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ARTICLES

Case Report

- Oral cancer: The Nigerian experience** 210
Claudia Cordoba-Carrillo, José Nieves-Nieves, Viridiana Salinas-González,
María Christina Betancourt-Quiles, Rosángela Fernández-Medero, Ricardo
Fernández-González, José Lozada-Costas, Melissa Sepúlveda-Ramos,
Román Vélez-Rosario

Research Articles

- Occupational xylene exposure and respiratory impairment of paint
manufacturing workers** 214
Zailina, H, Mohd Hafiz Syazwan, T, Naing, L, Jamal, H. H and Rusli, N.
- Sonographic quantification of basal gastric antral area, gastric motility
and gastric emptying time of a liquid meal in healthy subjects: A pilot study** 221
Ugwu Anthony Chukwuka, Erondy Okechukwu Felix and Shu Elvis Nepa
- Effectiveness of dry *Moringa oleifera* leaf powder in treatment of anaemia** 226
Madukwe E. U, Ugwuoke A. L. and Ezeugwu J. O
- Evaluation of the incidences of dermatophilic infection in Rajasthan: Case
studies from Rajasthan, India** 229
Sarika Gupta and B. Lal Gupta

ARTICLES

Research Articles

- Analyzing long-term mortality among female alcoholics and matched controls: Accounting for age and follow-up time** **233**
Rolf Gjestad, Johan Franck and Brit Haver
- Maternal and neonatal seroprevalence of hepatitis B surface antigen in a hospital based population in South-South, Nigeria** **241**
J. O. Alegbeleye, T. K. Nyengidiki and J. I. Ikimalo

Case Report

A very rare and aggressive lung tumor

Claudia Cordoba-Carrillo^{1*}, José Nieves-Nieves¹, Viridiana Salinas-González¹, María Christina Betancourt-Quiles¹, Rosángela Fernández-Medero¹, Ricardo Fernández-González¹, José Lozada-Costas², Melissa Sepúlveda-Ramos³, Román Vélez-Rosario³

¹Pulmonary Medicine Department, San Juan City Hospital, P. M. B. 463 P. O. Box 70344 San Juan, PR 00936 San Juan, Puerto Rico.

²Hematology and Oncology Department, San Juan City Hospital, San Juan, Puerto Rico.

³Pathology Department, University of Puerto Rico School of Medicine, San Juan, Puerto Rico.

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Rhabdoid phenotype is an uncommon histopathologic malignancy found in <1% of pulmonary large cell carcinomas and is associated with an extremely poor prognosis. To our knowledge, merely forty-six cases have been reported in the literature with reasonable immunohistochemical discoveries allowing differentiation from other rhabdoid-like neoplasms though none acknowledged effective treatment of this exceedingly aggressive tumor. At this moment, there is no specific treatment for large cell cancer with rhabdoid phenotype for this subpopulation and most cases have been treated as non-small cell lung cancer. Herein presented is a case of a 63 year-old male non-smoker diagnosed with this aggressive tumor phenotype which had an aggressive behavior and unfortunate prognosis. The main characteristics of the tumor and the most recent treatment options are discussed.

Key words: Pleural effusion, large cell lung carcinoma, rhabdoid phenotype, immunohistochemical stains, lung cancer.

INTRODUCTION

Large Cell Carcinoma with Rhabdoid Phenotype (LCCRP) is a rare histological variant of lung tumors. Approximately 10% of its cells contain distinctive eosinophilic whorled perinuclear inclusions composed of intermediate filaments, giving the cells a resemblance of a rhabdomyosarcoma (Travis et al., 2004). This variant was first reported in 1995 and recognized by the World Health Organization in 1999 (Tamboli et al., 2004; Brambilla et al., 2001). To our knowledge there have been only 46 reported cases worldwide. Saini et al. (2009) published a literature review of this malignant process and reported 38 cases in the English literature until 2009. Since then, Izquierdo-Garcia et al. (2010) reported 7 new cases of infrequent histological types, and Dettmer et al. (2012) described the first case of an exon 19 deletion in epidermal growth factor receptor (EGFR) in a

LCCRP associated with a poorly differentiated pulmonary adenocarcinoma.

Tobacco smoking and male gender have been the most common risk factors identified for the development of this rare condition. We report a case where interestingly, our patient had no significant risks factors for this uncommon variant.

The objective of this article is to provide relative medical literature concerning therapeutic management options available today for this aggressive tumor.

CASE

A 63 year-old man that worked as a gardener and who had a history of arterial hypertension and bronchial asthma

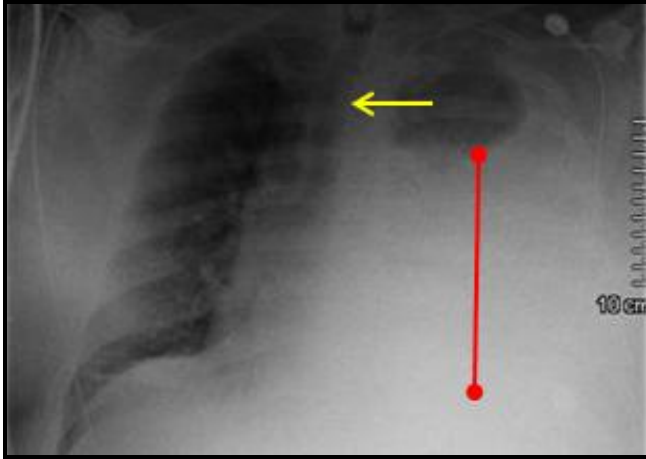


Figure 1. Chest XRAY demonstrating opacity of the left lung (red bar) and deviation of the trachea to the right (yellow arrow).



Figure 2. Chest computed tomography representing left lung tumor (red arrow).

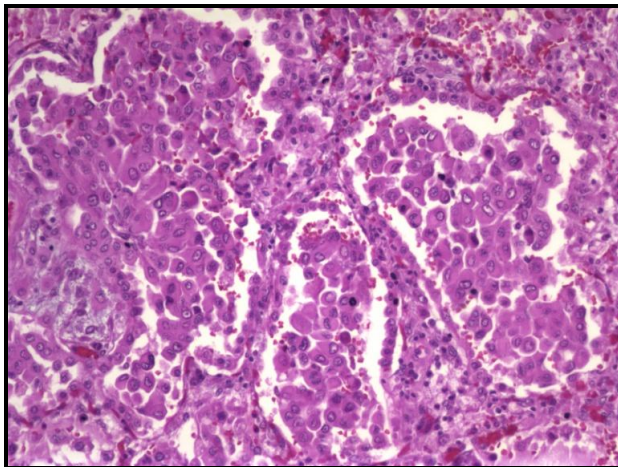


Figure 3. Lung involved by rhabdoid carcinoma showing large cells with eosinophilic cytoplasmic inclusions. (H&E, 200x).

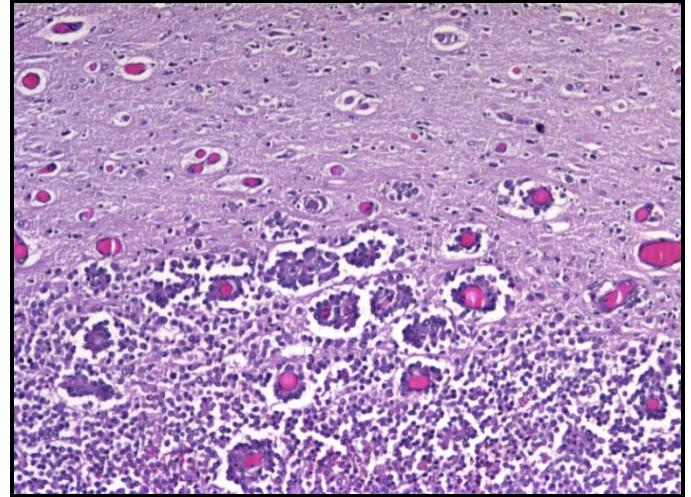


Figure 4. Rhabdoid carcinoma of lung metastatic to brain; tumor in lower half and uninvolved brain in upper half. (H&E, 100x).

asthma was evaluated after presenting a non productive cough and shortness of breath associated with a left sided pleuritic chest pain of one week evolution. He had neither history of tobacco smoking nor any other identifiable environmental exposures. Pertinent clinical findings included tachypnea, decreased breath sounds, dullness to percussion and tactile fremitus on his left hemithorax. The laboratories were unremarkable except for respiratory alkalosis and hypoxemia on the arterial blood gases. Chest images demonstrated a complete opacification of his left lung due to a large pleural effusion (Figures 1 and 2). A diagnostic as well as therapeutic thoracentesis was performed showing an exudative fluid with impression remarkable for atypical reactive mesothelial cells with inflammatory infiltrates. Partial symptomatic relief was obtained until the reaccumulation of the pleural effusion leading to a chest tube placement for fluid drainage. However, the patient's condition deteriorated rapidly, consequently requiring mechanical ventilator support and eventually died from complications of septic shock and multi-organ failure. An autopsy gave the diagnosis of LCCRP features, along with alveolar hemorrhage and metastatic lesions to the heart, liver, pancreas, adrenals, kidneys, thyroid, lymph nodes, and brain (Figures 3 and 4). The lung tissue immunohistochemical staining was positive for cytokeratin, vimentin and pankeratin lastly providing the diagnosis of metastatic rhabdoid carcinoma of the lung (Figures 5 and 6). Due to the nature of the advanced disease and diagnosis made post mortem, no treatment protocols of chemotherapy or radiotherapy were initiated.

DISCUSSION

As in our case, LCCRP has been found to be very

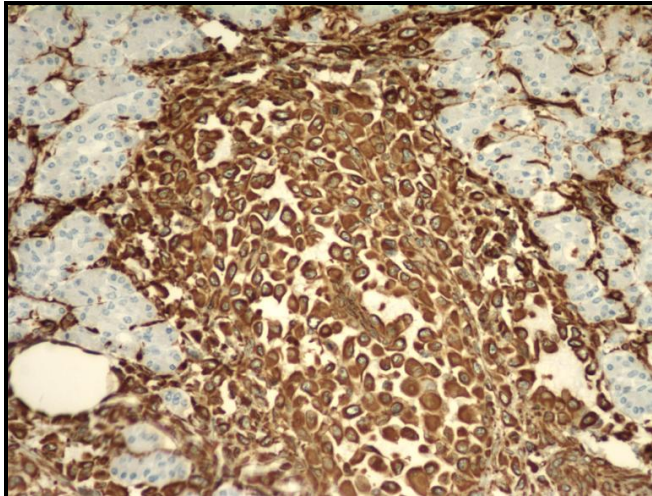


Figure 5. Vimentin positive (brown) rhabdoid carcinoma metastatic to pancreas (light blue) (200x).

