore, the middle aged subjects had highest prevalence of “two or more risk factors” (33.9%) compared to the elderly (27.9%; \(P = 0.70\)), and then the young who had the least prevalence (16.1%; \(P = 0.05\)). It, therefore, seemed that the prevalence of multiple risk factors increased with age and then began to drop after the middle age in this community; a trend which recent studies have demonstrated for individual CVDs in Nigerians.\(^{[2,32]}\)

As can be seen in Table 3, aside hypertriglyceridemia, the females had higher prevalence of all other lipid abnormality compared to the males and as shown in Table 4a and b, they also had higher prevalence of multiple risk factors both in the general population and above and below 55 years age groups. A study conducted about a decade ago in Nigerians\(^{[3]}\) indicated that gender was not a modifier of cardiovascular risk
in Nigeria. Our data tend to suggest that CVD risk prevalence was higher in females and the degree of risk in them tended to increase further in older age when all the risk factors were considered either singly (“only one risk factor”) or collectively (“two or more risk factors”). These findings suggest that the females in this study (mean age: 53.1 years) seemed to run higher risk of CVD than the males who were significantly older (mean age: 59.4 years; $P < 0.01$).

**Limitations of study**

Due to financial constraint and other logistics, contact with each participant was once. Those with grade I hypertension should have had follow-up checks but this first time and only visit was taken as their BP as in other epidemiologic studies.

**Conclusion**

Cardiovascular disease risk factors are prevalent even in rural communities in Southeast Nigeria and the chances of a female having CVD after menopause seemed to outweigh that of the age-matched male. There is a need for health education at the primary/community level as a means to curtailing the increasing morbidity and eventual mortality from CVDs.

**Recommendation**

Periodic screening of individuals at risk by clinicians and other health workers using the parameters studied in this research work is advocated. Education and lifestyle modification are important measures to be deployed by responsible health professionals in addressing the rising trend of CVDs in Nigeria and nations with similar setting.

**References**


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Conflict of Interest: None declared.
Possible Hematological Changes Associated with Acute Gastroenteritis among Kindergarten Children in Gaza

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Abstract

Background: Gastroenteritis is considered one of the leading causes of morbidity and mortality in children especially in developing countries. It is a major childhood problem in Gaza and one of the most common etiologic agents of iron deficiency anemia (IDA).

Aim: This study was conducted to investigate possible changes in blood parameters that are associated with gastroenteritis infection among kindergarten children in Gaza.

Subjects and Methods: A cross-sectional case–control study was performed including kindergarten children suffering from gastroenteritis and matched healthy control group. Types of etiological agents were identified using standard microbiological and serological procedures. Blood samples were collected for estimation of complete blood count and for determination of serum iron, total iron binding capacity (TIBC), and transferrin saturation. Independent sample t-test was used for comparisons and performed using SPSS software version 17 (Chicago Illinois USA).

Results: The prevalence of enteric pathogens among cases (88.5% [85/96]) was significantly higher than in asymptomatic controls (11.1% [6/54]). The most common enteric pathogens isolated were Entamoeba histolytica (28% [42/91]) and Giardia lamblia (26.7% [40/91]). Blood tests revealed that 21.8% (21/96) of cases and 14.8% (8/54) of controls had IDA, which were not significantly different. Meanwhile, a significant difference was found between the TIBC and hemoglobin in cases compared to controls. Conclusion: This study indicates that gastroenteritis infection could be considered as a common health problem in kindergarten children in Gaza, and it is possibly associated with changes in hemoglobin concentration and TIBC.

Keywords: Acute gastroenteritis, Gaza, hematological changes, Iron deficiency, Kindergarten

Introduction

Worldwide, diarrheal diseases are continued to be a common health problem that increase the financial burden on the health systems particularly in developing countries. Acute gastroenteritis is one of the leading causes of morbidity and mortality in children <5-years.1-3 especially in developing countries.4-7 Gastroenteritis is a serious burden to public health. Its etiologic agents include a wide variety of bacteria, viruses, and parasites8-10 Serious outcomes are associated with these intestinal pathogens include malnutrition, impaired physical development, and reduced school achievement in children.11 In Gaza, acute gastroenteritis is a common infection among children, associated with high morbidity and mortality rates when left without a proper treatment.12

Anemia is a major public health problem in both developed and developing countries that needs a national and international interventions to correct it and its underlying causes.13,14 It is currently estimated that anemia affects about one-quarter of the world’s population and estimated that 36% of developing world’s population suffers from this health condition.13-16

Iron deficiency is recognized as the primary cause of anemia worldwide and is the most prevalent form of micronutrient malnutrition in the world. The etiology of anemia is multifactorial including nutritional habits, the bioavailability of micronutrients, parasitic infections, inflammation, and genetic
Gaza Strip (geographic coordinates 31°252 N, 34° 202 E) is a narrow region (365 km²) of land along the Mediterranean coast, just 41 km long and 6–12 km wide. It is one of the most heavily populated areas (4383 persons/km²) of the world with a total population about 1.7 million people. In the present work, a prospective cross-sectional case–control study was conducted in Gaza city to determine the type and prevalence of enteropathogens causing acute gastroenteritis among kindergarten children and to assess their possible effect on blood parameters and body iron status.

**Subjects and Methods**

**Study population and sample collection**

This study is a cross-sectional case–control study conducted in Gaza from 1st of January to the 30th of June 2011. Sample selection was randomly done for 15 kindergarten in Gaza but covering all different localities and population clusters in Gaza. Sample size was determined using Sample Size Calculator according to the number of enrolled children in Gaza kindergartens (http://www.surveysystem.com/sscalc.htm) to define the enteropathogens causing gastroenteritis among kindergarten children and to find out their possible effect on blood parameters.

One hundred and ninety written requests were sent to the parents or guardians of the symptomatic cases and asymptomatic carriage (apparently healthy controls) in 15 kindergartens to have their approval to involve their child in the study. Thankfully, 96 of 115 (response rate 83.5%) and 54 of 75 (response rate 72.0%) of symptomatic cases and asymptomatic carriage, respectively, agreed to participate in the study and to provide stool and blood samples for further investigations. The 15 kindergartens are in geographically different localities and population clusters in Gaza.

The study protocol was approved by the ethical committee of our biology department and performed in accordance with the ethical standards established in the 1964 and 1975 Declaration of Helsinki and the modifications thereafter. All ethical considerations were followed including respect for the privacy of the subject. A signed consent form was obtained from the parents or guardians providing their acceptance and full understanding of this study and its aims. Stool samples were collected from cases and controls in clean, dry plastic containers in their kindergarten by the help and under the supervision of the investigator. They were tested using standard microbiological procedures according to the guidelines of Clinical and Laboratory Standards Institute (CLSI). Furthermore, blood samples were collected on the same time by expert laboratory technologist for estimation of complete blood count (CBC) and serum samples were tested for determination of iron concentration and total iron binding capacity (TIBC).

**Inclusion and exclusion criteria**

All samples were collected from different kindergarten regions representing all parts of Gaza. Any child who is suffering from any chronic disease or was hospitalized was excluded. Children with gastroenteritis symptoms including watery, nonbloody or bloody diarrhea, abdominal cramps, nausea, vomiting or low-grade fever considered as cases and apparently healthy matched controls (asymptomatic) were included.

**Microbiological methods**

Standard microbiological procedures and guidelines, as described by CLSI,[26], were used. For the identification of parasites, fresh stool specimens prepared with saline and/or iodine were directly examined. In addition, flotation and sedimentation techniques were performed on all samples that gave negative result using direct microscopic examination. For detection of bacterial agents, fresh stool samples were plated directly or after enrichment step onto Salmonella-Shigella, Heektoen enteric, and Sorbitol MacConkey agars (HiMedia, India) and incubated for 24 h at 37°C. Suspected colonies were identified by standard laboratory procedures. For detection of viral agents, enzyme immunoassay (GastroVir-Stripcolor kit) was used for the detection of Group A Rotavirus and Adenovirus serotype 40 and 41 according to the manufacturer’s instructions (Cori BioConcept, Belgium).

**Complete blood count**

About 2½ ml of venous blood were drawn into sterile ethylenediaminetetraacetic acid tubes, mixed and processed within 1 h by (Sysmex-Kx-21). The following parameters were calculated: White blood cells (WBC), red blood cells (RBC), platelets (PLT), hemoglobin, hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH), and MCH concentration (MCHC). Serum iron (SI) and TIBC were performed using Mindray Bs-200, chemistry analyzer (Shenzhen Mindray Bio-Medical Electronics, Shenzhen, China) on samples with hemoglobin concentration <11 g/dL.

**Evaluation of iron deficiency anemia**

In the present research, and due to the inadequate financial and laboratory resources, as well as the politically constrictive environment in the Gaza Strip, which negatively affects the availability of medical, laboratory equipment, chemicals and diagnostic kits to perform laboratory investigations, iron deficiency anemia (IDA) were considered in the kindergarten children; when transferrin saturation (SI/TIBC) was <16%[27] in combination with low hemoglobin...
concentration <11 g/dL for ≤4-year-old children, and <11.5 g/dL for 5-year-old children.[28]

**Determination of serum iron**
Serum iron was determined by the colorimetric method according to the manufacturer instructions of DiaSys, Diagnostic Systems GmbH, Germany kit by using computerized chemical chemistry auto analyzer equipment (Mindray BS-200).

**Determination of total iron binding capacity**
Total iron binding capacity was determined by the colorimetric method according to the manufacturer instructions of Linear Chemicals, Spain kit by using computerized chemical chemistry auto analyzer equipment (Mindray BS-200).

**Calculation of transferrin saturation**
Transferrin saturation was calculated as a percentage of the ratio of SI to total TIBC.

**Data analysis**
The results that obtained from laboratory investigations were tabulated, encoded and statistically analyzed using Statistical Package for Social Sciences (SPSS) program version 17 (Chicago, IL, USA). Descriptive analysis and independent t-test were performed. The differences were considered statistically significant at $P \leq 0.05$.

**Results**

**General characteristics of the study population**
The present study is a prospective cross-sectional case–control study included 150 kindergarten children from Gaza city. The stool and blood samples were collected from children of both genders (56% [84/150] males and 44% [66/150] females). The ages of children enrolled in this study were 3 (8% [12/150]), 4 (34% [51/150]), and 5-years (58% [87/150]). According to the study protocol, 96 diarrheal stool samples were collected from 51 boys (53.1% [51/96]), and 45 girls (46.9% [45/96]) and they considered as symptomatic cases. The asymptomatic carriage (controls group) included 33 boys (61.1% [33/54]) and 21 (38.9% [21/54]) girls.

**Prevalence and type of enteropathogens isolated from cases and controls**
Overall, there were 91 samples (60.7% [91/150]) positive for parasitic, bacterial, and/or viral enteric pathogens. The parasitic etiologic agents had the highest prevalence rate (54.7% [82/150]) in comparison to the bacterial (4% [6/150]) and viral (2% [3/150]) etiologic agents. More than half of samples collected were positive for one or more parasitic agent. The highest isolated enteric pathogen was *Entamoeba histolytica* (28% [42/91]), followed by *Giardia lamblia* (26.7% [40/91]). *Adenovirus* and *Salmonella* were not isolated from all tested samples [Figure 1]. The overall prevalence of positive stools from symptomatic cases was significantly higher (88.5% [85/96]) than that isolated from asymptomatic controls (11.1% [6/54]).

**Hematological parameters of cases and controls**
Table 1 shows the CBC parameters of blood samples that collected from all enrolled children in this study. There was no significant differences between the mean score results of blood parameters tested for both, cases (diarrheal) and controls (nondiarrheal), except for the WBC which was significantly higher in cases (symptomatic) compared to control.

![Figure 1: Prevalence of parasitic, bacterial, and viral enteric pathogens causing acute gastroenteritis among tested stool samples](image-url)
group (asymptomatic) \( (P < 0.01) \). However, the mean score result of hemoglobin concentration is slightly lower in cases compared to controls, but this difference did not reach statistical significance \((P = 0.32)\). The other blood parameters (RBC, HCT, MCV, MCHC, and PLT) had almost the same mean score results.

Serum iron, TIBC, and transferrin saturation level were performed only on blood samples that have hemoglobin concentration <11 g/dL. SI level divided by the TIBC multiplied by 100 gives a transferrin saturation level. There were 21 (21.9% [21/96]) and 8 (14.8% [8/54]) children from the cases (diarrheal samples) and controls (no diarrhea) showing IDA with hemoglobin level <11 g/dL. Decreased hemoglobin, SI, and transferrin saturation levels were found in cases compared to controls, but this difference was not statistically significant. Nevertheless, the results showed a statistically significant difference between the level of TIBC of cases compared to controls \((P < 0.01)\) [Table 2].

**Possible effect of parasitic infection on blood parameters**

The mean level of blood parameters tested from children with different parasitic infections showed almost the same values irrespective of the type of parasite or even when double infection with two parasites were existed. Furthermore, comparing these results with the blood parameters values tested from children with negative parasitic samples revealed no clear differences. Yet, the mean value of WBC was found to be higher in blood samples of children with *E. histolytica* infections compared to blood samples of children with *G. lamblia* infection alone or children without any parasitic infection [Table 3]. These differences were statistically significant.

**Table 1: Differences in blood parameters among cases (diarrheal) and controls (no diarrheal)**

<table>
<thead>
<tr>
<th>Blood parameter</th>
<th>Diarrheal samples Mean (SD) (n=96)</th>
<th>Presence of diarrheal</th>
<th>Non-diarrheal samples Mean (SD) (n=54)</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (×10^12)/L</td>
<td>4.5 (0.3)</td>
<td>4.5-4.6</td>
<td>4.6 (0.4)</td>
<td>4.5-4.7</td>
<td>0.39</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>11.5 (0.9)</td>
<td>11.3-11.7</td>
<td>11.6 (0.8)</td>
<td>11.4-11.9</td>
<td>0.32</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>35.3 (2.3)</td>
<td>34.8-35.7</td>
<td>35.8 (2.2)</td>
<td>35.2-36.4</td>
<td>0.18</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>78.1 (4.1)</td>
<td>77.2-78.9</td>
<td>78.5 (5.7)</td>
<td>76.9-80.0</td>
<td>0.6</td>
</tr>
<tr>
<td>MCH (g/dL)</td>
<td>32.5 (1)</td>
<td>32.3-32.7</td>
<td>32.5 (1.1)</td>
<td>32.2-32.8</td>
<td>0.64</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>388.5 (99)</td>
<td>368.4-408.6</td>
<td>385.4 (92.4)</td>
<td>360.2-410.7</td>
<td>0.85</td>
</tr>
<tr>
<td>PLT (×10^9/L)</td>
<td>7.7 (3.2)</td>
<td>9.0-10.3</td>
<td>9.3 (2.7)</td>
<td>7.6-9.00</td>
<td>&lt;0.01*</td>
</tr>
</tbody>
</table>

*Significant at P≤0.05. RBC: Red blood cell, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, PLT: Platelets, WBC: White blood cell, SD: Standard deviation, CI: Confidence interval, HCT: Hematocrit

**Table 2: Hemoglobin, SI, TIBC, and transferrin saturation results of cases and controls having hemoglobin concentration <11 g/dL**

<table>
<thead>
<tr>
<th>Blood parameter</th>
<th>Presence of diarrheal Mean (SD) (n=21)</th>
<th>95% CI</th>
<th>Non-diarrheal samples Mean (SD) (n=80)</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>10.3 (0.6)</td>
<td>10.2-11.6</td>
<td>10.4 (0.6)</td>
<td>11.5-11.8</td>
<td>0.71</td>
</tr>
<tr>
<td>SI (µg/dL)</td>
<td>34.4 (10.5)</td>
<td>29.6-39.2</td>
<td>40.3 (2.4)</td>
<td>19.5-61.0</td>
<td>0.38</td>
</tr>
<tr>
<td>TIBC (µg/dL)</td>
<td>316.6 (23.6)</td>
<td>306.9-328.4</td>
<td>363.7 (57)</td>
<td>317.6-413.1</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>Transferrin saturation (%)</td>
<td>11.0 (3.8)</td>
<td>9.3-12.7</td>
<td>11.4 (7.2)</td>
<td>5.4-17.4</td>
<td>0.84</td>
</tr>
</tbody>
</table>

*Significant at P≤0.05. SI: Serum iron; TIBC: Total iron-binding capacity; SD: standard deviation, CI: Confidence interval

**Table 3: Mean values of blood parameters in association with different parasitic pathogens**

<table>
<thead>
<tr>
<th>Blood parameter</th>
<th>Negative Mean (SD) (n=61)</th>
<th><em>G. lamblia</em> Mean (SD) (n=40)</th>
<th><em>E. histolytica</em> Mean (SD) (n=42)</th>
<th><em>E. histolytica</em> and <em>G. lamblia</em> Mean (SD) (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (×10^12)/L</td>
<td>4.6 (0.4)</td>
<td>4.5 (0.3)</td>
<td>4.4 (0.3)</td>
<td>4.7 (0.2)</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>11.6 (0.9)</td>
<td>11.6 (8.1)</td>
<td>11.4 (1.0)</td>
<td>12.0 (0.6)</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>35.7 (2.3)</td>
<td>35.3 (2.1)</td>
<td>35.2 (2.5)</td>
<td>36.1 (1.7)</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>78.1 (5.4)</td>
<td>79.1 (3.5)</td>
<td>77.7 (5.0)</td>
<td>77.6 (2.2)</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>325.2 (2.4)</td>
<td>325.9 (1.4)</td>
<td>325.2 (2.2)</td>
<td>259.9 (2.0)</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>32.3 (1.1)</td>
<td>32.7 (0.9)</td>
<td>32.5 (1.1)</td>
<td>33.3 (0.7)</td>
</tr>
<tr>
<td>PLT (×10^9/L)</td>
<td>385.5 (95.3)</td>
<td>390.2 (102.3)</td>
<td>379.9 (90.3)</td>
<td>432.7 (114.6)</td>
</tr>
<tr>
<td>WBC (×10^9/L)</td>
<td>8.7 (3.1)</td>
<td>8.8 (2.3)</td>
<td>10.0 (3.4)</td>
<td>10.6 (2.6)*</td>
</tr>
<tr>
<td>SI (µg/dL)</td>
<td>37.5 (22.8)</td>
<td>36.9 (14.4)</td>
<td>34.3 (7.4)</td>
<td>ND</td>
</tr>
<tr>
<td>TIBC (µg/dL)</td>
<td>358.5 (53.2)</td>
<td>311.4 (20.8)</td>
<td>319.8 (25.9)</td>
<td>ND</td>
</tr>
<tr>
<td>Transferrin saturation (%)</td>
<td>10.7 (6.5)</td>
<td>12.0 (5.1)</td>
<td>10.9 (2.8)</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Significant at P≤0.05. RBC: Red blood cell; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; PLT: Platelets; WBC: White blood cell, SI: Serum iron, TIBC: Total iron binding capacity; SD: Standard deviation, ND: Nondetermined, HCT: Hematocrit, *G. lamblia*: G. lamblia, *E. histolytica*: Entamoeba histolytica
Discussion

Gastrointestinal diseases are a major public health problem for children in Gaza Strip, as in other developing communities around the world, and are among the most common causes of IDA due to gastrointestinal tract blood loss. Gastroenteritis can also be accompanied by malnutrition with reduced iron intake further contributing to the IDA. Many previous and most recent studies from Gaza Strip and other parts of Palestine (West Bank) revealed a significant prevalence of IDA among kindergarten, pre- and age-school children due to gastrointestinal infection and other nutritional factors.

In this study, CBC, SI, and TIBC were performed to investigate the possible effect of gastrointestinal infections on these blood parameters among kindergarten children suffering from community gastroenteritis and their comparable healthy controls. The findings of our study showed that 21.8% (21/96) of the diarrheal cases also had iron deficiency with hemoglobin concentration <11 g/dL, compared to only 14.8% (8/54) of non-diarrheal controls. The hemoglobin level with other blood parameters was slightly lower in diarrheal cases than in non-diarrheal controls. In the iron deficient children, SI, TIBC, and transferrin saturation were also slightly lower in diarrheal cases. Assuming no interference from other etiological factors like inflammation, the results suggest that IDA is increased in diarrheal cases, although this difference did not achieve statistical significance.

This could be due to the fact that the infection was not chronic enough to cause malabsorption or sufficient blood loss to effect iron levels. The results of this work are inconsistent with those noted in other studies, where the authors found that the high prevalence of enteropathogens was associated with anemia. Al-Zain studied the impact of socioeconomic conditions and intestinal parasitic infection on hemoglobin level among children aged 2–15 years in Um-Unnasser village, North Gaza, Palestine. He found that 25% were anemic, and the prevalence rate of anemia was highest in children aged below 6 years. In another study in Gaza Strip, Al-Agha and Teodorescu studied the prevalence of anemia, nutritional indices and intestinal parasitic infestation in primary school children aged 6–11 years. They found an association between anemia and intestinal parasitic infestation in those children who manifested mainly by intestinal parasites E. histolytica and G. lamblia. Furthermore, they showed a high prevalence of anemia in children suffered from malnutrition along with intestinal parasitic infestation.

Iron deficiency anemia seen in diarrheal and non-diarrheal cases suggests that nutritional anemia may be prevalent in this population, which may aggravate the clinical symptoms. Infections are a well-recognized cause of mild to moderate anemia. Several studies have demonstrated that even mild infections can induce a significant decrease in iron levels. The reduction in hemoglobin level during and after acute or chronic inflammatory diseases is due to many factors such as blocking the iron release, reduction in the intestinal absorption of iron and inhibition or inappropriate erythropoietin production. Levy et al. 2005 in a study, conducted in Israel examined the association between anemia and infection among Bedouin infants. They concluded that anemia is a significant and independent risk factor for diarrhea. In Ecuador, Sackey et al. found that children suffered from intestinal parasitic infections had significantly reduced mean hemoglobin levels compared with their noninfected counterparts. Their data indicated that G. lamblia infection had an adverse impact on child linear growth and hemoglobin levels. Moreover, in a recent study performed in Egypt, the frequency of parasites was significantly higher in cases with IDA compared to controls. Furthermore, a study conducted recently to compare hematological indices in cases with Blastocystis hominis infection against healthy controls revealed that the frequency of SI was significantly lower in cases with B. hominis infection compared to controls.