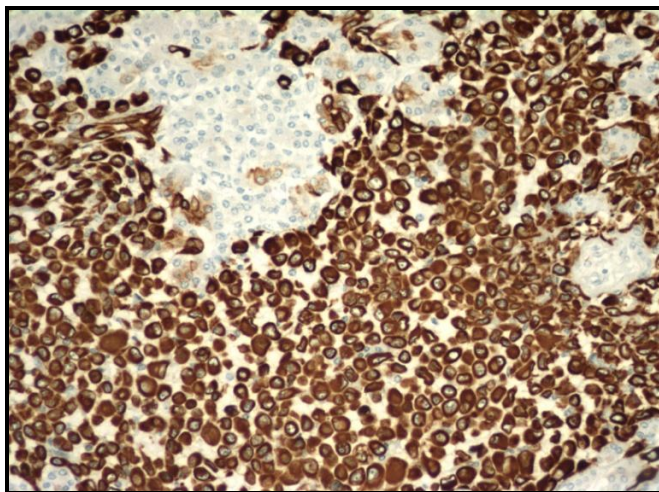


Figure 6. Cytokeratin positive (brown) rhabdoid carcinoma metastatic to pancreas (light blue) (200x).

aggressive. Although the cases reported in the literature have been related to heavy cigarette smoking our patient had no recognizable risk factors for this rare tumor. This entity generally when diagnosed is at an advanced stage, with an expected mortality within months of diagnosis. There have been reports suggesting that the presence of rhabdoid cells is a marker of poorer prognosis. Specific immunohistochemical stains are required for the diagnosis, such as vimentin, which is ubiquitously expressed and is often intermingled with cytokeratins. Other markers including neuron-specific enolase, epithelial membrane antigen, chromogranin, and synaptophysin, are frequently mentioned to some degree in a significant proportion of rhabdoid cells (Saini et al., 2009; Shimazaki

et al., 2001). The expression of thyroid transcription factor-1 (TTF-1), a commonly used marker for primary lung cancers, appears to be less common in this variant (Tamboli et al., 2004).

No clinical trials have been conducted specifically for treatment for LCCRP since most cases have been treated as non small cell lung cancer (NSCLC) following existent guidelines. As in NSCLC, patients with LCCRP treated with surgery at early stages of diagnosis have better survival outcomes. In selected cases, surgical resection of metastatic disease can also have a positive effect on survival (Saini et al., 2009; National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology, 2012; Kaneko et al., 2002; Otera et al., 2010).

The management of advanced disease NSCLC is based on histologic, molecular characteristic and patient performance. The initial therapy should be the one with the highest benefit and less toxicity. Most protocols are platinum-based but when adequate response is not achieved or the diagnosis is made at advanced stages, combination therapy has been mentioned as well.

Bevacizumab, a humanized monoclonal antibody that inhibits vascular endothelial growth factor (VEGF) inhibiting the process of angiogenesis and/or chemotherapy alone has been given to patients who have good performance status and advanced or recurrent NSCLC. Bevacizumab has been reserved for disease progression.

Another agent, Erlotinib, which specifically targets the epidermal growth factor receptor (EGFR) tyrosine kinase, which is highly expressed and occasionally mutated in various forms of cancer, is indicated as first-line therapy in patients with EGFR mutation. Other tyrosine kinase inhibitors include Crizotinib - the preferred therapy in anaplastic lymphoma kinase (ALK) positive patients.

If the disease progresses, the single-agent docetaxel with its well-established anti-mitotic chemotherapy properties, which interferes with cell division, is well recognized as a second-line agent. Erlotinib is also used as a second-line agent. Erlotinib is furthermore indicated as a third line agent; in reported cases it has been superior to supportive care (National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology, 2012).

Conclusion

Although the available information has been limited due to few reported cases, chest physicians should be aware of this very aggressive and uncommon malignancy. LCCRP may be considered in male patients without significant risk factors and an unusual hemithorax opacification. This rare tumor usually has catastrophic outcome however early intervention for tissue diagnosis is essential since therapies such as pneumonectomy and adjuvant chemotherapy may improve survival.

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ABBREVIATIONS

LCCRP, Large cell carcinoma with rhabdoid phenotype; **NSCLC**, non-small cell lung cancer; **TTF-1**, thyroid transcription factor-1; **VEGF**, vascular endothelial growth factor; **EGFR**, epidermal growth factor receptor, **ALK**, anaplastic lymphoma kinase.

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Occupational xylene exposure and respiratory impairment of paint manufacturing workers

Zailina, H.^{1*}, Mohd Hafiz Syazwan, T.¹, Naing, L.², Jamal, H. H.³ and Rusli, N.⁴

¹Department of Environmental and Community Health, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

²Institute of Medicine, Universiti Brunei Darussalam, Jalan Tungku Link, Gadong BE 1410, Brunei Darussalam.

³International Institute for Global Health, United Nations University, UKM Medical Center, Jalan Yaacob Latif, Bandar Tun Razak, 56000, Kuala Lumpur, Malaysia.

⁴Tan Sri Jeffrey Cheah School of Medicine, MONASH University, JKR 1235, Bukit Azah, 80100 Johor Bahru, Johor, Malaysia.

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A cross-sectional study was conducted among paint workers to determine the association between xylene exposures with respiratory health. Sixty-four exposed workers working with xylene and 47 unexposed administrative workers were selected. Air xylene (AX) were analyzed using the Gas Chromatography while urinary methyl hippuric acid (MHA) were analyzed using High Performance Liquid Chromatography. Lung functions were measured using Chestgraph HI-701 spirometer. The AX for the exposed was significantly higher than the unexposed workers ($p < 0.001$). The urinary MHA of the exposed was higher than the unexposed workers ($p < 0.001$). Among the exposed, more respiratory symptoms, higher lung functions abnormality and significantly lower FEV1% predicted and FVC% predicted were found. Findings showed significant correlations between AX and urinary MHA. AX significantly influenced the lung functions. Smoking years and education influenced the respiratory symptoms. Those exposed have early signs of lung impairment and respiratory symptoms. Smokers faced the risk of developing chronic irreversible respiratory diseases.

Key words: Solvent, xylene, methyl hippuric acid, lung functions, paint manufacturing industries.

INTRODUCTION

Solvent also defined as an organic substance that is liquid at room temperature, and under standard atmospheric conditions is able to dissolve a wide range of organic compound. Organic solvents are used for extraction of fats and oils, degreasing, dry cleaning, and the manufacturing of a wide range of products including paints, adhesives, plastics, textiles, electronics and semiconductors. Although solvents have many useful properties that make them ubiquitous in human activities, they have, however, many potential adverse health effects

(Schenker and Jacobs, 1994).

Solvent made up of mixtures of chemicals such as xylene, produced in very large quantities and is extensively employed in a broad spectrum of applications, primarily as a solvent for which its use is increasing as a "safe" replacement for benzene, and in gasoline as part of the BTX (benzene-toluene-xylene) component. There is a broad potential for exposure both to industrial workers in the production and use of the xylenes, and to the general public via vehicle exhausts, consumer products,

consumer products, and others (Fishbein, 1984). Technical (industrial) xylene is a mixture of the isomers plus ethylbenzene (6 - 15%) and occasionally toluene, trimethyl benzene and other trace components. Xylene is widely used as thinner for paints and varnishes, often in combination with other organic compounds and as a solvent in glues and printing inks (International Programme on Chemical Safety – IPCS, 1992).

Xylene exposure has been associated with effects in a number of organ systems including the lungs, skin and eyes, neurological system; heart and gastrointestinal system; kidney; and possibly the reproductive system. High levels of xylene exposure for short periods are associated with irritation of the skin, eyes, nose and throat (Agency for Toxic Substances and Disease Registry – ATSDR, 1992).

The general objective of the study was to determine if there is a significant relationship between xylene exposures with respiratory problems among the paint manufacturing workers.

SUBJECTS AND METHODS

This is a cross-sectional comparative study in a paint manufacturing factory. This factory is made up of two plants namely the Industrial Used Plant and the Trade Used Plant. The Industrial Used Plant produces paints for industrial purposes while the Trade Used Plant produces paints for home use. Both plants had the same work process and use xylene as solvent.

Thousands of chemical compounds are used in paint products as pigments, extenders, binders, solvents and additives. Painters are commonly exposed by inhalation to solvents and other volatile paint components; inhalation of less volatile and nonvolatile components is also common during spray painting. Painters are commonly exposed to solvents, mainly the petroleum solvents, toluene, xylene, ketones, alcohols, esters and glycol ethers. In this factory, the paint is made up of mixtures with thinner containing up to 30 - 40% xylene.

The sampling frame for exposed group was the list of name of workers obtained from the Human Resource Section. Those who work with solvent as the raw material in the manufacturing process were randomly selected. The unexposed group was made up of workers from other sections where they were not exposed. Majority of the workers were males, therefore, only male were selected as respondents for both the exposed and unexposed group in order to match the two groups. Matching was also on the basis of age, ethnicity, educational level and smoking habit. Statistical test were carried out to determine if the differences in these variables were significant.

Questionnaires were used to gather the demographic and socioeconomic background, educational level, work history, current work activities and other relevant information from the respondents. The questionnaires were pretested to ensure quality of the data collected. This pre-test involved 10% of the sample size among other working population that have similar characteristics with the study sample.

Spirometer was used to determine any early stage abnormalities to lung functions. Before the test, each worker was instructed on how the tests would be carried out. Measurements were made by recording the volume of air that a worker can forcibly blow out from the lungs after a full inspiration. A flow-volume curve generated and thus several values were derived from this maneuver, where the best value will be taken after three trials. The percentage of predicted

value for each spirometric value was calculated according to the worker's age, sex, weight and height. The spirometer used was according to the recommended method by American Thoracic Society (1991). The instrument was calibrated by using the method. The personal air sampling pump was calibrated each time before the measurement. The calibrations and measurements were carried by using NIOSH Analytical Method 1501.

For individual air sampling, portable air sampling pump PAS-500 model with solid sorbent tube containing coconut shell charcoal were used. The sampling was carried out during the 8-h shift at 2 h interval with four tubes. The sampling pump with flexible tubing was calibrated at an accurately known flow rate of 0.2 L/min for a total sample size of 0.25 to 12 L.

Urine samples were collected to determine the methyl hippuric acid (MHA) as the metabolite of xylene, measured using High Performance Liquid Chromatography (HPLC), according to NIOSH Manual of Analytical Method - NMAM 8301 (2003). The samples were collected at the end of the weekly work shift on alternate days for 1 work week. The urine was stored in 250-ml polyethylene bottle containing a few crystals of thymol. For storage, urine samples were bagged and refrigerated at a temperature of 4°C in which they were stable for a month.

RESULTS

The socio-demographic status of respondents, such as sex, marital status, educational level and ethnic background are shown in Table 1.

From Table 1, majority of the exposed workers were from Mixing and Canning Processing Sections. Both of these processes use solvents for mixing all of the chemicals into a big container while the canning process ensures all of the finished products were canned properly. Beside these, the workers in the Color Match Section were also exposed to the solvent. For the process workers, their task were to make sure all of the solvents and chemicals were mixed well, the ingredients balanced and to ensure the final product fulfill the company's criteria by comparing the final product's color with the standard. For other paint work processes, workers were also exposed to the xylene because of their work tasks as well as the work environment.

For the unexposed group, most of the workers were forklift drivers and storage workers who did not use xylene or other solvent in their work tasks. However, they were exposed to dust which can be a confounder in this study (Table 1).