Ali and Zuberi in Pakistan investigated the presence or absence of any association between low birth weights, recurrent diarrhea or recurrent acute respiratory infections with IDA in Pakistani children aged 1–2 years. They found that there was no statistically significant difference of recurrent diarrhea between anemic and nonanemic children at 1–2 years age. Another study in Pakistan conducted to determine the clinical pattern of infections in admitted malnourished children. A total of 112 admitted malnourished children with various types of infections aged 3–60 months were enrolled in the study. The authors found that the association of malnutrition and infections among children is an important health issue and the common infections found in malnourished children were acute gastroenteritis (44.6%), followed by enteric protozoa and helminthic infections (23%).

Study limitation

The generalizability of above results is subject to certain limitations. For instance, the difficulty in measuring serum ferritin levels and using low transferrin saturation as the marker of iron deficiency could interfere with anemia of inflammation.

Conclusion

Diarrhea remains a health problem in children in Gaza. This study highlights quite a number of the enteric pathogens that cause community gastroenteritis among kindergarten children in Gaza and their possible effect on hemoglobin concentration and TIBC. Another cohort study, on infected and healthy population is recommended to determine other causes of community gastroenteritis and their possible effect on hematological indices.
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Expression of Podoplanin in Different Grades of Oral Squamous Cell Carcinoma

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Abstract

Background: The expression of podoplanin is up-regulated in a number of different human cancers, including squamous cell carcinoma of the oral cavity and its relationship with tumor invasion raises the possibility that podoplanin expression could be used as a biomarker for diagnosis and prognosis. Aim: The aim of the present study is to evaluate the expression of podoplanin in different grades of squamous cell carcinoma (SCC) and to correlate the expression of podoplanin with relevant clinical features such as age, sex, site and associated habits. Materials and Methods: Retrospective study was carried on formalin fixed, paraffin embedded blocks of oral SCC (OSCC) from the archives of Department of Oral and Maxillofacial Pathology, Vishnu Dental College, Bhimavaram. Thirty diagnosed cases were included, of which 10 were well-differentiated SCC (WDSCC) (n = 10), 10 moderately DSCC and 10 poorly DSCC. Demographics including age, sex, gender and associated habit history, were recorded. Immunohistochemical staining was done with podoplanin anti D2–40 antibody, for all the cases of OSCC and assessed qualitatively. The data obtained were tabulated and subjected to statistical analysis. Results: In the present study, 27 cases of SCC showed podoplanin expression and remaining three cases showed no expression. The scoring criterion suggested by Yuan et al. was followed for semi-quantitative assessment. OSCC, seven cases presented weak expression (Immunoreactive score [IRS] 0–3), 15 presented moderate expression (IRS Score 4–7) and 5 presented high expression (IRS Score > 8). The assessment of podoplanin expression in the cytoplasm, the membrane and the cytoplasm and membrane (both) of tumor cells showed overall high positivity in the cytoplasmic followed by both and the membrane. Conclusion: Podoplanin could be a potent biomarker in assessing the cytoplasm/membrane staining of tumor cells. Furthermore, a high level of podoplanin expression is suggestive of high frequency of lymph node metastasis and immature status in the differentiation process of OSCC.

Keywords: Epithelial mesenchymal transition, Immunohistochemistry, Podoplanin, Squamous cell carcinoma

Introduction

Head and neck squamous cell carcinoma (HNSCC) is a devastating disease involving dysregulation of numerous pathways linked to cellular differentiation, cell cycle control, epithelial mesenchyme interaction, apoptosis, angiogenesis, and metastasis.[1] Abnormal proliferation of cell results from the accumulation of multiple genetic alterations influenced by genetic predisposition as well as by environmental influences, including tobacco, alcohol, chronic inflammation and viral infections. HNSCC is the sixth most common cancer accounting over 500,000 new cases annually worldwide that includes sites in the oral cavity, pharynx and larynx. Oral SCC (OSCC) is the most common malignant tumor of the HN regions and accounts for the two-thirds of the HNSCC cases occurring in developing countries.[2,3]

The potentially malignant disorders such as leukoplakia, erythroplakia, palatal changes associated with reverse smoking, oral submucous fibrosis, lichen planus, syphilitic glossitis, and sideropenic dysphagia have a high risk of malignant transformation, on average about 1% of oral lesions transform into cancer annually. Knowledge in genomic and basic research have made ease in understanding the molecular
process governing tumor formation and progression. The important factor which shows a direct impact on prognosis and therapeutic strategy in various types of cancer is that of lymph node status. Electron microscopic studies have revealed that the lymphatic spread of cancer cells will show the poor prognosis. Understanding the lymphatic system at the molecular level showed some loophole 15 years back, due to lack of lymphatic epithelium markers. The introduction of the markers that is, LYVE-1, Prox1 and podoplanin has explored the lymphatic vessels present in the tumor and peritumoral areas, and the prognosis of various malignancies such as malignant melanoma, SCC of the HN, breast cancer and gastric adenocarcinoma.

Podoplanin is a 38-KDa type-I transmembrane glycoprotein consisting of 162 amino acids, nine of which form the intracellular domain. The extracellular domain is highly O-glycosylated with sialic acid, α-2, 3 linked to galactose, forming the main part of the protein carbohydrate moieties. In normal human tissue podoplanin is expressed in kidney podocytes, alveolar type-1 cells, lymphatic endothelium, skeletal muscle, placenta, lung and heart, in the myofibroblasts of the breast and salivary glands, in the osteoblasts and mesothelial cells and in the basal layer of human epidermis. The expression of podoplanin is up-regulated in a number of different human cancers, including SCC of the oral cavity. In addition, recent experimental results have demonstrated that podoplanin mediates a pathway leading to collective cell migration and invasion in vivo and in vitro. The expression of podoplanin in human cancers and its relationship with tumor invasion raises the possibility that podoplanin expression could be used as a biomarker for diagnosis and prognosis.

Podoplanin is specifically expressed in lymphatic endothelial cells but not in blood endothelial cells. OSCC’s spread through the lymphatic route and podoplanin can be utilized for recognizing the lymphatic vessels in the tumor and peritumoral areas. The expression of podoplanin by the tumor cells can also provide a clue towards the tumor migration and progression. Hence, the present study aims to analyze and correlate the podoplanin expression in different grades of OSCC.

Materials and Methods

This study is an retrospective study, retrieved from the archives by MDSCC and 10 were of poorly differentiated SCC (PDSCC). Of 30 diagnosed cases were included, of which 10 were well differentiated SCC (WDSCC) (n = 10), 10 moderately differentiated SCC (MDSCC) and 10 were of poorly differentiated SCC (PDSCC).

Demographics including age, sex, gender and associated habit history, were recorded for all the 30 cases of OSCC. Diagnosis for the selected cases was also established on hematoxylin and eosin stained sections. Immunohistochemical staining was done with podoplanin anti D2–40 antibody, for all the cases of OSCC.

Interpretation and counting

Presence of brown colored end product at the site of target antigen was considered as positive immunoreactivity. Cytoplasmic and/or membrane staining was considered as positive immunoreactivity. Lymphatics were considered as internal control. Ten representative areas were selected in each slide. Hundred cells were counted in 10 different fields so as to assess the percentage of podoplanin expression in the stained slides. Also, the brown stained cells of each representative area were differentiated based on the podoplanin expression in the cytoplasm, membrane or both (cytoplasm and membrane). Quantity scores of 0–5 were given if 0%, 1% to 10%, 11% to 30%, 31% to 50%, 51% to 80%, and 81% to 100% of the tumor cells showing positivity. The staining intensity was rated on a scale of 0–3, with 0 = negative, 1 = weak, 2 = moderate, and 3 = strong. The raw data were then converted to a German immunoreactive score (IRS) by multiplying the quantity and staining intensity scores. The final scores were put on the scale ranging from 0 to 15 as 0 to 3 = Weak, 4 to 7 = Moderate, >8 = Strong.

Results

Of 30 cases of OSCC, 19 were male, and 11 were female with an age range of 20–80 years with a mean age of 50 years. The predominant cases were observed in fourth to sixth decade of life. OSCC recorded were observed to affect all the sites of the oral mucosa with buccal mucosa being the predominant site followed by alveolar mucosa, tongue, palatal mucosa. The least affected site includes retromolar trigone, floor of the mouth and vestibule.

The related history of the tobacco habit in smokeless or smoking form was recorded. Another habit including alcohol was also assessed. Present study showed 19 cases with a history of smoking, nine cases with alcohol habit, seven with pan chewing and remaining eight did not reveal any habit history [Figure 1]. Podoplanin was highly positive for the lymphatic vessels within the tumor tissue and were considered as internal control. The expression of podoplanin in the tumor cells were observed in the cytoplasm, the membrane and the cytoplasm/membrane (both) and these varied expressions by the tumor cells in different grades of carcinoma were suggestive of prognostic importance [Figures 2, 3 and 4].

Immunostaining of podoplanin was assessed in all the grades of SCC. WDSCC showed positivity in nine cases, MDSCC in all the 10 cases and PDSCC in eight cases. Three cases, one in WDSCC and two in PDSCC showed negative staining. Podoplanin expression in the cytoplasm was highest in PDSCC (71%) followed by WDSCC (56.5%) and then by MDSCC (53.86%). Expression in membrane was also more in PDSCC (6.7%) then in WDSCC (5.1%) and least
in MDSCC (1.31%). The variability in the expression of the podoplanin in the cytoplasm and membrane (both) was observed, which revealed highest in MDSCC (44.79%), followed by WDSCC (38.4%) and least in PDSCC (22.2%). The expressions of podoplanin by tumor cells were observed to be focal and diffuse [Figures 5 and 6]. The focal expression was observed in the tumor nests of WDSCC whereas diffuse expression was observed in MDSCC and PDSCC. IRS scoring.
of MDSCC showed three cases as strong, four as moderate and three as weak whereas PDSCC showed one as strong, five as moderate and two as weak. WDS CC revealed one as strong, six as moderate and two as weak IRS score [Graph 1].

The correlation of habit with the IRS scoring of OSCC revealed major number of cases (15) with moderate scoring out of which 13 cases were associated with habit. Seven cases were with a weak score all associated with habit. Five cases showed strong scores out of which three were associated with habit. Pearson’s correlation of IRS with sex and habit were not statistically significant.

Discussion

Oral cancer development is a multistep process with accretion of genetic, epigenetic and metabolic alterations resulting from exposure to carcinogens. Metastatic spread to regional lymphnodes through the lymphatic system is considered one of the major pathways by which SCC disseminates.[1,5,6]

The incidence of OSCC is approximately 2–10/100,000 population/year that could be due to the environmental differences or life style or habits such as betel quid, snuff dipping or reverse smoking. Tobacco in various forms is regarded as the main cause of oral cancer. Apart from tobacco the other associated factors include alcohol, ultraviolet light, Vitamin A and C deficiency, immuno-compromised patients, viral infections and to the least extent trauma. OSCC shows a male predominance with 2:1 ratio except for the carcinoma arising on the vermillion border of the lower lip and with the wide age range of fourth to seventh decades.[7] The present study observed occurrence of OSCC predominantly in male with a peak incidence in fifth to seventh decades and buccal mucosa as the most common site followed by alveolar mucosa, tongue and palatal mucosa. 22 cases of OSCC were associated with a history of smoking, alcohol and pan chewing. Eight cases presented without adverse habits such as tobacco and alcohol, which could be attributed to the combined effect of genetic, epigenetic and environmental factors.

Podoplanin is a mucin like transmembrane glycoprotein that is expressed in lymphatic endothelium and various normal human tissues.[8] The podoplanin expression was found in tumor cells of various types of cancer such as vascular tumors, malignant mesothelioma, central nervous system tumors and SCC. The presence of this protein in tumor cells is helpful for pathological diagnosis and is reported to be expressed in aggressive tumors with higher invasive and metastatic potential.[9,10] The present study assessed the expression of podoplanin in different grades of OSCC as this heterogeneous malignancy is characterized by the increased proliferation of the tumor cells which initiates cellular migration and invasion.

Podoplanin expression shows two patterns (a) diffused expression in the tumor cells and (b) focal expression in proliferating periphery with no expression in the central areas of the tumor cell nests.[9] The hierarchical distribution pattern of podoplanin was observed within the tumor nests of WDS CC. The podoplanin positive tumor cells appear to be more localized to the periphery of the tumor nests whereas the central area appeared negative.[11,12] The expression at the periphery of the tumor cells suggests its higher proliferative and self-renewal capacity, whereas the central cells are suggestive of terminal differentiation of tumor cells resulting from maturation and/or degenerative changes. These findings were in correlation with various authors including Martin-Villar et al.[11] (2005), Shimada et al.[13] (2009), Carvalho et al.[9] (2010), Rodrigo et al.[11] (2010), Margaritescu et al.[14] (2010) and Chuang et al.[10] (2013). The podoplanin expression pattern was different in MDSCC and P DSCC when compared to WDS CC. Most of the cases of MDSCC showed a diffuse pattern except for one which shows focal expression whereas all the cases of PDSCC showed diffuse expression. The diffuse expression of podoplanin in MDSCC and PDSCC suggests some cytoskeletal alterations within the tumor cells thereby suggesting increased cellular migration and a role in carcinogenesis.[9] This finding is in contrast to Rodrigo et al. who showed highly significant podoplanin expression in well differentiated tumors when compared to poorly differentiated tumors and Franchi et al. who reported no staining of tumor cells in their study from HNSCC.[1,12]

Of 30 cases of SCC, three cases lacked podoplanin expression, and 27 showed expression that was scored by the criteria suggested by Yuan et al.[2] The overexpression of podoplanin was observed in MDSCC with three cases showing high IRS score. High levels of podoplanin may be suggestive of high frequency for lymph node metastasis than those which expressed a lower level of podoplanin. The difference in the scoring of podoplanin expression was not statistically significant probably because of small sample size and technique sensitivity.

An attempt has been made to assess the habit relationship and podoplanin expression in OSCC. The finding of which suggested that the habit alone may not cause cellular changes instead it may act as an accelerating factor in the tumor
progression. Apart from the habit other factors like genetic, epigenetic, transcriptional and post-transcriptional mechanisms may co-exist so as to promote tumorigenesis.[2,10]

Schacht et al. in his study showed predominant cytoplasmic podoplanin positivity in PDSCC, enhanced membrane podoplanin expression in MDSCC and no expression in WDSCC. Our study is in slight correlation with Schacht et al. in the cytoplasmic podoplanin expression which was high in PDSCC followed by WDSCC and then MDSCC.[17] The diverse podoplanin expression in the cytoplasm, the membrane and the cytoplasm and membrane (both) of tumor cells of SCC cases may show enhanced propensity for tumor progression. The present study also showed both the cytoplasmic and membrane positivity being higher in all the grades of SCC when compared to the only membrane positivity and this high expression could be attributed to the expression of podoplanin at the protein and mRNA levels.[19] Overall podoplanin expression was high in MDSCC when compared to PDSCC and WDSCC thereby reflecting the immature status in the differentiation process of SCC.

The limitations drawn from the present study is that the expression of podoplanin was studied exclusively on the tumor cells with a limited number of OSCC cases. The localization of podoplanin expression also raised the question whether it was truly induced by the tumor cells or by factors secreted from stromal cells, as the tumor cells present close to surrounding stromal cells and the tumor microenvironment interactions might collectively play a decisive role in tumor progression.

In the present study, the podoplanin expression was studied mainly in the tumor cell nests without involving the invasive front and observed that the podoplanin expression was highly expressed by the epithelial tumor cells along with the stroma surrounding the tumor cells. This finding is in correlation with Astarita et al.[19] and Yamanashi et al.[18] On the other hand we also stress that podoplanin expression alone cannot be sufficient for tumor progression because many lesions exhibited the protein expression only in the basal cell layers.[11,21] Hence, to assess the prognostic role of podoplanin in different grades of SCC needs further investigation with large sample size and considering the staining pattern so as to assess its accurate role in the morbidity and mortality.

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Audit of Childbirth Emergency Referrals by Trained Traditional Birth Attendants in Enugu, Southeast, Nigeria

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Abstract

Background: The essence of training traditional birth attendants (TBAs) is to attend to women in uncomplicated labor and to refer them immediately to hospitals when complications develop. Aim: The aim was to audit childbirth emergency referrals by trained TBAs to a specialist hospital in Enugu, Nigeria. Subjects and Methods: A retrospective study of 205 childbirth emergencies referred to Semino Hospital and Maternity (SHM), Enugu by trained TBAs from August 1, 2011 to January 31, 2014. Data analysis was descriptive and inferential at 95% confidence level. Results: Most of the patients (185/205, 90.2%) were married and (100/205, 48.8%) had earlier booked for antenatal care in formal health facilities. There were obstetric danger signs or previous bad obstetric histories (pregnancies with unfavorable outcome) in 110 (110/205, 53.7%) women on admission at SHM. One hundred and fifteen (115/205, 56.1%) women walked into the hospital by themselves while 50 (50/205, 24.3%) could not walk. The fetal heart sounds were normal in 94 (94/205, 45.6%), abnormal in 65 (65/205, 31.8%) and absent in 42 (42/205, 20.4%) of the women on admission. Five healthy babies were delivered by the TBAs before referring their mothers. Delays of more than 12 h had occurred in 155 (155/205, 76.6%) of the women before referrals. Prolonged labor (100/205, 48.8%), obstructed labor (40/205, 19.5%), attempted vaginal birth after previous cesarean delivery (40/205, 19.5%) and malpresentation (30/205, 14.6%) were the common indications for referrals. The maternal mortality and perinatal mortality ratios were 610/100,000 live births and 228/1000 total births respectively. Conclusion: Delays at TBA centers are common before referral and most patients are referred in poor clinical state. Further training and re-training of the TBAs with more emphasis on recognition of obstetric danger signs and bad obstetric histories may help in screening high-risk patients for prompt referral to hospitals before complications develop.

Keywords: Childbirth emergencies, Delay, Outcomes, Referrals, Trained traditional birth attendants

Introduction

About 15% of pregnant women worldwide will have life-threatening complications that will require emergency obstetric care services.[1] The risk of developing these emergencies is highest during childbirth and the first few hours after delivery. These emergencies are not always predictable and should be anticipated, recognized early and promptly referred to hospitals with comprehensive obstetric emergency facilities to prevent adverse fetomaternal outcomes. Early recognition of danger signs, previous pregnancies with unfavorable outcome (bad obstetric histories) and childbirth complications by the patients and their families, traditional birth attendants (TBAs), and skilled birth attendants (SBAs) may prompt early presentation of patients for life-saving interventions. The presence of an SBA at every delivery is one...
of the key interventions for reducing maternal and perinatal mortality and morbidity.\(^5\) World Health Organization (WHO) defined a SBA as “an accredited health professional such as a midwife, doctor or nurse who has been educated and trained to proficiency in the skills needed to manage uncomplicated pregnancies, childbirths and the immediate postnatal period and in the identification, management, and referral of complications in women or newborns.”\(^3\)

On the other hand, a TBA is a person who assists the mother during childbirth and who initially acquired her skills by delivering babies herself or through an apprenticeship to other TBAs.\(^4\) The services of the TBAs ought to be limited to uncomplicated pregnancies and childbirths, however a large number of complicated deliveries have been reported.\(^5\) Deliveries conducted by TBAs and other unskilled birth attendants have been shown to be associated with 4.67 times higher morbidity and mortality when compared with deliveries supervised by SBAs.\(^4\) The high rate of utilization of TBA care in Nigeria may contribute to the very low rate (36%) of SBA utilization in Nigeria despite the 60.1% rate of antenatal care uptake.\(^11\) This may also contribute to the currently high maternal mortality rate of 576/100,000 live births in Nigeria.\(^11\)

This high level of TBA-services utilization prompted WHO to promote training of TBAs as a major public health strategy to reduce these tragic and preventable fetomaternal deaths and countless complications. The training usually involve; recognition of previous “bad obstetric histories” and “obstetric danger signs” of common causes of maternal and neonatal deaths during pregnancy, labor and puerperium, and the need for prompt referral of such cases. While some authors observed improvements in maternal and neonatal outcome following training,\(^12\) others could not establish any potential benefits.\(^13\)

Despite some concerns about the usefulness of TBA training,\(^5,14\) the Enugu state Ministry of Health embarked on training of TBAs in the state with a view to improve the overall maternal and neonatal health indices of the state. This study, therefore, aims at auditing the childbirth emergency referrals to a specialist hospital in Enugu East Local Government Area (LGA) of Enugu state by trained TBAs in the LGA by assessing the conditions of the referred women at presentation, indications for their referrals, obstetric delays at the TBAs, and the fetomaternal outcome of such pregnancies.

**Subjects and Methods**

The ethical clearance of the study was obtained from the institutional review board of the Enugu state university teaching hospital (ESUTH), Parklane, Enugu. The study took place at Semino Hospital and Maternity (SHM), Enugu between August 1, 2011 and January 31, 2014. The SHM is located at Abakpa Nike, Enugu, the center of Enugu East LGA of Enugu state. It is a private health facility that provides comprehensive emergency obstetric care services 24 h of every day.

This study was a retrospective study that audited 208 consecutive cases of childbirth emergency referrals to SHM by trained TBAs in Enugu East LGA, Enugu state. The patients for the study were consecutively selected as already stated in the methodology. Pregnant women in labor referred to SHM from a trained TBA home and requiring emergency obstetric intervention were included in the study. Exclusion criteria included women referred to a teaching hospital from SHM because of medical or labor complications that required intensive care or multidisciplinary approach and women with incomplete data.