There was no significant difference in the height and weight between the exposed and unexposed group. The comparison between the two groups on the number of children, total years of formal education, employment years and the total household income, did not show any significant difference. However, there was a significant difference in age ($p=0.010$), whereby, the unexposed group was made up of older workers. As much as possible, the two groups were matched (Table 2).

The main confounder in this study was their smoking habit. The majority of the exposed workers was smokers and, was matched with the unexposed workers. No significant difference was observed in the number of cigarette

Table 1. The background information of respondents.

Variable	Exposed group {n=64; Frequency (%)}	Unexposed group {n=47; Frequency (%)}
Marital status		
Single	18 (28.1)	8 (17.0)
Married	46 (71.9)	39 (83.0)
Educational level		
Primary	3 (4.7)	4 (8.5)
SRP/PMR	12 (18.8)	7 (14.9)
SPM	49 (76.6)	31 (66.0)
STPM/Diploma	0 (0)	4 (8.5)
Degree	0 (0)	1 (2.1)
Ethnicity		
Malay	57 (89.1)	41 (87.2)
Chinese	3 (4.7)	2 (4.3)
Indian	2 (3.1)	4 (8.5)
Smokers	46 (71.9)	30 (63.8)
Consume carbonate drink	39 (60.9)	19 (40.4)
Drink alcohol	0 (0)	0 (0)
Consume seafood	37 (57.8)	25 (53.2)
Take medication	12 (18.8)	11 (23.4)
Job classification		
Mixing workers	17 (26.6)	
Canning workers	17 (26.6)	
Color matcher	9 (14.1)	
Grinder	3 (4.7)	
Packer	1 (1.6)	
Quality controller	5 (7.8)	
Paint work processors	10 (15.6)	
Supervisor in exposed sections	2 (3.1)	
Managers		3 (6.4)
Forklift driver		15 (31.9)
Lorry driver		1 (2.1)
Security guard		3 (6.4)
Product distributor		8 (17)
Maintenance personnel		1 (2.1)
Picking cans worker		1 (2.1)
Supervisors of unexposed section		1 (2.1)
Storage workers		12 (25.5)
Transportation personnel		2 (4.2)

N=111, PMR=Lower Certificate of Education, SPM= Malaysian Certificate of Education, STPM=Higher School Certificate.

cigarette smoked daily and the years of smoking between the exposed and unexposed group (Table 2). Spirometric test were conducted for a few parameters such as FVC% predicted, FEV₁% predicted and FEV₁/FVC% predicted. The lung functions were classified into normal, mild, moderate, severe and very severe based on a previous study

(NIOSH Manual of Analytical Methods - NMAM, 2003).

Table 3 shows the classification of lung functions of the two groups. From Table 3, the exposed group showed a higher number of respiratory symptoms than the unexposed group. The four main symptoms observed were cough, phlegm, chest tightness and shortness of breath.

Table 2. Comparison of socio-economic variables

Variable	Exposed group (n=64)	Unexposed group (n=47)	Statistical test		
	Mean ^a (SD) /Median (IQR)	Mean ^a (SD) /Median (IQR)	t	z	p
Age (log ₁₀)	1.50 ^a (0.11)	1.56 ^a (0.11)	-2.58		0.010**
No. of children	1.00 (3.0)	2.00 (3.0)		-0.930	0.353
Total years of education	11.00 (0)	11.00 (0)		-0.845	0.398
Total employment years	11.50 (10.8)	13.00 (11.00)		-1.456	0.145
Total income	1800.00 (1400.5)	1870.00 (1100.00)		-0.836	0.403

N=111, ** Significance at $p \leq 0.01$, t = t-test, z = Mann-Whitney U test.

Table 3. Classification of lung functions between the groups.

Abnormality of the lungs	Exposed {n=64; Frequency (%)}	Unexposed {n=47; Frequency (%)}
FVC% predicted		
Normal	55(85.9)	43(91.5)
Mild	4(6.3)	3(6.4)
Moderate	5(7.8)	1(2.1)
Severe	0	0
Very severe	0	0
FEV₁% predicted		
Normal	55(85.9)	46(97.9)
Mild	8(12.5)	1(2.1)
Moderate	1(1.6)	0
Severe	0	0
Very severe	0	0
FEV₁/FVC% predicted		
Normal	64(100)	47(100)
Mild	0	0
Moderate	0	0
Severe	0	0
Very severe	0	0
Respiratory symptom		
Cough	10 (15.6)	7 (14.9)
Cough with phlegm	25 (39.1)	14 (29.8)
Chest tightness	13 (20.3)	11 (23.4)
Shortness of breath	6 (9.4)	3 (6.4)

There was no significant correlation between all parameters for lung functions and respiratory symptoms such as cough, phlegm, chest tightness and shortness of breath with the air xylene concentrations. However, the correlation between urinary MHA and air xylene was significant (Table 4).

The lung functions values for FVC% predicted and FEV₁% predicted of the exposed group were significantly lower than unexposed group, while for the urinary MHA

and personal air xylene, the exposed group was significantly higher than the unexposed group. However, for the FEV₁/FVC% predicted, there was no significant difference between the groups (Table 5).

General Linear Model was used to determine which of the variable significantly influenced the lung functions. All the related variables such as smoking, age, total income, frequency of exposure, air xylene exposure, daily work duration, overtime hours, hours of handling and exposure

Table 4. Correlation between lung functions, air xylene concentrations and respiratory symptoms with urinary MHA per creatinine (g/g) among the exposed group.

Variable	Concentration of urinary MHA (g/g creatinine)		
	r	χ^2	p
FVC% predicted	-0.123		0.333
FEV ₁ % predicted	-0.176		0.165
FEV ₁ /FVC% predicted	-0.016		0.900
Air xylene conc. (ppm) ^a	0.387		0.009**
Cough		0.166	0.920
Cough with phlegm		1.003	0.606
Chest tightness		0.709	0.701
Shortness of breath		1.124	0.570

N = 64, r = Spearman Rho correlation test, χ^2 = Chi square test, ** Significance level of $p \leq 0.01$, ^a N = 45.

Table 5. Comparison of lung functions, urinary MHA and air xylene concentrations between two groups.

Variable	Exposed (n=64)	Unexposed (n=47)	t	Z	p
FEV ₁ % predicted	98.03 (14.58)	105.40(13.90)	-2.70		0.008**
FVC% predicted	92.79(14.59)	98.22(13.01)	-2.06		0.040*
FEV ₁ /FVC% predicted	95.38(9.76)	96.95*(11.15)		-0.687	0.492
MHA per creatinine (g/g)	7.4x10 ⁻³ (0.019)	1.5 x10 ⁻⁴ *(0.0004)		-4.695	0.001***
Air xylene conc. ^a	0.25 (0.32)	-0.15 (0.02)	8.199		0.001***

N=111, *Significance level of $p \leq 0.05$, z = Mann-Whitney U test, ^a n exposed = 45, unexposed = 3, t = student t test, **, *** Significance level of $p \leq 0.01$ and $p \leq 0.001$, respectively.

Table 6. Variables that influenced the lung functions and respiratory symptoms of the exposed group.

Independent variable	FEV ₁ % predicted			FVC% predicted			Cough		
	Mean square	F	p	Mean square	F	p	Mean square	F	p
Solvent exposure (h)	263.6	9.7	0.029*	218.2	2.9	0.164	-	-	-
Income (RM)	1180.5	43.5	0.003*	1253.2	16.8	0.015*	-	-	-
Weight (kg)	246.4	9.1	0.039*	541.4	7.3	0.054	-	-	-
Smoking (yr)	-	-	-	-	-	-	1.482	10.241	0.015*

N=64, General linear model significance at $p \leq 0.05$.

to solvent, number of cigarettes smoked daily, years of smoking, height, weight, urinary MHA concentrations, and air xylene concentrations were included. The results in Table 6 shows that the hours of handling and exposure to solvent during work, total income, and weight were the most significant variables influencing the FEV₁% predicted. While the FVC% predicted was influenced only by the total income.

The variables that significantly influenced the respiratory symptoms were the total years of smoking (Table 6).

DISCUSSION

This study was an attempt to minimize the influence of confounding factors by using matched controls who were

also smokers but not exposed to solvent in their daily work tasks. Majority of the exposed (71.9%) as well as the unexposed group (63.8%) were smokers. The two groups were quite comparable. Results showed that for the FVC% predicted, 6.4% of both the exposed and unexposed group have mild lung functions, and 7.8 and 2.1% of the exposed and unexposed group have moderate classification respectively.

As for the FEV₁%, 12.1% of the exposed group and only 2.1% of the unexposed group had mild classifications while all respondents were normal for the FEV₁/FVC% predicted. Therefore, the exposed workers are experiencing obstructive airways as indicated by the reduction in the FEV₁% predicted. Traditionally, the severity of chronic obstructive pulmonary disease is graded by FEV₁% predicted rather than the FEV₁/FVC ratio

(Jakeways et al., 2002).

Meo et al. (2008) conducted a study on 20 workers exposed to crude oil spill in sea water, have significant reduction in FVC and FEV₁ compared to their matched controls. Crude oil is a complex of mixtures of paraffin and aromatic hydrocarbon such as benzene, toluene and xylene as well as polycyclic hydrocarbon. Petrol pump workers exposed to gasoline also experienced reduced mechanical properties of breathing. In this study, the exposed group also experienced more respiratory symptoms such as cough, cough with phlegm, chest tightness and shortness of breath (Table 5).

There was no significant correlation between air xylene concentrations with all parameters of lung functions and the respiratory symptoms. The air xylene in the individual breathing zone sampled, were very low and therefore, the association between these parameters were not seen. There were limitations in sampling the individual air since the sampling duration was short and the samples stored for about 2 - 3 weeks before analysis of xylene tend to vaporize. These low results may also be due to the effectiveness of the workplace ventilation. The employer seems to be concerned about the safety and health of the workers. However, there was a significant direct correlation between the air xylene and the urinary MHA. This result was supported by Kawai et al. (1991) study in which 121 male workers engaged in dip-coating of metal parts who were predominantly exposed to 3 xylene isomers that were o-, p- and m-xylene. Findings showed that there was a linear relationship between the exposure concentrations to xylene with the urinary metabolite MHA.

Results showed no significant correlation between urinary MHA per creatinine (g/g) with lung functions and respiratory symptoms among respondents. Probably urinary metabolite MHA is the product of acute exposure while lung function impairment and respiratory symptoms were usually chronic health outcome which would not be seen immediately after exposure. Inoue et al. (1993) found that smoking habit could also decrease the urinary excretion of MHA. This study groups were made up of healthy young males; very few of them had respiratory symptoms, however, majority of the exposed workers smoked.

No significant association between respiratory symptoms with lung functions was found. Jakeways et al. (2003) study conclude that FEV₁% predicted appears to be the measure of airflow impairment most closely associated with chronic respiratory symptoms in the general population. Jedrychowski and Krzyzanowski (1990) study conducted on the respiratory symptoms in men with normal lung functions, showed associations with a lower FEV₁%. Based on another study by Jakeways et al. (2003), there was a significant relationship between FEV₁ declines with respiratory diseases. In this study, the lung function in men decreased steeply after pneumonia infection. The acceleration of FEV₁ decline due to pneumonia was greater than in normal population.