Prior to the study, the Enugu State Ministry of Health had trained 45 TBAs in the LGA. The training included; the recognition of “danger signs” or “bad obstetric history” of common causes of maternal and neonatal deaths during pregnancy, labor and puerperium, and the need for prompt referral of such cases. They were also trained on clean safe delivery, universal precautions, and avoidance of harmful traditional practices. The TBAs in the LGA have regular meetings in Abakpa health center every 3 months. During these quarterly meetings, they decide among other things the hospitals to refer their patients whenever indicated. The decision on the hospitals to refer cases is determined by a number of factors including availability of efficient maternal services, ability to provide emergency cesarean delivery and other operative deliveries, affordability of cost of care by the patients, and willingness of the health facility to reimburse the TBAs the transport fare and other costs already incurred in the management of the patient before the referral. Consequently, the TBAs in the LGA had an understanding with the management of SHM to refer their patients to the hospital whenever indicated. However, there are some other health facilities in the LGA where the TBAs sometimes refer their patients when indicated. Most of the TBAs in the LGA are domicile at Abakpa Nike in Enugu urban, and at Ugwuowo Nike, a rural community in Enugu East LGA. The TBAs usually phoned the hospital prior to accompanying the patient to such hospital via private or public vehicle. Those in Abakpa Nike urban occasionally accompanied their patients to SHM by trekking. Most of the referrals were at night. The roads in Abakpa Nike (urban community) and Ugwuowo Nike (rural community) are good. The major barrier to referral is patient’s refusal to be referred to hospital usually because of an unaffordable cost of care.

During the study period, a total of 219 women were referred to SHM from the TBAs in the LGA. The case records (folders) of these women were retrieved and relevant data extracted using case record forms (data entry profoma) specifically designed for the study. Data sought included patients’ sociodemographic characteristics, presence of danger signs (surgical scars on the abdomen, leg/facial swelling, vaginal bleeding, and drainage of liquor), bad obstetric history (previous pregnancies with unfavorable outcome), fetomaternal condition on admission, delays by TBAs before referral, and fetomaternal outcome following delivery.

Functional ability to walk, maternal vital signs, and fetal heart sounds were used to assess the fetomaternal condition of the
patients on admission at SHM. The maternal condition on admission was said to be good if she was able to walk into the hospital (SHM) unaided and/or if her vital signs including the blood pressure, pulse rate, and temperature were within normal limits. It was said to be poor if she was supported or carried into the hospital and/or if her vital signs including the blood pressure, pulse rate, and temperature were deranged. On the other hand, the fetal condition was said to be good if the fetal heart rate was within normal limits (120–160 beats/min), and regular. It was said to be poor if it was deranged and/or irregular. A woman is said to have developed a postpartum morbidity if she had wound infection, urinary tract infection or any other adverse conditions in the postpartum period.

Eleven women out of the 219 women initially admitted were referred out from SHM to either University of Nigeria Hospital or Enugu State University Teaching Hospital (ESUTH) – Parklane due to certain complications at presentation. Three women’s folders that were delivered at SHM had incomplete medical history and were also excluded. Thus, a total of 205 women’s results were analyzed. The study was approved by the Ethics Committee of the ESUTH-Parklane, Enugu.

Data analysis was done by both descriptive and inferential statistics using the Statistical Package for Social Sciences (SPSS) Software version 16 (SPSS Inc., Chicago, IL, USA). Proportions were tested using the Pearson’s Chi-square and relationship expressed using the odds ratio (OR) and confidence interval (CI). A $P < 0.05$ was considered to be statistically significant.

**Results**

During the 2½ years (30 months) study period, childbirth emergency referrals from the trained TBAs accounted for 208/358 (58.1%) of the deliveries. However, 205 case notes had complete data and were analyzed. One hundred and eighty six 186/205 (90.7%) of the patients were between 20 and 40 years of age, 185/205 (90.2%) were married, 120/205 (58.5%) were unemployed and 115/205 (56.1%) were primigravidae. Details of the sociodemographic characteristics of the women are as shown in Table 1.

One hundred 100/205 (48.8%) of the patients had booked for antenatal care in formal health facilities and were seen at least once by SBA in the index pregnancies before presentation to the TBAs in labor. However, 51.2% (105/205) did not receive any form of antenatal care in a formal health facility prior to presentation to the TBAs in labor.

There were obvious dangers signs or bad obstetric histories in 155/205 (75.6%) of the patients. One hundred and fifty-five 155/205 (75.6%) of the women were admitted walking unsupported while 50/205 (24.4%) could not walk at the time of admission. The maternal vital signs were normal in the 100/205 (48.8%) that could walk on admission, and deranged in the 50 (24.4%) that could not walk. The fetal heart sounds were normal in 94/205 (45.6%), abnormal in 65/205 (31.8%), and absent in 42/205 (20.4%) of the patients on admission. Five healthy babies had been delivered by the TBAs before referring their mothers due to certain postpartum complications. Delays of more than 12 h at the TBAs before referrals occurred in 155 (75.6%) of the patients. Details are as shown in Table 2.

Prolonged labor (100/205, 48.8%), obstructed labor (40/205, 19.5%), attempted vaginal birth after cesarean delivery (40/205, 19.5%) and fetal mal-presentations (30/205, 14.6%) were the common indications for referrals during childbirth. Details of the indications for referral to SHM are as shown in Table 3. Oxytocin injections (65/205, 31.7%), native concoctions (45/205, 22.0%), fundal pressure (35/205, 17.1%), and rectal misoprostol insertion (10/205, 4.9%) were the common treatments given by the TBAs before referrals.

Following admission at SHM, most of the patients 171/205 (83.4%) had emergency cesarean section while 32/205 (15.6%) were delivered vaginally. Delays at SHM of more than 1 h prior to intervention occurred in 116/205 (56.6%) of the patients while 89/205 (43.4%) received appropriate treatment within 1 h of admission. Inability to pay the initial financial deposit (50/205, 24.4%), delay in giving consent for operations (20/205, 9.8%), nonavailability of anesthetist (20/205, 9.8%) nonavailability of obstetrician (15/205, 7.3%), nonavailability of blood (10/205, 4.9%) and nonavailability of power or light supply (1/205, 0.5%) were the identified causes of delays in SHM.

Following intervention at SHM, 204 (204/205, 99.5%) of the mothers survived while one (1/205, 0.5%) died of uncontrollable postpartum hemorrhage. Forty-eight (48/205, 23.4%) mothers developed some postpartum complications. Delays in the administration of antibiotics (30/205, 14.6%) and in the delivery of neonates (30/205, 14.6%) was noted in some cases.

**Table 1: Socio-demographic characteristics of the women**

<table>
<thead>
<tr>
<th>Socio-demographic variables</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (in years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>5</td>
<td>2.4</td>
</tr>
<tr>
<td>20-30</td>
<td>123</td>
<td>60.0</td>
</tr>
<tr>
<td>31-40</td>
<td>63</td>
<td>30.7</td>
</tr>
<tr>
<td>41-50</td>
<td>14</td>
<td>6.8</td>
</tr>
<tr>
<td>Residence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural residence</td>
<td>85</td>
<td>41.5</td>
</tr>
<tr>
<td>Urban residence</td>
<td>120</td>
<td>58.5</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>185</td>
<td>90.2</td>
</tr>
<tr>
<td>Unmarried</td>
<td>20</td>
<td>9.8</td>
</tr>
<tr>
<td>Employment status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employed</td>
<td>85</td>
<td>41.5</td>
</tr>
<tr>
<td>Unemployed</td>
<td>120</td>
<td>58.5</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nullipara</td>
<td>115</td>
<td>56.1</td>
</tr>
<tr>
<td>Multipara</td>
<td>90</td>
<td>43.9</td>
</tr>
</tbody>
</table>
There were 206 babies (including a set of twin) out of whom 42 were stillbirths and 164 live births giving a maternal mortality ratio (1/164 live births × 100,000) of 610/100,000 live birth. Fetal asphyxia with 5 min Apgar score of <7 occurred in 104/206 (50.7%) babies out of whom 65/206 (31.6%) were admitted for newborn care and 5 (2.4%) died within 7 days of newborn admission. The perinatal mortality ratio was (47 × 1000/206 total births) 228/1000 total birth.

**Discussion**

An audit of the referred cases revealed the presence of obstetric danger signs or bad obstetric histories in 53.7% of the patients that were ignored or not noticed by the trained TBAs. Forty (40/100, 40%) of such patients had one or two previous cesarean deliveries but defaulted from planned cesarean delivery in formal health facilities where they were receiving antenatal care to risk vaginal delivery in TBA centers. These patients should have been referred by the trained TBAs before the complications developed. Previous report from the study area observed that “pregnant women abhor cesarean delivery, regard it as social calamity, a slur on their reproductive integrity and would do everything possible to resist further cesarean delivery including defaulting hospital delivery, taking native concoctions, and starving in order to reduce the fetal size so as

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**Table 2: Clinical history/signs observed in the referred women on admission at SHM and the duration of obstetric delays at the TBA homes before referral to SHM**

<table>
<thead>
<tr>
<th>Clinical history/signs</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of danger signs or bad obstetric history</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous one or two cesarean delivery</td>
<td>40</td>
<td>19.5</td>
</tr>
<tr>
<td>Defaulted cesarean delivery</td>
<td>40</td>
<td>19.5</td>
</tr>
<tr>
<td>Previous history of obstructed labor</td>
<td>10</td>
<td>4.9</td>
</tr>
<tr>
<td>Previous history of birth asphyxia</td>
<td>5</td>
<td>2.4</td>
</tr>
<tr>
<td>Fetal macrosomia</td>
<td>15</td>
<td>7.3</td>
</tr>
<tr>
<td>Absence of any danger signs or bad obstetric history</td>
<td>95</td>
<td>46.4</td>
</tr>
<tr>
<td>Total</td>
<td>205</td>
<td>100</td>
</tr>
<tr>
<td>Maternal conditions on admission</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother walking unsupported</td>
<td>155</td>
<td>75.6</td>
</tr>
<tr>
<td>Mother not walking</td>
<td>50</td>
<td>24.4</td>
</tr>
<tr>
<td>Normal vital signs</td>
<td>105</td>
<td>51.2</td>
</tr>
<tr>
<td>Abnormal vital signs</td>
<td>100</td>
<td>48.8</td>
</tr>
<tr>
<td>Shock</td>
<td>20</td>
<td>9.8</td>
</tr>
<tr>
<td>Severe anemia</td>
<td>15</td>
<td>7.3</td>
</tr>
<tr>
<td>Fetal conditions on admission</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delivered before arrival to SHM</td>
<td>5</td>
<td>2.4</td>
</tr>
<tr>
<td>Normal heart rate</td>
<td>94</td>
<td>45.6</td>
</tr>
<tr>
<td>(including one set of twin)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal fetal heart rate</td>
<td>65</td>
<td>31.6</td>
</tr>
<tr>
<td>Fetal heart sound absent</td>
<td>42</td>
<td>20.4</td>
</tr>
<tr>
<td>Total</td>
<td>206</td>
<td>100</td>
</tr>
<tr>
<td>Duration of obstetric delays at the TBA homes before referral to SHM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤12 h</td>
<td>50</td>
<td>24.4</td>
</tr>
<tr>
<td>12-24 h</td>
<td>80</td>
<td>39.0</td>
</tr>
<tr>
<td>&gt;24 h</td>
<td>75</td>
<td>36.6</td>
</tr>
<tr>
<td>Total</td>
<td>205</td>
<td>100</td>
</tr>
</tbody>
</table>