In another study by Inoue et al. (1993), among 175 Chinese workers who had been predominantly exposed to xylene, there were correlations between exposure to three xylene isomers and the resulting urinary excretion of MHA isomers. Non-exposed controls (281 men and women) were also studied to determine the background level of urinary MHA. From the study, the concentration of each MHA isomer correlated significantly with the time weighted average intensity of exposure to the corresponding xylene isomer.

There was a significant difference in the air xylene concentrations between the two groups with a higher mean among the exposed. Table 5 also shows there are significant differences for FVC% predicted and FEV₁% predicted between exposed and unexposed, with the exposed group having lower values than unexposed group. However, for the FEV₁/FVC% predicted, there was no significant difference because both groups are normal.

Results from Shin et al. (2005) study proved that exposure to organic solvents such as xylene can reduce the lung function and induce asthma related symptoms or attacks. For income and weight, Cakmak et al. (2004) study supported the results in which with good income, the lung growth and functions improved with good nutritional intake. Weight also affected the lung function whereby lung function would decrease with obesity (Eagan et al., 2004). For other variables, no significant relation was found. Smoking can induce a decline in pulmonary functions (Miller et al., 2005) as well as aggravate throat irritation which usually gives rise to severe dry cough (Table 6).

Conclusion

From the results, the mean urinary MHA concentrations and air xylene for exposed workers was significantly higher and they also have significantly lower FEV₁% predicted and FVC% predicted compared to the unexposed group. Significant correlations between air xylene and urinary MHA found. The exposure to the solvent vapors during work, and confounders such as total income, and weight significantly influenced the FEV₁% predicted and FVC% predicted while the confounding factors such as total years of smoking and years of education significantly increased coughing among the exposed workers. The exposed workers should quit smoking because exposure to tobacco smoke together with the solvent will further impair the lung functions and elevate respiratory symptoms such as coughing among them.

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Full Length Research Paper

Sonographic quantification of basal gastric antral area, gastric motility and gastric emptying time of a liquid meal in healthy subjects: A pilot study

Ugwu Anthony Chukwuka¹, Erondu Okechukwu Felix^{2*} and Shu Elvis Nepa³

¹Department of Medical Radiography, Nnamdi Azikiwe University Nnewi campus, Anambra State, Nigeria.

²Department of Clinical Imaging, Image Diagnostics, Port Harcourt, Rivers State, Nigeria.

³Department of Pharmacology and Therapeutics, University of Nigeria, Enugu Campus, Enugu State, Nigeria.

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This study was designed to sonographically measure the basal gastric antral area, gastric motility and gastric emptying time of a standardized meal in healthy Nigerian subjects. The gastric antral area was measured pre-prandially using the antero-posterior and the longitudinal diameters in 24 healthy subjects. These measurements were repeated every 5 min after the ingestion of liquid meals for 30 min. The gastric basal antral area, gastric emptying rate (GER), gastric emptying ratio and gastric emptying time were calculated. Anthropometric variables were measured. Statistical analysis was conducted using SPSS version 16.0 with $P < 0.05$ as criterion of statistical significance. The mean \pm standard deviation for basal gastric antral area and gastric emptying ratio were $384 \pm 187 \text{ mm}^2$ and 1.56 ± 0.2 , respectively. The stomach emptied completely between 25 and 30 min. Body surface area significantly correlated with the 15th minute gastric emptying ratio (GERA₁₅). A simple linear regression equation for GERA₁₅ was derived as $0.64\text{BSA} + 0.46$. This study attempted to establish a standard model for assessing gastric emptying of a liquid meal. The normal values established in this study can be used alongside the prediction formula in the assessment of gastric motility and morbidity in patients with impaired gastric accommodation and early satiety. This small sample survey has provided a novel data which would serve as a pilot and justify the need for future research in this direction.

Key words: Sonographic quantification, gastric basal antral area, gastric emptying rate (GER), gastric emptying ratio, gastric emptying time

INTRODUCTION

The study of gastric motility has continued to evoke interest among medical researchers. Cross-sectional studies have shown that gastric emptying is delayed in as many as 50% of diabetic patients, causing symptoms such as postprandial early satiety, abdominal fullness, nausea, vomiting and early postprandial hypoglycemia (Vantrappen, 1994). Gastric emptying is delayed in 30 to 50% of patients with functional dyspepsia (Malagelada, 1996), including liquid emptying (Kellow, 1992), solid emptying (Stanghellini et al., 1996) and non-digestible emptying

(Chang et al., 1996). Upper gastrointestinal function is a critical determinant of postprandial blood glucose concentrations by influencing the absorption of ingested nutrients. Acute changes in blood glucose concentration have a major reversible effect on oesophageal, gastric, intestinal, gallbladder, and anorectal motility in both healthy subjects and diabetic patients. Interventions that reduce postprandial hyperglycemia, by modulating the rate of gastric emptying, have the potential to become mainstream therapies in the treatment of diabetes (Rayner

*Corresponding author. E-mail: okeron@yahoo.com.

et al., 2001). In 1999, Darwiche and co- authors (Darwiche et al., 1999) reported higher values of median fasting (basal) gastric antral area in diabetic patients than in healthy subjects which is most likely a correlate of antral motility. An inverse relationship between gastric motility and body surface area and between gastric motility and body weight has been reported (Lavigne et al., 1978).

Several methods have been used to assess gastric emptying in humans. Barium contrast examinations, scintigraphy, manometric technique, and intubation methods have been used to assess gastric emptying in humans (Vantrappen, 1994). Barium contrast examinations, scintigraphy, manometric techniques, and intubation methods have the disadvantages of being invasive and potentially harmful. An alternative, safe, reproducible and reliable non invasive technique would therefore be preferable. Scintigraphy is still regarded as the gold standard for clinical measurement of gastric emptying (Horowitz and Akkermans, 1989). Real time ultrasonography provides another valid method for dynamic imaging of gastric peristaltic activity and its effect on gastric emptying (Darwiche et al., 2003) and has the advantage of being cheap, easily accessible and does not make use of ionizing radiation. Other non-invasive methods of assessing gastric motility include bio-impedance (Zhang-Young et al., 2011) and electrogastrogram (Parkman et al., 2003). Studies on gastric emptying rate using a standard meal in our African locality have been scarcely documented. Earlier studies using scintigraphic techniques were not done in this locality. Racial and dietary peculiarities and differences may provide additional rationale for this study among Africans of Igbo descent. This can be deduced from previous studies, which show dietary influences on gastric emptying rates in animals (Trout et al., 1977; Trout et al., 1978). This study was designed to investigate the normal values of basal gastric antral area, gastric emptying (motility) and gastric emptying time among adults of Nigerian/ African descent.

MATERIALS AND METHODS

In line with Helsinki Declaration, approval for this study was obtained from the human research ethics committee of St. Charles Borromes hospital, Onitsha, Anambra State. The procedure was explained to the participants (subjects) and each subject signed a content form before enrolling into the study. Twenty four (24) apparently healthy male subjects were recruited for this study as volunteers. Subjects had mean ages of 33.75 ± 4.12 years. The mean body mass index (BMI) was 23.12 ± 2.21 kg/m².

The preference for male subjects is due to the fact that majority of volunteers were males. Moreover, previous studies found no established difference between the parameters for males and females.

Potential study subjects underwent medical history, fasting blood sugar tests, occults blood tests and physical examination. Exclusion criteria included history of gastro-oesophageal reflux, peptic ulcer disease and irritable bowel syndrome. The subjects had no previous

abdominal surgery (except for appendectomy). The subjects were instructed not to take drugs affecting gastrointestinal motility (Arienti et al., 1994) at least 10 days before the examination. All subjects were of Ibo (tribe) ancestry.

The fasting blood sugar was conducted using a portable blood glucose meter (companion 2 m; medisense, Waltham, MA). Subjects were advised to report to the department after an over night fast and not to drink or eat any other thing after 7.30 am and to avoid alcohol and cigarette smoking on the day of the examination. All the examinations were conducted between 9.00 and 10:00 am.

On arrival to the department, the basal (fasting) antral measurements were obtained according to established protocols (Darwiche et al., 2003). Immediately before the procedure, each subject took a tin of full cream peak brand milk [157 ml, 170 g; contents: vitamins and iodine. Carbohydrate (12.6 g), protein (8.7 g), milk fat (9.7 g), milk solids not fat 22%, milk stabilizer E 339, brand of Friesland foods, WAMCO Nig PLC] immediately before the procedures. This was immediately followed by the drinking of 30 cL of ion free water (Eva water produced by Coca Cola co, plc) (Arienti et al., 1994). 1 min was allowed for both milk and water intake. Both water and milk were stored in a large flask at room temperature.

The subjects were examined with a Siemens sonoline – SL 2 machine (Issaquah, USA), using a 3.5 MHz curve, linear probe. Gastric emptying was monitored indirectly by determining the longitudinal and antero posterior diameters of a single section of the gastric antrum using the abdominal aorta and the left lobe of the liver as internal land marks (Figure 1) to obtain the same standardized scanning level consistently according to Darwiche et al. (2003).

At this level, the scan showed the gastric antrum shape as either a circle or an ellipse, so the gastric antral cross- sectional area (GAA) was calculated with the formula for calculating the area of a circle (πr^2) with the diameter being the anteroposterior and longitudinal diameters; the area of the gastric antrum will be:

$$\pi \times d_1/2 \times d_2/2$$

Note: $d/2 = r$

The gastric emptying rate (GER) was estimated and expressed as the percent reduction in antral cross- sectional area (10) from the 5th min after ingestion of water to the 15th min after ingestion thus:

$$GER = [1 - (A_{15 \text{ mins}} / A_{5 \text{ mins}})] \times 100$$

The subjects were studied in supine position with the ultrasound transducer applied with minimal abdominal compression.

Between examinations, the subjects were raised seated in a chair. Measurements were taken immediately before the test meal as well as at intervals of 5, 10, 15, 20, 25 and 30 min after the ingestion of the test meal. This decision for 30 min timing was based on a pilot study which showed that the test meal emptied completely from the stomach in about 25 to 30 min.

The pilot study showed maximal antral area at the 5th min and decreased, with a relative plateau at 15th to 20th min, hence the decision to use 5th and 15th min timing for calculation of GER. The subjects laid on the couch for transabdominal sonography and sat after each measurement. The methods for this procedure have been previously described and have been validated in healthy controls, correlating well with scintigraphic measurement (Darwiche et al., 1999; Darwiche et al., 2003). The gastric emptying ratio (GERA) was calculated as gastric antral area at a given observation divided by the fasting area (Holt et al., 1986). At the end of each procedure, subjects height was measured on a calibrated vertical wall and the weight measured on a weighing scale (model H 89 by Hanson Scales Coy), as well as obtaining their age. The body mass index (BMI) was measured in kg/m² while the body surface area (BSA) was calculated using the Du Bois and Du Bois (Arienti et al.,

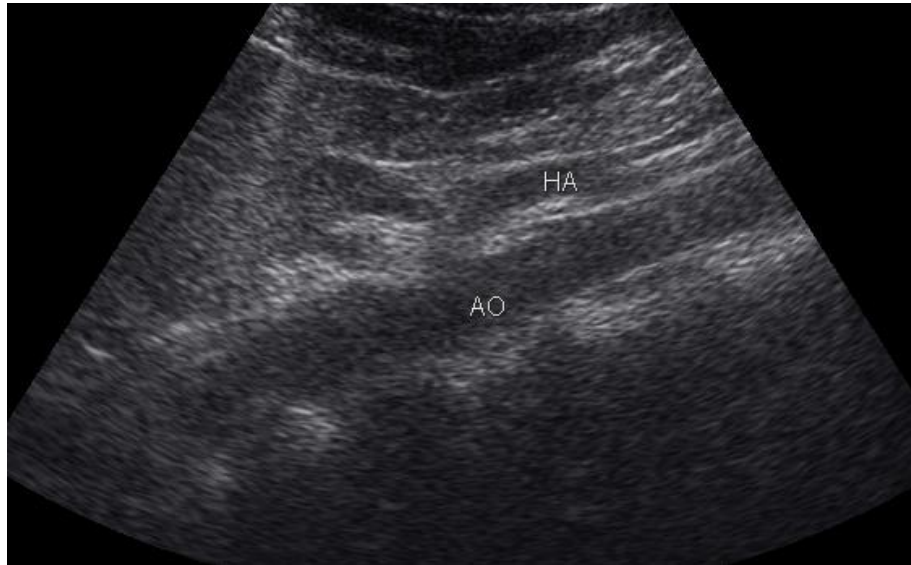


Figure 1. Ultrasonographic vertical scan through, hepatic artery (HA) and aorta (AO).

1994) equation, thus:

$$BSA = (\text{Weight}^{0.425} + \text{Height}^{0.725}) \times 0.007184$$

Statistical analysis were conducted using SPSS software version 16.0 (SPSS INC, Chicago, Illinois, USA) and graph drawn on Microsoft Excel. Both inferential (Pearson's correlation) and descriptive statistics were applied to the data namely BSA and BMI. $P < 0.05$ was used as a criterion of statistical significance.

RESULTS

The mean \pm 2 S.D. of fasting (basal) gastric antral area (FGAA) was $384 \pm 187 \text{ mm}^2$ with a range of 237 to 485 mm^2 . The basal gastric antral area was compared with gastric antral areas (GAAs) at the 5th, 10th, 15th, 20th, 25th and 30th min using a dependent t- test. Significant differences were noted in all comparisons except for the 30 min GAA which indicates that the stomach emptied this liquid meal completely after 25 min. A significant correlation existed between FGAA and weight ($r = -0.67$, $p = 0.00$), height ($r = -0.50$, $p < 0.01$) and age ($r = -0.95$, $p < 0.01$). No significant relationship existed between FGAA and BMI. However, FGAA correlated with BSA as may be seen later in the results.

The gastric emptying rate (GER) was noted to be highly variable as shown by its high coefficient of variation which was 47.8%. It ranged from 23.8 to 72.5% with a mean value of 40.7%. The GERA values were also noted to be highly variable. The least variation in GERA values was obtained with the 15th minute GERA value (6.5% coefficient of variation) and hence it was adopted as the measure of gastric emptying in this study. The mean \pm 2 standard deviation (SD) for GERA₁₅ was 1.56 ± 0.2 (range = 1.36 to 1.76) using a 2 sigma rule and a 5% false

positive (type 1) error limit. The graph of GERA against time (Figure 2) gives the gastric emptying curve. Higher values of GERA indicate lower emptying rate. GERA₁₅ significantly correlated with age ($r = 0.61$, $r^2 = 0.224$, $P = 0.02$) and BSA ($r = 0.61$, $r^2 = 0.37$, $p = 0.002$), but showed no significant correlation with height, weight and BMI. This indicates that as BSA or age increases, gastric emptying (motility) reduces as low GERA values indicates high emptying rate. A simple linear regression (predictive) equation for GERA₁₅ was derived as: $GERA_{15} = 0.64BSA + 0.4$. The shape of the gastric emptying curve (Figure 2) did not follow the well known 3 phase cycle: filling, lag and emptying. This could be due to the liquid nature of the test meals. Liquid meals empty faster than solid meals.

DISCUSSION

The result of the study shows a normal FGAA (mean \pm 2 SD) of $384 \pm 187 \text{ mm}^2$ and a mean \pm 2 SD of 1.56 ± 0.2 for GERA. The stomach emptied completely between the 25th and the 30th min with the standardized liquid meal used for this study. The use of mean \pm 2 SD and a 5% false positive error (2 sigma rule) was adopted as these values were normally distributed. This false positive rate (type 1 error) might be lower in symptomatic subjects. This is because healthy subjects have a low pretest probability, while patients clinically referred for gut sonography have a pretest likelihood of disease. These patients have undergone extensive workup prior to referral to exclude other diseases. The Bayes theorem indicates that in healthy subjects who have a low pretest probability of diseases, a positive test result is likely to be false positive, that is, the positive predictive value is low.

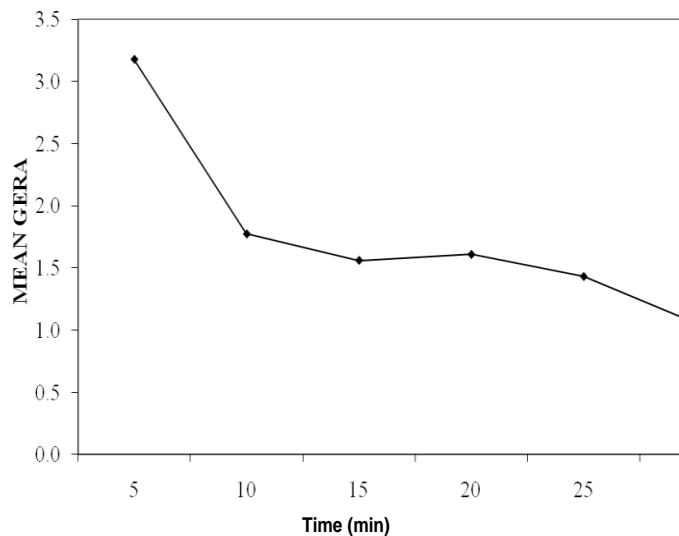


Figure 2. Gastric emptying curve

The sample size (Brown, 1995) for establishing these normal values of FGAA, GERA 15 and gastric emptying time (25 to 30 min) was small. The result can be validated on account of the normal distribution pattern of the values as proved by Lyapunov central limit theorem.

Final emptying time calculated in relation to start of meal was considered to be the time at which the antral area returned to baseline value. Reduced gastric emptying has been noted in many pathologies including diabetes (Arienti et al., 1994) and in 30 to 50% of patients with functional dyspepsia (Holt et al., 1986). A significantly higher degree of dilation of gastric antrum was found in dyspeptic patient (Xu et al., 1998) and type 1 diabetes mellitus (Malagelada, 1996). In a previous study by Darwiche et al. (1999), the basal antral area ranged from 126 to 263 mm² with a median value of 214 mm².

These Caucasian values are obviously below the range and median values established in this study among Ibo of south east Nigeria. Higher values of GERA₁₅ indicate reduced gastric emptying hence the positive and significant correlation between GERA₁₅ and age indicates that as age increases, gastric motility reduces. This finding is contrary to that of a previous study by Darwiche et al. (1999) which indicated that there is no significant relationship between age and gastric emptying while still acknowledging a possible loss of autonomic function and decrease in gastric motility with increasing age (above 70 years). Darwiche et al. (1999) noted no significant difference between the gastric emptying rates of males and females. Hence reports obtained from this study which recruited only male subjects can be applied widely in clinical environment. The significant positive relationship between GERA₁₅ and BSA indicated that subjects with higher values of BSA will have lower gastric emptying rate. This finding agrees with reports by previous researchers (Lavigne et al., 1978). Hence, variation in body size should

be taken into account when measurements of gastric emptying of liquid meals are performed. The finding in this study which showed no significant relationship between GERA₁₅ and BMI, disagrees with a previous study (Bolondi et al., 1985) in literature. A 0.37 (37%) coefficient of determination (r^2) which translates to a 63% (100 to 37) coefficient of non determination was deduced for BSA as it affects gastric motility. The high coefficient of non determination (63%) indicates that there are other variables, cumulatively of stronger impact on gastric motility than BSA.

Scintigraphy is still the method of choice when measuring gastric emptying, although it is considerably more expensive than ultra sonography and involves exposure to ionizing radiation. In our environment, scintigraphic facilities are majorly unavailable, thus justifying a simple, cheap and available method of assessment. Until now, the sonographic technique for measuring gastric emptying has been time consuming (Vantrappen, 1994). This study has simplified the procedure and optimized time spent by using liquid meal which emptied faster than solid meal, hence saving time and resources. A variety of different test meals have been used to visualize the stomach on ultrasonography. The test meal should be appetizing, easy to ingest, and an ordinary meal since the gastric motor response could be influenced by the cephalic phase (Darwiche et al., 1999). Furthermore, it is important to choose an anechoic test meal to achieve clear images for reliable measurements of the gastric antrum (Figure 1). The volume of the meal should be limited to the quantity that can be taken with ease by both healthy and gastroparetic subjects. This is because, postprandial early satiety can occur with as little as 1.5 dL in some patients with delayed gastric emptying (Darwiche et al., 1999). Considering these points, a tin of milk, taken alongside 35 cL of ion free water provides an excellent test meal for gastric emptying assessment with ultrasonography. This method is also easy as standardized milk (peak brand) and water can easily be taken in clinical settings unlike solid meals. The use of purified and ion free water is also necessary to avoid a possible effect of ionized or carbonated water on gastric emptying (Gasbarrini et al., 1991).

Changes in meal temperature also influence gastric emptying (Sun et al., 1995). Postprandial antro-pyloro-duodenal motility in healthy subjects is retarded if the temperature is either raised or lowered from 37 to 50 and 4°C respectively (Brognac et al., 1998). As the water and milk used as test meals in this study were stored at room temperature (21 to 23°C), a possible influence of temperature on gastric emptying was obviated and hence interpreted as insignificant in our study. Between the examinations, the subjects rested, seated in a chair since gastric emptying maybe affected by the posture and gravity as well as exercise (Brown, 1995). Each subject's usual defecation frequency was taken into consideration as a reference before evaluating presence of constipation or diarrhea (Powell, 1995). Voluntary suppression of defecation

delays gastric emptying in normal subjects (Tjeerdsma et al., 1993) and this “cologastric brake” is believed to be involved in the pathogenesis of upper abdominal symptoms in constipated patients.

A fairly great range in GER (41 to 91%) was reported by Darwiche et al. (1999). Though Darwiche and co-authors (Darwiche et al., 1999) used solid meal unlike the present study which adopted the use of a liquid meal, a great congruency exists in the range established by the two studies. Whereas the study by Darwiche et al. (1999) gave a range of 41 to 91% (50%), the present study established a range of approximately 24 to 73% (49%). The lower cut off (minimum value) in this present study was obviously due to the timings (points/periods at which measurements were obtained). Normal values in this baseline study show great concordance with those of the previous study. This concordance in range indicates that liquid and solid meals could have equal diagnostic value in the assessment of GER or GERA. Furthermore, liquid meal has the advantage of easy availability, standardization and acceptability by among subjects.