SHM: Semino Hospital and Maternity, TBAs: Traditional birth attendants

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**Table 3: Indications for referrals of childbirth emergencies to SHM by the trained TBAs**

<table>
<thead>
<tr>
<th>Indications</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prolonged labor</td>
<td>100</td>
<td>48.8</td>
</tr>
<tr>
<td>Obstructed labor</td>
<td>40</td>
<td>19.5</td>
</tr>
<tr>
<td>Attempted vaginal birth after cesarean delivery</td>
<td>40</td>
<td>19.5</td>
</tr>
<tr>
<td>Retained placenta</td>
<td>5</td>
<td>4.9</td>
</tr>
<tr>
<td>Chorioamnionitis</td>
<td>25</td>
<td>12.2</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>10</td>
<td>4.9</td>
</tr>
<tr>
<td>Big baby</td>
<td>15</td>
<td>7.3</td>
</tr>
<tr>
<td>Abnormal fetal heart rate</td>
<td>10</td>
<td>4.9</td>
</tr>
<tr>
<td>Shoulder dystocia</td>
<td>2</td>
<td>0.98</td>
</tr>
<tr>
<td>Trapped after coming head of the breech</td>
<td>3</td>
<td>1.46</td>
</tr>
<tr>
<td>Multiple pregnancy</td>
<td>1</td>
<td>0.49</td>
</tr>
<tr>
<td>Abnormal lie/mal-position/presentation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breech presentation</td>
<td>15</td>
<td>7.3</td>
</tr>
<tr>
<td>Transverse lie</td>
<td>15</td>
<td>7.3</td>
</tr>
<tr>
<td>Persistent occipito posterior</td>
<td>5</td>
<td>2.4</td>
</tr>
<tr>
<td>Hand prolapse</td>
<td>4</td>
<td>1.9</td>
</tr>
</tbody>
</table>

SHM: Semino Hospital and Maternity, TBAs: Traditional birth attendants

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**Table 4: Association between certain variables and development of postpartum morbidities in the women**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Postpartum morbidity (%)</th>
<th>OR 95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (in years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35</td>
<td>37 (23.3) 122 (76.7)</td>
<td>0.97 0.45-2.09 0.93</td>
<td></td>
</tr>
<tr>
<td>≥35</td>
<td>11 (24.0) 35 (76.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employment status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employed</td>
<td>15 (17.6) 70 (82.4)</td>
<td>0.90 0.44-1.86 0.78</td>
<td></td>
</tr>
<tr>
<td>Unemployed</td>
<td>23 (19.2) 97 (80.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nullipara</td>
<td>29 (25.2) 86 (74.8)</td>
<td>1.26 0.65-2.43 0.49</td>
<td></td>
</tr>
<tr>
<td>Multipara</td>
<td>19 (21.1) 71 (78.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal condition on admission</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>11 (7.1) 148 (92.9)</td>
<td>0.03 0.01-0.66 &lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>37 (74.0) 13 (26.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delay at SHM before intervention</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤1 h</td>
<td>18 (20.2) 71 (79.8)</td>
<td>0.73 0.37-1.41 0.35</td>
<td></td>
</tr>
<tr>
<td>&gt;1 h</td>
<td>30 (25.9) 86 (74.1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SHM: Semino Hospital and Maternity, CI: Confidence interval, OR: Odds ratio

morbidities including wound infections (43/205, 21.0%) and urinary tract infection (5/205, 2.4%). Maternal condition on admission (good or poor) was strongly associated with development of postpartum morbidities (OR: 0.03; 95% CI: 0.01–0.06; P < 0.001). Other factors including maternal age, parity, employment status, and delay at SHM had no significant association with the development of postpartum morbidities (P > 0.05). Details are as shown in Table 4.
to achieve vaginal delivery”.[15] This observation was evident in this study as 17.2% (20/116) of the facility delays were due to patients’ refusal to give consent for cesarean delivery. Similar to previous reports from Nigeria,[16,17] many women in labor referred by TBAs had multiple antenatal booking in formal health facility but elected to deliver outside the modern facility.

Prolonged delays of more than 24 h in TBA homes occurred in 36.6% of the patients in this study with 48.8% of the patients presenting in poor clinical conditions. This observation though not very impressive is a lot better than the reports from Ebony state, Southeast Nigeria[5] where 74.1% of the referrals presented in poor clinical conditions. This could be attributed to earlier referrals by the trained TBAs in the present study compared to the above previous study where the TBAs were not trained on the importance of prompt referral. The feasibility of improving timely referral of emergency obstetric cases via training of TBAs has been documented by previous African authors.[18] Mal-presentations, previous cesarean delivery, and scarred uterus are complicated and high-risk labors that should be managed in comprehensive emergency obstetric facilities and not at TBA centers.[19] Any labor that lasts for more than 12 h in the active phase in a TBA center should be referred without further delay.

Financial constraints and refusal to give consent (which could be due to lack of funds) accounted for 29.3% and 12.2% of facility delays, respectively, in our study. Okafor et al. in 2001[20] documented a tremendous uptake of facility-based delivery under SBAs and marked reduction in mortality and morbidity when maternity services were made free in Enugu state. Childbirth services should, therefore, be provided free of charge in government hospitals in developing countries in order to enable women have access to SBAs. The maternal mortality ratio of 610/100,000 live births in this study is remarkably lower than 3385/100,000 live births reported in a similar study from Ebony state in 2010.[5] This could be attributed to the improvement in early referrals by the trained TBAs, and a better enabling environment such as availability of comprehensive obstetric emergency services in the study population, good access roads, and telecommunication services.

Lack of universal access to SBAs and emergency obstetric care services as seen in this study is particularly challenging to most developing countries because of poor infrastructure, acute shortage of SBAs, nonavailability of blood, drugs, and equipment.[21,22] It has been estimated that an additional 180,000 midwives are needed in the next 10 years in Africa to overcome the current shortage.[21] This requires substantial funding, staff motivation, and further professional development. The availability of high quality maternal and newborn services that are free has been documented to increase tremendously the utilization of SBA services.[20] The major challenge in Nigeria is the provision and decentralization of such high-quality services to the communities.

The limitation of this study includes the utilization of the referrals by the TBAs to only one center (SHM) thereby limiting the generalization of the findings to the entire population. However, the study has commenced the process of filling the gap in the performance of the trained TBAs in our environment. This study being a retrospective one is also limited to the extent to which records about the patients were previously collected and kept.

**Conclusion**

Delays at trained TBA centers are common before referral and many patients are referred in poor clinical state. There was a failure of the TBAs to recognize and promptly refer cases with obvious “obstetric danger signs” and “bad obstetric histories.” Further training and re-training of the TBAs with more emphasis on recognition of obstetric danger signs and bad obstetric histories may help in screening high-risk patients for prompt referral before complications develop.

**Acknowledgments**

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**References**


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Ulcerative Colitis Prone to Delayed Diagnosis in a Nigerian Population: Case Series

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Abstract

Inflammatory bowel disease is an emerging disease burden in the developing world. In Nigeria there is a persisting perception among physicians that it is a very rare disease, and publications on it are sparse. Early manifestations of ulcerative colitis (UC) are therefore likely to be missed at many health institutions. This publication aims to contribute to the growing literature on UC among Nigerians. We present 3 cases of UC that were diagnosed at very late stages. It took a range of 2–7 years for the diagnosis to be made from onset of symptoms. UC was confirmed in the first patient after bowel resection for massive gastrointestinal haemorrhage. The other two had colonoscopy and biopsy for confirmation. An increased awareness about UC is necessary in Nigerian population, because the condition may be commoner than hitherto thought. Provision of colonoscopy services to a wider population will assist in early discovery of this disease.

Keywords: Delayed diagnosis, Nigeria, Ulcerative colitis

Introduction

Inflammatory bowel disease (IBD) has emerged as a global disease. Temporal trends indicate an increasing incidence and prevalence across various regions of the world.¹ However this increase has largely been attributed to environmental influences consequent on urbanization and industrialization, better diagnostic tools and increased awareness by physicians have been contributory. The disease burden may be plateauing in Western nations, but this is not the case in previously low incidence regions like Eastern Europe, Asia and other developing nations.² In Nigeria and indeed Africa, there is still the perception among physicians that ulcerative colitis (UC) is a rare disease. However, the increasing reports from Nigeria of this subtype of IBD does suggest that the few case reports and case series in literature may just be the tip of the iceberg.³⁻⁵ This condition is most likely an under diagnosed problem in our environment. We report a case series of UC seen over an 18 months period at two South-eastern Nigerian centres with active gastrointestinal endoscopy services: Federal Medical Centre Owerri and Carez Clinic Owerri. This will serve as a contribution to the wider body of knowledge about UC in Nigeria.

Case Reports

Case 1

A 64 Nigerian male was referred to our general surgery outpatient clinic on account of 6 months history of progressive weight loss, frequent stooling 5–7 times per day and haematochezia. He had similar bowel complaints 2 years prior to presentation which subsided on unspecified medications. He has had no previous surgical operation. He looked cachetic and anaemic on examination with tenderness on the right upper quadrant of the abdomen. Abdominal computed tomography (CT) done showed thickened bowel which was initially suggested to be a neoplasm of the colon. However, further evaluation of the CT images showed circumferential and diffuse thickening of the rectum as well as the colon; features which are indicative of an inflammatory condition [Figure 1]. On account of the ongoing blood loss he had an emergency laparotomy. There was no tumour found intraoperatively, rather gross inflammation of the entire colon, more pronounced on the descending and sigmoid and transverse colon was noticed. Subtotal colectomy was done. Photomicrograph of the resected bowel segment is as shown [Figure 2]. Postoperatively patient had anastomotic

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leakage that was managed conservatively. Subsequently, the patient has been maintained on intermittent sulphalazine for 14 months. During an episode of relapse he was placed on prednisolone but has to be stopped on account of diabetes mellitus. Infliximab was prescribed for him but it has been difficult to access it.

**Case 2**

A 30-year-old lady presented with a 7 years history abdominal cramps, 4–9 bowel motions per day that warranted the use of diapers. At the disease onset she was managed in a teaching hospital in neighbouring state where she was admitted for 35 days. During this period she developed widespread suppurative skin lesions. No definitive diagnosis of her condition was made. Subsequently she has been in and out of hospitals on account of febrile illness and abdominal complaints that usually required intravenous fluid and antibiotic resuscitation. A suspicion of UC was made and barium enema requested. It showed loss of hastral markings over the entire length of the colon with evidence of pseudopolyps [Figure 3]. This was confirmed at colonoscopy. She has been on sulphalazine with marked relief of her symptoms.