Several observations indicate that feedback from gastric mucosal receptor can be influenced by prior nutrient intake (Cunningham et al., 1991) resulting in adaptive changes in gastric emptying. This could be an underlying factor that can partially explain day-to-day variability of gastric emptying within subjects.

Probably other factors exist that could affect day-to-day variability such as acute stress (Thompson, 1983). These are possible limitations to the present study as diet intake of the subjects were not moderated prior to and during the study. The stress levels in the subjects were also not considered in our study. Future studies in this area with diet moderations and predetermined stress levels should be considered. This study has provided baseline values of gastric antral area (pre-prandial), gastric emptying ratio and gastric emptying time which could guide clinicians in evaluating patients with suspected cases impaired gastric emptying, increased duodenogastric reflux and insufficient post prandial accommodation of the proximal stomach.

The mean \pm 2SD for FGAA, mean \pm GERA₁₅ the gastric emptying time, the predictive equation for GERA₁₅ and BSA and the predicative equation for GERA₁₅ and GBCI₃₅ (when gall bladder function is normal) should be combined in the assessment of morbidity in functional dyspepsia, diabetic gastroparesis, duodenogastric reflux and other forms of insufficient postprandial accommodation.

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Full Length Research Paper

Effectiveness of dry *Moringa oleifera* leaf powder in treatment of anaemia

Madukwe E. U.* Ugwuoke A. L. and Ezeugwu J. O.

Department of Home Science, Nutrition and Dietetics, University of Nigeria Nsukka, Enugu State, Nigeria.

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This study evaluated the effectiveness of dry *Moringa oleifera* leaf powder in the management of anaemia in adult albino rats. The proximate, mineral, vitamin and phytochemical composition of dry *M. oleifera* leaf powder were analysed. Twelve adult albino rats grouped into three were used. Cyclophosphamide was used to induce anaemia into them. The percentage proximate values were protein (26.28%), ash (7.69%), carbohydrate (49.35%), crude fibre (7.48%) and moisture (7.05%). The rats whose feed were supplemented with *M. oleifera* leaf powder showed superior attributes to the unsupplemented group. The study showed dry *M. oleifera* leaf powder is promising in the management of anaemia.

Key words: Effectiveness, anaemia, treatment, *Moringa oleifera* leaf powder.

INTRODUCTION

Anaemia is a widespread public health problem associated with an increased risk of morbidity and mortality, especially in pregnant women and young children. It is a condition caused by both nutritional (vitamin and mineral deficiencies) and non-nutritional (infection) factors. One of the most contributing factors is iron deficiency which is considered the number one contributor to the global burden of diseases (Brandy, 2007). Anaemia can result in impaired cognitive development, reduced physical work capacity and in severe cases, increased risk of mortality, particularly during the perinatal period. Anaemia may also result in reduced growth and increased morbidity (WHO/UNU/UNICEF, 2001).

Green leafy vegetables are a great source of minerals such as zinc, iron and potassium. Leafy vegetables also contain bioactive phytochemicals that have been linked to protection against cardiovascular and other degenerative disease (Okeno and Chebert, 2003). The plant *Moringa oleifera* is the most widely cultivated species of a monogetic family, the Moringaceae that is native to the

Sub-Himalayan part of India, Pakistan, Bangladesh and Afghanistan (Palada and Chang, 2003). It is now cultivated and has become naturalised in many locales in the tropics. *M. oleifera* is reported to prevent malnutrition because of the high protein and micronutrient content of the leaves (Anjorin et al., 2010). The minerals contents in *M. oleifera* and their bioavailability have been a subject of tremendous studies. There are however limited reports on the influence of variation in geographical locations or agro-ecology of *M. oleifera* on the mineral composition in various organs of the plant in Nigeria. Confirmation of minerals content of plant materials across varied agro-ecologies is necessary in the selection and formulation of plant-based mineral supplement in animal and human nutrition (Anjorin et al., 2010). The leaves of this plant contain a profile of important trace elements and are a good source of proteins, beta-carotene, amino acids and various phenolics. The leaves contain phytochemicals which protect plants from predators that include alkaloids, anthraquinone, coumarins, flavones, phenols, quinines and

*Corresponding author. E-mail: happyeze01@yahoo.com

Table 1. Chemical composition of dry *Moringa oleifera* leaf powder (per 100 g).

Nutrient	Composition
Moisture (%)	7.05 ± 0.17
Protein (%)	26.28 ± 0.06
Fat (%)	2.16 ± 0.01
Ash (%)	7.69 ± 0.13
Crude fibre (%)	7.48 ± 0.12
Carbohydrate (%)	49.35 ± 0.15
Iron (mg)	19.42 ± 0.13
Calcium (mg)	171.6 ± 5.66
Beta-carotene (RE)	7.30 ± 0.01
Vitamin C (mg)	18.72 ± 0.13
Flavonoid (%)	3.29 ± 0.03
Alkaloids (%)	1.18 ± 0.05

Means ± SD of three determinations.

and tannins (Kasolo et al., 2011). The plant has been said to be a promising remedy for anaemia especially iron deficiency anaemia. The plant *M. oleifera* (fresh) has been used to combat malnutrition, especially among infants and nursing mothers. Countries like Senegal, India, Benin and Zimbabwe are now using *Moringa* leaves for programmes to fight malnutrition (Fahey, 2005). This study aims to evaluate the effectiveness of dry *Moringa* leaf powder as remedy to manage anaemia.

MATERIALS AND METHODS

Sample collection and preparation

The leaves of *M. oleifera* were harvested from Orba Nsukka to ensure uniformity and to avoid variation. They were shade-dried for 4 days after which they were milled into fine powder with the aid of electric blender.

Chemical analysis

The proximate values of the dry *M. oleifera* leaf powder (moisture, crude protein, fat, crude fibre and ash) were determined by the AOAC (1995) method. However, carbohydrate was determined by difference as follows: 100 – (% ash + crude fibre + protein + fat + moisture). Iron and calcium and ascorbate content were analysed using the AOAC (1990) method. Beta-carotene was determined according to the method of Pearson (1976). Alkaloids and flavonoids content were determined by the method of Harborne (1973).

Metabolic (anaemia) study

Twelve rats used for the study were bought from Department of Veterinary Medicine, University of Nigeria, Nsukka. They were allotted individually into metabolic cages equipped to separate urine and faeces and were fed animal feed and water *ad-libitum* for five days to get them acclimatised to both the feed and environment. Cyclophosphamide was used to induce anaemia intraperitoneally in

the three groups of rats. On establishment of anaemia, they were grouped into three (3) according to their body weight in such a way that the difference between each group was not more than 5 g. They were subsequently fed their respective diets and water (*ad libitum*) for five (5) days. The first group (A) served as the control. This group continued to receive only commercial animal feed. The second group (B) was fed the commercial animal feed supplemented with *M. oleifera* leaf powder (at 5% protein level). The third group was fed the commercial animal diet supplemented with *M. oleifera* leaf powder (at 10% protein level).

On the fifth day, they were weighed and blood samples were drawn from the rats through the ocular vein to determine their packed cell volume (PCV), haemoglobin and red blood count level.

Statistical analysis

Means and standard deviation were calculated from three determinations. One-Way Analysis of Variance (ANOVA) was calculated and Duncan's New Multiple Range Test was used to separate and compare means (Steel and Torrie, 1960).

RESULTS

Table 1 presents the chemical composition of dry *M. oleifera* leaf powder. The moisture content was 7.05%, protein 26.28%, while fat, ash and crude fibre were 2.16, 7.69 and 7.48%, respectively. Iron was 19.42% and vitamin C was 18.72 mg. Flavonoids and alkaloids contents were 3.29 and 1.18%, respectively.

Table 2 presents the levels of PCV, HB, RBC and weight gain of anaemic rats. Rats in group B had the highest PCV (37.50%), HB (13.00 g/dl) and RBC (10.69) when compared with the other groups. Rats in group C had higher PCV (34.00%), HB (10.38 g/dl) and RBC (910.58) than the control (group A). Group A had the lowest PCV (22.75%), HB (6.73 g/dl) and RBC (6.52). The PCV, HB and RBC of rats in the control differed significantly from the test groups ($P = 0.013$). The weight gain of rats in groups A, B and C were 10.00, 15.06 and 12.05 g, respectively. Rats in the control group differed significantly from the test groups ($p < 0.05$) in terms of weight gain. In all the attributes assessed, group A showed inferior values.

DISCUSSION

The moisture content of dry *M. oleifera* leaf powder was low. This explains the higher values of protein, fat, carbohydrate and crude fibre. The lower the moisture contents of a food, the higher the nutrient density (Udofia and Obizoba, 2005). The low moisture content suggests that the storage life will be high. Dry *M. oleifera* leaf powder can be classified as carbohydrate rich food because of its high carbohydrate contents. The protein found in the dry leaf powder was lower than that reported by Fahey (2005). This might be due to differences in processing methods and environmental conditions. However the fat, ash and crude fibre contents were similar to literature

Table 2. Packed cell volume (PCV), Haemoglobin (HB), Red Blood Cell (RBC) and weight gain of anaemic rats after treatment.

Experimental group	PCV	HB	RBC	Weight gain
Group A (control)	22.75 ± 3.59 ^a	6.73 ± 0.57 ^a	6.52 ± 2.63 ^a	10.00 ± 1.39 ^a
Group B (5% <i>Moringa oleifera</i> powder)	37.50 ± 6.25 ^b	13.00 ± 1.75 ^b	10.69 ± 0.84 ^b	15.06 ± 0.71 ^b
Group C (10% <i>Moringa oleifera</i>)	34.00 ± 3.27 ^c	10.38 ± 1.18 ^c	10.58 ± 0.63 ^b	12.00 ± 0.76 ^c

Means ± SD of three determination, Means followed by the same letters on the vertical column are not significantly different from each other (P<0.05).

reports (Fahey, 2005). The iron, calcium, beta-carotene and vitamin C contents were also high. This accounts for its effectiveness as a remedy for malnutrition. The presence of phytochemicals (flavonoids and alkaloids) in the leaves shows that it may have antioxidant property.

Results of the metabolic (anaemia) study revealed true to literature reports dry *M. oleifera* leaf powder is effective in treatment of anaemia (Fahey, 2005). This evidently is because of its content of quality protein, iron, vitamins A and C. It also implies that the nutrients are bioavailable in rats. Rats are monogastric just like humans. If the nutrients are biologically available in rats, it is an indication or prediction of their bioavailability in man. Anaemia is caused by mineral (for example iron) and vitamin (for example Vitamins A and C) deficiencies as well as infections. Proteins being a fundamental component of blood are found in high quantity in the leaf powder. Rats whose diets were supplemented with dry *M. oleifera* leaf powder had higher mean weight gain than the control. This confirms the report of (Nambiar and Seshadri, 2001) that supplementation of food with *M. oleifera* leaf powder results both in increased food intake and weight gain. Supplementation of feed with *M. oleifera* leaf powder at 5% protein level produced better results than at 10% protein level.

Conclusion

Dry *M. oleifera* leaves are rich in essential nutrients and might be used in food supplementation to improve the nutritional status of individuals and communities especially the vulnerable groups. The leaf powder was also effective in management of anaemia in adult rats.

RECOMMENDATIONS

Dry *M. oleifera* leaves could be used as a nutrient supplement in foods to improve the nutritional status of people both in terms of micronutrient nutrition and weight gain. However, confirmatory studies should be carried out in humans to establish a sound evidence-based nutrition advice.

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Full Length Research Paper

Evaluation of the incidences of dermatophilic infection in Rajasthan: Case studies from Rajasthan, India

Sarika Gupta^{1*} and B. Lal Gupta^{1,2}

¹Dr. B. Lal Institute of Biotechnology, Jaipur, India.

²Dr. B. Lal Clinical laboratory, Jaipur, India.

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Dermatophytosis accounts for the majority of fungal skin diseases. Sixty (60) case studies (45 males and 16 females) of dermatophyte infection were carried out. Out of the 60 cases, 13% cases were healthy and 87% cases were found infected with one or more fungi. In 95% of cases, the fungal species recovered were from the infected symptomatic area like inflammatory lesions redness, dry patches itching, flaky rings, and 6.7% of cases from pain. The pattern of distribution of site of infection was recorded maximum at internal parts (54%) followed by hand (15%), neck (12%) and leg (6%). The incidence of *Aspergillus niger* (19%), *Cladosporium* sp. (14%), *Aspergillus flavus* (13%), *Trichophyton* sp. (13%) and *Microsporum* sp. (5%) was high and *Fusarium* sp., *Curvularia* sp., *Penicillium* sp., *Trichothecium roseum*, *Epidermatophyton* sp., *Drechslera* sp. and *Alternaria* sp. was low.

Key words: Evaluation, incidence, dermatophilic infection, clinical isolates.

INTRODUCTION

Dermatophytes cause infections of the skin, hair and nails because of their ability to obtain nutrients from keratinized material. These organisms colonize the keratin tissues and in response to their metabolic by products host experiences, and inflammatory reactions results in the penetration and establishment of the fungi in epidermal and subepidermal tissue. They are usually restricted to the non-living cornified layer of the epidermis because of their inability to penetrate viable tissue of an immunocompetent host. Acid proteinases, elastase, keratinases, and other proteinases reportedly act as virulence factors. Dermatophytes are: (i) anamorphic (asexual or imperfect fungi) with three genera that is, *Microsporum*, *Epidermophyton* and *Trichophyton*; (ii) telomorphic (sexually reproducing) and (iii) Arthroderma, of the ascomycota. Diagnosis of these mycoses is made

from mycologic studies, direct examination, stains, isolation, and identification of the fungi. Treatment includes systemic antifungals, topical antifungals, and keratolytics (Bonifaz et al., 2010).

Tinea infections are among the most common dermatologic conditions throughout the world. To avoid a misdiagnosis, identification of dermatophyte infections requires both a fungal culture on Sabouraud's agar media, and a light microscopic mycologic examination from skin scrapings. Preventative measures of tinea infections include practicing good personal hygiene, keeping the skin dry and cool at all times and avoiding sharing towels, clothing, or hair accessories with infected individuals (Gupta et al., 2003). The National Skin Centre is a tertiary referral centre for dermatological diseases in Singapore and observed more than 2,500

*Corresponding author. E-mail: blalbiotech@gmail.com.

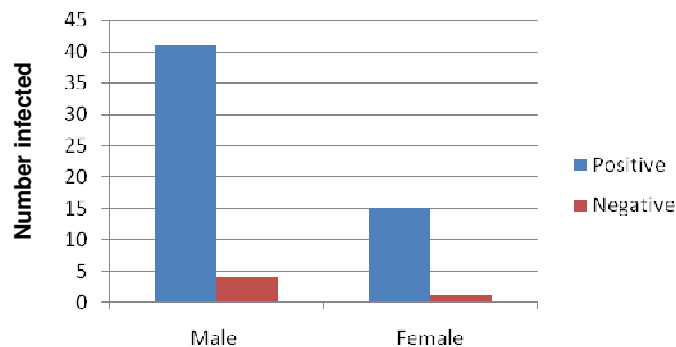


Figure 1. Histogram showing results of KOH test in the cases susceptible to dermatophytic infection.

cases of superficial fungal infections annually.

Trichophyton rubrum was the most prevalent fungal pathogen isolated from all cases of superficial fungal infections of the skin, except for *Tinea pedis*, in which *Trichophyton interdigitale* was the most frequently isolated organism (*T. rubrum* is the most common isolate in the superficial fungal infection whereas in *Tinea pedis* that is, infection in feet, *T. interdigitale* is the most common isolate). Dermatophytes remain the most commonly isolated fungal pathogens isolated in toenail onychomycosis, whilst *Candida* species accounted for the majority of isolates in fingernail onychomycosis (Tan, 2005). This is now a common infection among Rajasthan, although the climatic conditions are not favorable for the fungal growth during most of the time of the year (2009 to 2010). Due to some injuries and inappropriate approach for the treatment of trauma situations in rural population, the incidences of dermatophytic infections are increasing. The present work aims at evaluation of the rate of incidences of dermatophytic infections (group wise) and the associated symptoms.

MATERIALS AND METHODS

Sample selection

A detailed record of dermatophytic patients visiting Dr. Rishi Bhargav Girdhar Hospital, Malviya Nagar, Jaipur and Dr. B. Lal. Clinical Laboratory, Malviya Nagar, Jaipur for treatment or clinical diagnosis were taken as case study on the basis of their susceptibility for dermatophytic infection. Patient proforma is filled during the collection of sample to obtain information on duration of the lesion, clinical picture, prior therapy as well as demographic data such as age, sex, nationality and family status of the respondents. 60 cases were finally selected, consisting of 45 males and 16 females.

Sample collection

Two sample collection methods were used in this study. On one method, samples consisting of epidermal scales and infected hairs were scraped from the scalp/rim of lesions using a sterile scalpel

blade (using the blunt side, care was taken so that it did not rupture the tissue) following cleaning of the affected sites with 70 v/v isopropyl alcohol. The scrapings were collected on a piece of sterile brown paper Griffin (1960) (sterile brown paper is used for the transportation of clinical scrapping from the site of collection to the centre for microbiological analysis as per the reference or it was transported in the culture bottle containing sterile SDA slant. Sterile petriplate was not used). Moist cotton swabs were used to collect pus from inflammatory lesions. The samples were divided into two portions: one for microscopic examination and one for culture. The collected samples were transported to the laboratory within 2 h for microscopic and cultural analysis.

Sample processing

Direct microscopic examination

Direct microscopic examination of the scrapping placed on a microscope slide with one or two drop of 20% potassium hydroxide (KOH) and a cover slip was performed. The sample was warmed for 5 min over a flame as described by Hainer (2003). Each treated slide was then carefully examined under low ($\times 10$) and high ($\times 40$) power objective for the presence of hyphae and/or arthroconidia.

Fungal culture

Each scraping was cultured into Sabouraud dextrose chloramphenicol actidione agar (Ajello et al., 1966). A duplicate inoculation of the specimen was also cultured on sabouraud's dextrose cycloheximide agar. The plates were incubated at 28°C for up to 4 weeks and examined at 2 to 3 days interval for fungal growth. Fungal isolates were subcultured onto plates of sabouraud's agar and potato glucose agar. The isolates were examined visually and microscopically for morphology of fungi using lactophenol cotton blue stain by slide culture technique (as per the standard protocol). The dermatophytes species were identified by gross and microscopic morphology and by *in vitro* culture (cultured in laboratory). Evaluation of the relative percent occurrence (RPO) of the fungi and sensitivity of KOH test was done. Different fungi alone and in combination were observed. The clinical isolates were further maintained in Sabouraud's dextrose cycloheximide agar slants.

RESULTS

Sixty (60) case studies (45 males and 16 females) of dermatophyte infection were carried out under the guidance of Dr. B. Lal Gupta and Dr Rishi bhargava. Out of 60 cases, 13% cases were with no traces of fungal infection as per confirmation by KOH test and culture test whereas 87% cases were found to be infected with one or more fungi. On the basis of KOH test, positive reactions were seen in 93.3% cases and negative reactions were found in 8.3% cases (Figure 1). In 95% of cases, the fungal species recovered were from the infected symptomatic area like inflammatory lesions redness, dry patches itching, flaky rings and 6.7% of cases from pain (Figure 2). Figure 3 shows maximum infection in internal part (54%) followed by hand (15%), neck (12%), leg (6%), back, eyes, face, hair, head, nail and thighs. Culture of the scrapings after 7 days of incubation yielded different

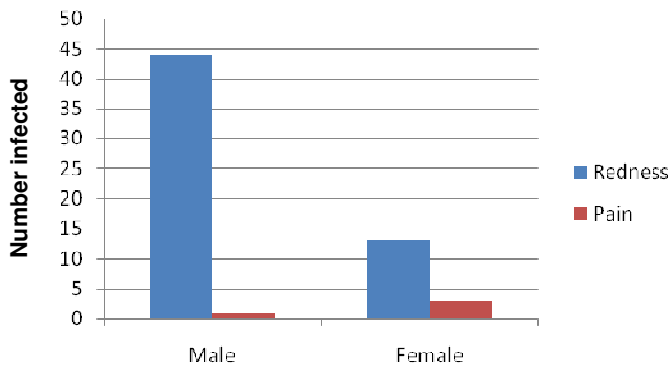


Figure 2. Histogram showing symptoms associated with the dermatophytic infection.

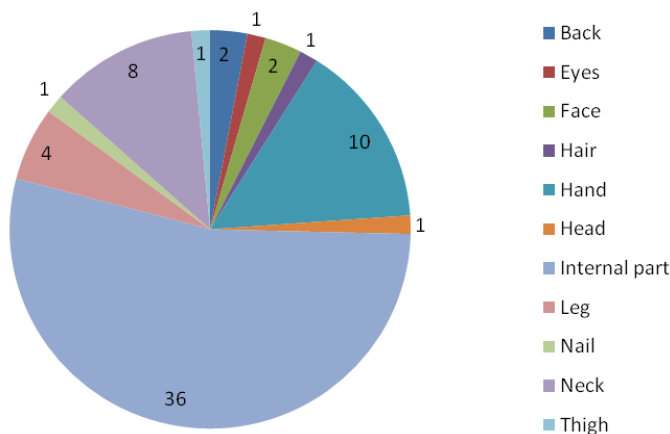


Figure 3. Histogram showing site of infection for the recovery of skin scrapings. Values are in percentages.