**Case 3**

A 41-year-old Nigerian woman presented with a 32 months history of passage of loose stools, 7–8 motions per day, mixed with fresh blood. She occasionally passes blood clots per rectum and has been losing weight. She has received medications at various clinics for diarrhoeal disease without relief. She had erythema nodosum on the dorsum of the left foot. A diagnosis of IBD was suspected and she underwent colonoscopy. Findings included friable mucosal inflammation of the entire rectum and colon as well as a pedunculated 1.5 cm diameter polyp at the descending colon. Histologic sections of the intestinal mucosa tissue fragments showed heavy infiltration of the lamina propria by neutrophils and occasional lymphocytes and plasma cells. Features of crypt abscesses, cryptitis, epithelial damage (mucin depletion) and crypt distortion were also seen [Figure 4]. She was placed on sulphalazine, which she takes irregularly on account of difficulty in sourcing it. However, stool frequency has decreased to 2–3/day, although it is still bloody.

**Discussion**

Ulcerative colitis is reportedly very rare in black Africans compared to Western populations. The appreciable data from the disease is mainly from South Africa. This could be attributed to the better health system infrastructure in that country juxtaposed with other sub-Saharan African countries. There are no large patient series from Nigeria despite its population of about 160 million. This becomes more worrisome when neighbouring smaller countries seem to have comparatively larger reported cases. A report from Senegal revealed 32 cases of UC over a 7 years period while from Burkina Faso, Bougouma published 20 cases seen between 1st January 1995 and 21st May 2006. Apart from the compounding problems of absence of population based health surveys and adequate medical recording system, the perception that UC is a disease of the Caucasian population points to condition that is potentially under-diagnosed.
Diagnostic delay is a topical issue in the management of IBD in several countries. The Swiss IBD cohort study group reported a median diagnostic delay of 4 months from the onset of symptoms to a diagnosis of UC which is significantly shorter than that for Crohn’s.\(^{[9]}\) The alarming nature of bloody diarrhea may be a contributory factor for the earlier diagnosis of UC. However, the delay in the diagnosis of our cases is unduly too long. This may be the reason why all our patients presented with severe disease and associated pancolitis. Patients are likely to self-medicate when they have mild bloody diarrhea as was the case in our first patient. Physicians are also more likely to attribute bloody diarrhea to amoebic colitis, other bacterial infective causes prevalent in the tropics, human immunodeficiency virus and less common to colonic neoplasia.\(^{[10]}\) Thus it is the severe case that gets to be investigated more comprehensively. Even in this regard options are limited because colonoscopy facilities are still rudimentary in many parts of Nigeria. In our centre, it is barely 1-year. The poor radiologic service in our practice is also being highlighted by this study. The initial CT scan report for Case 1 suggested colonic malignancy as cause of the haemorrhage. It was only in hindsight that another radiologist was able to outline the diffuse thickening of the bowel that is in keeping with an inflammatory process.\(^{[11]}\)

As an aid in diagnosis, it needs to be reinforced among our physicians that the skin is the most common extraintestinal organ to be affected in IBD.\(^{[12,13]}\) Two of our patients had skin manifestation which heightened our suspicion of UC. A drawback in this regard is that there is a huge dearth of dermatologists in our country.

Treatment of patients with UC has profound challenges in our society. It may be difficult sourcing the basic drug, sulphasalazine. The national drug agency does not seem to have any company that is licensed to import it. Patients occasionally get supplies from neighbouring countries. In order to access infliximab a special application had to be made to the same agency. Ultimately this makes costs prohibitive for patients. We believe that establishing a national database on UC is pertinent at the moment; as the disease condition becomes increasingly diagnosed and reported in our environment. This will make some sense from the various cases reported from some various health institutions. It will also provide the opportunity for timely and indigenous research on a condition that will likely compound the various health challenges in a sub-Saharan African environment.

References


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Candidiasis, A Rare Cause of Gastric Perforation: A Case Report and Review of Literature

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Abstract

Fungi are unusually rare causes of gastric perforation, with most cases of gastric perforation occurring as complications of peptic ulcer disease (PUD), nonsteroidal anti-inflammatory drugs (NSAIDs) and gastric neoplasms. Here, we report the case of a 70-year-old Nigerian male who presented with severe epigastric pain, with no associated history of PUD, NSAIDs use or gastric neoplasm. An emergency exploratory laparotomy was performed and a gastric perforation was discovered and repaired. Histopathological examination of the gastric perforation edge biopsy revealed an intense Candida growth consisting of numerous fungal spores and hyphae invading and destroying the gastric wall. He was subsequently treated with fluconazole antifungal and discharged home after an uneventful postoperative period.

Keywords: Candidiasis, Fluconazole, Gastric perforation, Peritonitis, Surgical pathology

Introduction

Most cases of gastric perforation occur as complications of peptic ulcer disease (PUD), nonsteroidal anti-inflammatory drugs and gastric neoplasms.¹ Though Candida species are regarded as normal commensals of the gastrointestinal tract, infections of the gastrointestinal tract do very rarely occur and have been reported as very rare causes of gastric perforation, seen mostly in immunocompromised and debilitated patients as well as in healthy persons who indulge in habitual use of strong antacids.¹² A prudent search of literature show that this condition has not been previously reported in Nigeria. Here we report the first Nigerian case of gastric perforation from invasive gastric candidiasis in a 70-year-old Nigerian male and review the relevant literature.

Case Report

A 70-year-old Nigerian man presented at the emergency unit of our hospital with a 2 weeks history of abdominal pain. The pain was epigastric; severe, deep-seated, progressive, non-radiating, not relieved by food intake and not associated with vomiting or diarrhea. His condition worsened 5 days to presentation with generalized abdominal pain and abdominal wall rigidity. There was no change in bowel habit, no blood in stool, no abdominal distension, no associated fever, weight loss, alcohol binge or trauma. No history of previous medication, PUD or abdominal surgery, not a known diabetic, hypertensive, epileptic, asthmatic or sickler but has a history of moderate alcohol and cigarette use of more than 20 years. On clinical examination was an elderly man, conscious and alert but in obvious painful distress. He was afebrile, not pale, anicteric, not cyanosed, no pedal edema but was moderately dehydrated. The respiratory rate was 60 breaths per minute, pulse rate – 120 beats per minute and blood pressure - 100/70 mmHg. The abdomen was flat, tense, and with minimal movement on respiration, marked generalized abdominal tenderness, rebound tenderness, and rigidity. The examination of the liver, spleen, kidneys, and rectum were unremarkable but the prostate was moderately enlarged. An impression of
peritonitis 2° to perforated PUD was made and he was placed on omeprazole, tramadol, paracetamol, flagyl, ceftriaxone, hydrocortisone, and fluid hydration. The preoperative laboratory investigations were hemoglobin concentration - 13 g/dl, serum electrolyte concentration - sodium (Na⁺) - 138 mmol/L, potassium (K⁺) - 4.1 mmol/L, bicarbonate (HCO₃⁻) - 20 mmol/L, chloride (Cl⁻) - 108 mmol/L, and negative HIV 1 and 2 antibodies. Abdominal ultrasonography showed intraperitoneal echo-rich collection with dilated small bowel loops. The patient measured 1.67 m tall, weighed 60 kg, and had a body mass index of 21.5 kg/m². An emergency exploratory laparotomy was performed and a 3.1 cm by 1 cm gastric perforation covered with fibrinous exudate was seen with vegetative material within the peritoneum. An edge biopsy of the ulcerated perforation was taken and an omental patch repair subsequently treated with fluconazole, clindamycin, ciprofloxacin, levofloxacin, imipenem, tamsulosin, and discharged home after an uneventful postoperative period.

**Discussion**

_Candida_ species are a major constituents of the normal commensal flora of the gastrointestinal tract in humans. They cause infections mostly in immunocompromised and severely ill patients, patients on steroid therapy and chemotherapy, diabetics, and HIV/AIDS patients,

![Figure 1: Photomicrograph showing numerous Candida spores (S) and budding hyphae (H) in the gastric perforation edge (H and E, ×400)](Image)

and budding hyphae

![Figure 2: Photomicrograph showing numerous Candida spores (S) and budding hyphae (H) invading and destroying adjacent gastric wall (PAS, ×400)](Image)}
Candida demonstrated on microscopy [Figures 1 and 2]. The unavailability of immunohistochemistry, in situ hybridization and direct sequencing (polymerase chain reaction) for fungal studies in our center and our inability to do microbiological culture because the perforation edge biopsy was formalin fixed limited further Candida classification into species. The absence of previously reported associations in our patient could point to the presence of other predisposing factors or the presence of a more virulent Candida strain in the etiopathogenesis of gastric perforation from gastrointestinal candidiasis.

**Conclusion**

This report underscores fungal etiology as an unusually rare but important cause of the gastric perforation even in apparently healthy patients. Early diagnosis and treatment would reduce the significant mortality associated with this condition which is hitherto unreported in our environment.

**References**

Multiple Unerupted Permanent Teeth Associated with Noonan Syndrome

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Abstract

The present report describes a case of Noonan’s syndrome from a dental viewpoint. Noonan syndrome is an autosomal dominant multisystem disorder. Congenital heart deformities, short stature, thoracic deformities, short neck with webbing, hypertelorism, and malocclusions are some of the frequently observed clinical features. Atypical dental anomalies such as multiple unerupted permanent teeth, multiple submerged and retained deciduous teeth, and supernumerary teeth were found in the present case. Oral prophylaxis and preventive resin restorations were done following which the supernumerary teeth were extracted. 54, 55, 64, 65, 74, 75 and 84 were extracted after orthodontic consultation to facilitate the eruption of unerupted permanent teeth. The patient is undergoing fixed orthodontic therapy for forced eruption of unerupted permanent teeth. General dentists should correlate dental anomalies with other systemic features in the diagnosis of such syndromes because of the variability in presentation and the need for multidisciplinary care.

Keywords: Hypertelorism, Noonan syndrome, Submerged teeth, Supernumerary teeth, Unerupted teeth

Introduction

Noonan syndrome (NS) is a clinically and genetically heterogeneous condition characterized by distinctive facial features, short stature, chest deformity, congenital heart disease, and other co-morbidities. The distinctive facial features consist of a broad forehead, hypertelorism, down-slanting palpebral fissures, a high-arched palate, and low-set, posteriorly rotated ears. Cardiac involvement is present in up to 90% of patients. Pulmonic stenosis and hypertrophic cardiomyopathy are the most common forms of cardiac disease, but a variety of other lesions are also observed. Additional relatively frequent features include multiple skeletal defects (chest and spine deformities), webbed neck, mental retardation, cryptorchidism and bleeding diathesis. Oral findings in patients with NS include a high arched palate (55–100%), dental malocclusion (50–67%), articulation difficulties (72%), and micrognathia (33–43%).

Some individuals with NS develop mandibular cysts, which can mimic cherubism

This condition affects both males and females, and most cases are sporadic, but occasionally autosomal dominant inheritance occurs. The incidence of NS is estimated to be between 1:1000 and 1:2500 live births. Other disorders with significant phenotypic overlap with NS include William’s syndrome, Leopard syndrome, fetal alcohol syndrome, and Aarskog syndrome. The present case report describes the atypical dental anomalies such as multiple unerupted permanent teeth; multiple submerged and retained deciduous teeth and supernumerary teeth associated with other known clinical features in a child with NS.

Case Report

A 13-year-old boy reported to the Department of Pediatric Dentistry with the chief complaint of irregularly placed upper and lower front teeth and unerupted teeth. Though his medical history was noncontributory, the child experienced an epileptic attack during childhood. Dental history reveals that he visited a private dental practitioner 1-year back with the same problem, and his lower front teeth were extracted. Physical examination revealed hypertelorism, flat nasal bridge, saddle nose, mild webbing of neck, pectus excavatum
(funnel-shaped chest) [Figure 1], arachnodactyly (long and narrow toes and fingers) and clubbing of nails [Figure 2]. No marked cardiac abnormality was observed, and the patient had a normal electrocardiogram. Hand wrist radiograph revealed a missing carpal bone with no fusion of the present carpal bones [Figure 2]. Hematological findings showed an increase in alkaline phosphatase levels (179 U/L).

Intraoral examination revealed multiple submerged and retained deciduous teeth (53, 54, 55, 63, 64, 65, 73, 74, 75, 84, 85) [Figure 3], deep bite, high arched palate, gingival overgrowth with supernumerary teeth in the lower anterior region with grade I mobility of lower permanent incisors. Many permanent teeth were unerupted (12, 14, 15, 22, 24, 25, 33, 34, 35, 43, 44, 45, 46) [Figure 3] and all the erupted teeth were caries free.

Orthopantomograph revealed three supernumerary teeth in relation to lower anteriors, congenitally missing (12, 22, 38, and 48) and multiple impacted permanent teeth.

Mandibular right first permanent molar (46) was impacted and exhibited divergent roots [Figure 4]. Cephalometric analysis confirmed skeletal class I malocclusion with orthognathic maxilla and mandible associated with horizontal growth pattern and retroclined upper and lower anteriors [Figure 5].

The differential diagnosis includes William’s syndrome, Leopard syndrome, fetal alcohol syndrome, and Aarskog syndrome.[4,5] The absence of cardiovascular abnormalities in our patient has ruled out the possibility of William’s syndrome.[6] The absence of maternal alcohol consumption during pregnancy and lack of central nervous system abnormalities that are the cardinal features of fetal alcohol syndrome excludes it from the diagnosis.[7] The present case could not be attributed to Aarskog syndrome due to lack of digital and genital findings.[1] The presence of only one cardinal feature of Leopard syndrome that is, ocular hypertelorism in our patient excludes it from our diagnosis.[1] The dental anomalies in the present case are closely associated to that of cleidocranial dysplasia, but the lack of skeletal anomalies affecting the skull and clavicle eliminates it from our diagnosis. Based on the clinical and radiographic features observed a
provisional diagnosis of Noonan’s syndrome was established after ruling out other differential diagnosis.

Comprehensive treatment was planned which included oral prophylaxis, fissure sealants, and preventive resin restorations. All the supernumerary teeth were extracted. The submerged primary teeth 54, 55, 64, 65, 73, 74, 75 and 84 were extracted to facilitate the eruption of permanent successors. After the extractions, only 35 has erupted into occlusion [Figure 6]. The patient has been referred for orthodontic consultation to address the delayed eruption of permanent teeth. Presently, the patient is undergoing fixed orthodontic therapy for forced eruption of unerupted permanent teeth.

**Discussion**

Noonan syndrome (MIM number: 163950) was described by Noonan and Ehmke in 1963 as a multisystem disorder, characterized by broad forehead, hypertelorism, down-slanting palpebral fissures, a high-arched palate, and low-set, posteriorly rotated ears. Noonan syndrome is an autosomal dominant disorder characterized by short stature, facial dysmorphism, and a wide spectrum of congenital heart defects.[2] The general body appearance is that of Turner syndrome, and hence NS is also known as “Pseudo-Turner syndrome” in females and “Male Turner syndrome” in males.[3] In NS, no consistent chromosomal abnormality has been found.[8] Recently, PTPN11 that encodes the nonreceptor protein tyrosine phosphatase SHP-2 (senescence or modulation region 2-domain phosphatase-2) was identified as the defective gene.[9] Peripheral blood chromosomal analysis of this patient showed a normal male karyotype with no structural or numerical chromosome abnormalities. In view of the fact that NS is genetically heterogeneous disorder,[10] we have established the diagnosis based on salient clinical features observed in this case.

The facial appearance is most characteristic in infancy and early-to-middle childhood and becomes more subtle in adulthood.[3] It is proposed that orofacial features of NS result from edema of face and neck as a result of developmental disturbances of 3rd and 4th pharyngeal arches.[11] Many adults have features that differ little from those in the general population. Other adults are recognizable because of continuing ptosis and wide-spaced eyes, low-set, posteriorly rotated ears with a fleshy helix, inverted triangular face that is broad at the temples and tapers to a small chin, and long and broad or webbed neck.[4] In the present case, the child showed hypertelorism, flat nasal bridge, saddle nose, clubbing of nails, mild webbing of neck, pectus excavatum and arachnodactyly.

Despite the reported frequency of 50–80% cardiovascular alterations among patients with NS no cardiac abnormality was found in this patient, he had a normal electrocardiogram.[12] Bleeding disorders are of variable clinical severity and stem from different defects in the coagulation and platelet systems in NS patients. It has been estimated that roughly one-third of the patients have some sort of bleeding disorder.[13] Hematological findings showed mild increase in clotting time and alkaline phosphatase levels. Derbent et al. (2011) concluded that patients with NS should have a thorough coagulation evaluation, but complications related to coagulation are unlikely, and extensive testing is unnecessary.[2]

Very few studies have investigated changes in hand wrist radiographs in patients with NS. Emral and Akcam reported a case where hand wrist radiography demonstrated a slightly retarded bone age (13 years old) according to the standards of Greulich and Pyle.[10] Hand wrist radiograph of this patient revealed a missing carpal bone with no fusion of carpal bones. Oral features of Noonan’s syndrome include micrognathia, high arched palate, dental malocclusion, dental anomalies, bifid uvula and rarely cleft palate.[3,5] Distinctively this child exhibited multiple submerged, retained deciduous teeth and
multiple unerupted permanent teeth compared to previously reported cases in the literature.

Though many case reports did not confirm the presence of supernumerary teeth, Ortega et al. and Toureno and Park reported the presence of supernumerary teeth.[13,14] In this case, the child had three supernumerary teeth. An interesting finding in this case was the presence of divergent roots in the unerupted mandibular right first permanent molar (46).

Full spectrum of dental anomalies in both deciduous and permanent dentition makes this case distinctive. The dental treatment was focused on extracting the submerged and retained deciduous teeth and supernumerary teeth and to aid the eruption of the unerupted permanent dentition. The need for early diagnosis should be emphasized in these cases because of the high incidence of cardiac, ophthalmic, growth, orthopedic and dental abnormalities. Growth hormone therapy has been tried in few children with NS with the aim of promoting growth.[14] The pediatric dentist serves as the coordinator of overall oral health care and disease prevention during an extended treatment regimen that includes both surgical and orthodontic interventions.

References


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Cerebral Malaria Complicated by Blindness, Deafness and Extrapyramidal Tract Manifestation

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Abstract
Cerebral malaria is a severe manifestation of a parasitic infection caused by Plasmodium falciparum. The sequelae of this disease such as blindness, deafness, loss of motor function could be emotionally traumatic and physically disabling. We, therefore, present this case of an 8-year-old boy who presented with high-grade intermittent fever associated with multiple convulsions and prolonged coma. He regained consciousness after 12 days of treatment with intravenous quinine but was found to have blindness, sensory-neural deafness and extrapyramidal sign. This extrapyramidal sign regressed following treatment with chlorpromazine. He also regained his sight and auditory function before he was discharged though not completely. This report is aimed at emphasizing these rare complications of cerebral malaria as well as reminding clinicians working in malaria endemic areas of the world on the need for early diagnosis and prompt treatment.

Keywords: Blindness, Cerebral malaria, Coma

Introduction
Malaria is a parasitic disease affecting about 1 billion people globally and causing about 1.24 million deaths annually especially in the developing countries.[1,2] About 1% of the patients with Plasmodium falciparum develop severe manifestations culminating in multi-organ failure.[1] Cerebral malaria is one of these severe manifestations with its attendant sequelae such as cerebral palsy, cortical blindness, sensory-neural deafness and rarely extrapyramidal manifestations.[1‑6] Diagnosis of cerebral malaria requires demonstration of asexual form of P. falciparum in peripheral blood smear, in thick and thin blood smear films stained by Giemsa stain.[4] The histopathological hallmark of cerebral malaria is engorgement of cerebral capillaries and venules with parasitized red blood cells (PRBCs) and non-PRBCs.[17] This case report is intended to remind clinicians working in malaria endemic areas of the world that the disease is still deadly.

Clinical Presentations
An 8-year-old child presented in the children emergency room of the Federal Teaching Hospital, Abakaliki, Nigeria, with a 5 days history of high grade intermittent fever and a 3 days history of multiple, generalized convulsions associated with loss of consciousness. Examination of the patient on admission revealed a comatose child with an axillary temperature of 39°C and pallor. The child had hypertonia and hyperreflexia in all the limbs, and the pupils were dilated and reacting sluggishly to light. The liver was enlarged 3 cm below the right costal margin. Lumbar puncture done after a dose of mannitol yielded clear and colorless cerebrospinal fluid that was not under pressure, and its analysis and culture were normal.

Blood film for malaria parasite was positive for asexual form of P. falciparum with a density of 29,920 parasites/mm³. Cranial computerized tomography was not done because of unavailability of the facility. A diagnosis of cerebral malaria was made, and the patient was commenced on intravenous quinine and parenteral paracetamol. He was transfused while on admission in the emergency room. The patient regained consciousness after 12 days of treatment but was found to have blindness and deafness following reviews by ophthalmology and ear, nose and throat teams respectively. Extrapyramidal tract manifestations evidenced by purposeless involuntary movements of the limbs and biting of the fingers.
were documented by the pediatric neurology team. Based on the above neurological features, the patient received prednisolone for the blindness. He was subsequently placed on chlorpromazine when diazepam could not stop the involuntary movements, and the symptoms subsided markedly after 3 days. He was discharged from the hospital after 30 days of admission following stable clinical condition though blindness and deafness have not resolved completely.

Discussion

Several hypotheses have been documented to explain the pathophysiology of cerebral malaria. These hypotheses include cytoadherence of parasitized red cells,[7] up-regulation of vascular endothelial ligands.[8-10] These hypotheses are said to be responsible for clinical features of malaria such as coma, convulsions, and neurological deficits. Sensory-neural deafness in this patient may be argued to result from quinine effect. However, it is not common to recover from deafness due to quinine. This patient recovered significantly before discharge implying that the deafness could be a complication of cerebral malaria that the child suffered. This was also confirmed by the ophthalmologist when fundus examination was done.

References