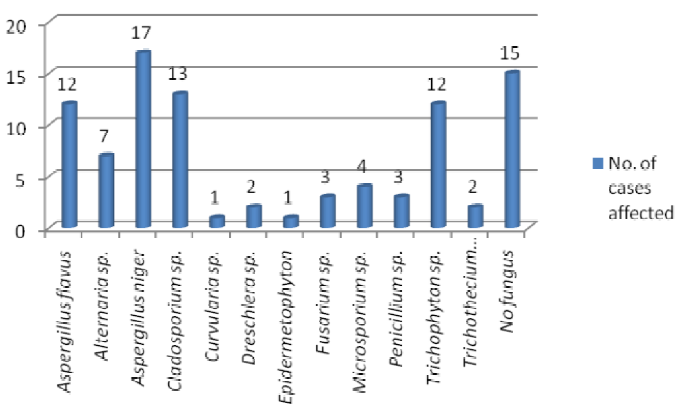


Figure 4. Histogram showing the incidence of fungi isolated from clinical samples.

alone and in combination. The incidence of *Aspergillus niger* (19%), *Cladosporium sp.* (14%), *Aspergillus flavus*

(13%), *Trichophyton sp.* (13%) and *Microsporium sp.* (5%) was high and *Fusarium sp.*, *Curvularia sp.*, *Penicillium sp.*, *T. roseum*, *Epidermatophyton sp.*, *Drechslera sp.* and *Alternaria sp.* was low (Figure 4). These clinical isolates were further maintained in agar slants.

DISCUSSION

During the study, 60 samples were collected and diagnosed for superficial fungal infection. Their cultural and microscopic characteristics yielded 12 fungal species causing dermatophytic infection. Eighty seven (87%) cases were affected by these organisms and 13% cases were free from dermatophytic infection as no trace of fungal infection was recorded during the culture test. However, accurate assessment of the prevalence of etiological agents is useful to estimate the size of problem and prevent its transmission. It was inferred that the males had more infections than females (primarily, males are more exposed to dust and external environment so more prone to infection and secondly, females are not paying attention to the lesion they have, as in most of the communities females do not bother about their health and they do not get enough time to visit the doctor). Certain factors may influence the distribution of dermatophytosis. According to Ekanem and Gugnami (1987), age influences susceptibility to dermatophytosis because of the changes in immunity. The pattern of site of infection was maximum in internal part (curved area of the body, the tissue in and around genital area), followed by hand, neck, leg and other body parts.

The common symptoms that were found to be associated with the infection were inflammatory lesions, redness, dry patches, itching, flaky rings and pain. The incidence of *A. niger*, *Cladosporium sp.*, *A. flavus*, *Trichophyton sp.* and *Microsporium sp.* fungi was high and *Fusarium sp.*, *Curvularia sp.*, *Penicillium sp.*, *T. roseum*, *Epidermatophyton sp.*, *Drechslera sp.* and *Alternaria sp.* was low (based on the present study). The presence of other non-dermatophytes (particularly *Aspergillus* and *Penicillium* species) may be due to the ubiquitous nature of their spores in our environment, carried transiently on healthy skin (from the environment the infection propagules may be transmitted on the skin where it colonizes due to the production of elastases proteinases and keratinases by the fungi) (Oyeka and Ugwu, 2002). *Fusarium solani* was isolated from scrapings from skin lesions. In our study, this organism was recovered in mixed with other organism. *Alternaria alternata* is a soil saprophytes and common pathogen. Cutaneous infections caused by *Alternaria* species are often associated with debilitating diseases or conditions Cabanes et al. (1988). During the past decade, there has been a significant increase in the number of phaeohyphomycotic infections recognized in humans reported by De Hoog and Rubio (1982) and Ernst (1983).

Majority of the isolated dermatophytes according to percentage of occurrence were *Microsporium audouinii* (18.88%), *Trycophyton mentagrophytes* (13.33%) and *Trycophyton terrestre* (3.33%). *M. audouinii* and *T. mentagrophytes* were the most frequently isolated dermatophytes. Other skin mycoses isolated include *Fusarium moniliforme* (6.66%), *A. flavus* (5.55%), *Fusarium oxysporum* (5.55%) and *Penicillium funiculosum* (4.44%). Infection was mainly due to *M. audouinii*, *Chrysosporium keratinophilum* and *T. mentagrophytes* as reported by Maruthi et al. (2008).

Conclusion

The infection was pronounced in males as compared to females, based on 60 patient cases studied. Utmost infection was recovered from internal parts followed by hands, neck and legs. Most frequently occurring fungus was *Aspergillus* sp. followed by *Cladosporium* sp., *Trichophyton* sp., and *Microsporium* sp.

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Full Length Research Paper

Analyzing long-term mortality among female alcoholics and matched controls: Accounting for age and follow-up time

Rolf Gjestad^{1*}, Johan Franck² and Brit Haver^{2,3}

¹Department of Clinical Medicine, Section of Psychiatry, Haukeland University Hospital, Division of Psychiatry, Research Department, Sandviken, Pb 23, N-5812 Bergen, Norway.

²Karolinska Institutet, Department of Clinical Neuroscience, Division of Psychiatry, Stockholm, Sweden.

³Department of Clinical Medicine, Section for Psychiatry, University of Bergen, Norway.

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Studies focusing on mortality data use a wide variation of strategies for data analyses, making comparison between studies difficult. The research problems focus upon different statistical analyses of mortality among patients and matched controls regarding clustered data and relations over different levels of age and follow-up time. Four hundred and twenty (420) treated female alcoholics were compared to 2,036 matched controls and public register data for a follow-up period of 27 years were used. The statistical analyses are multilevel, structural equation modeling (SEM) level-and-difference analyses, multilevel Cox regression analysis, interaction Cox models, time-dependent Cox survival models, proportional and non-proportional latent discrete-time survival models. The multilevel analyses confirm the success of the matching procedure. The interaction model adds more information to the main effect model and shows the mortality estimate to be dependent on age. Continuous time-dependent Cox regression models and latent discrete-time survival analyses show the mortality estimates to differ with time and age. Different results depend on statistical models. This illustrates how mortality as a construct not only represents hard and unequivocal evidence given by the samples studied, but also includes factors related to the statistical model used. Such methodological factors need to be incorporated in the scientific discussion of mortality studies generally.

Key words: Continuous and discrete time survival analysis, interaction models, matched data, mortality.

INTRODUCTION

Mortality statistics are one of the most important methods of reporting the health of general populations and the seriousness of a disorder. In addition, mortality is widely used in medicine to report the efficacy and risks of treatment procedures. The construct "mortality" seemingly represents hard evidence, making comparison between studies simple and unequivocal. Thus, a discussion of the applied outcome measurement may be seen as superfluous or unnecessary. However, methods of sampling and

analyses vary and the comparison of results between studies may be problematic or even misleading. Some studies report frequency of death without including time to death in the analyses. Information about this is important to take into consideration in order to explain why a group difference is found or not (Singer and Willett, 2003). Another topic is confounding variables, which more or less are taken into consideration in different studies. Regression models accounting for relevant variables,

both as main and interaction terms, may be used to control factors which themselves also may be related to mortality. A third factor is about sampling. Mortality risks are estimated in relation to a control sample. Control subjects may be randomly sampled from the general population (Gerdner and Berglund, 1997), or from a matched control population in order to control mortality related variables (Rosenbaum and Rubin, 1985).

Matching increases the efficiency of the estimation of difference between cases and controls and creates equivalence in the samples regarding relevant covariates (Smith, 1997). Increasing the numbers of matched subjects increases the accuracy of population estimates, while confounding effects may be controlled by increasing the number of matching variables. However, several matched controls for each study subject makes clustered data. In this study, up to five controls were matched for each study subject. Within cluster, women were almost identical, regarding age, civil status, socio-economic status and level of education and data may therefore be almost perfectly correlated within clusters on the matched variables and somewhat less perfectly correlated regarding other related variables. Thus, observations are not independent and statistical tests not accounting for data clustering may give biased results (Norušis, 2005; Brown and Prescott, 2006; Breslow, 1996). Multilevel analysis is a method that gives unbiased estimates and tests the success of the matching procedure (Drukker et al., 2008).

Matching variables may themselves be related to mortality (Rogers et al., 2010; Saarni et al., 2008). In addition to making cases and controls equal by matching, less unbiased estimates is achieved if such confounding matching variables also are included in the statistical model (Card et al., 2003; Jackson et al., 2007). For example, one study showed how a group difference changed from lower to higher mortality rates after accounting for age, race, gender, and major comorbidities (Yuan et al., 2001). In the present study, group difference is analyzed with statistical control for age and educational level. However, assuming mortality difference between patients and controls to be equal over all levels of age represents an oversimplification for the statistical model, especially in samples with large variation in age. The interaction term between group and age should also be considered for the model. Interaction terms are often not considered for inclusion in regression models (Cohen et al., 2003; Pedhazur and Schmelkin, 1991), nor in Cox regression models.

Mortality difference between two groups may vary during the follow-up interval, particularly if this interval is of long duration. Follow-up interval may vary between studies from months to decades, and comparisons over studies are difficult. To divide the number of deaths on the duration of follow-up time in order to compare findings from different studies may be a problematic procedure (Timko et al., 2006), as this strategy represents an average number of deaths for each unit of time and mask potential differences between groups regarding when deaths

occur. For example, a treatment study may show the preventive effect to be stronger right after the intervention than in the long run (Cuijpers et al., 2004). This will be reflected in varying mortality rates over time, a situation that represents a threat against the proportional hazard assumption (Norušis, 2005). Time-dependent interaction effects should be considered in order to explore this research problem (Willett et al., 1998). Also, time may be treated as a continuous variable or as discrete-time interval variables (Masyn, 2003).

The present research problems focuses on statistical analyses of mortality among patients and matched controls regarding clustered data with several matched controls for each study subject, the effect of statistical models including the group mortality difference and predictor relations over different levels of age and follow-up time. The data analyzed is from an alcohol study (Haver et al., 2009). However, the discussion of alcohol related mortality is not the substantial theme here, since this topic of methodology extends to mortality studies in general and to studies of long follow-up duration in particular.

MATERIALS AND METHODS

Participants

The subjects were 420 women not previously treated for alcohol use disorders, who participated in the European Workplace and Alcohol (EWA) project at the Karolinska Hospital, Stockholm, Sweden (Haver et al., 2009). This sample consists of four sub groups (sequence strata); one pilot study sample from 1981 to 1982 (N = 100), another randomized controlled trial (RCT) study sample with two groups receiving different treatments from 1983 to 1984 (N = 200), and a comorbidity study sample from 1991 to 1993 (N = 120). In 2009, a matched general population control (MGPC) group was obtained from the Swedish Causes of Death Register (N = 2036), with up to five matched controls for each study woman. The follow-up period was up to 27 years.

Measures

Variables used for analyses are group (addicted versus MGPC women), age and time since intake to treatment, mortality status, and education level. The education variable was ordinal with 3 categories: primary school, high school and college/university. Two Helmert contrast variables were constructed, specifying the difference between the low level and the sum of the two other levels (Edu_H1 = 1, -.5, -.5) and the difference between the two last education categories (Edu_H2 = 0, 1, -1).

Analyses

Due to strata and clustering of data, bootstrapping with stratified resampling was used to estimate confidence intervals (Timmerman et al., 2009; Barber and Thompson, 2000). Bootstrapping handles deviation from normal distribution well (Hair et al., 1998; Wehrens et al., 2000), and gives more precise estimates in samples smaller in size (Haukoos and Lewis, 2005). Clustered data may be analyzed with multilevel models, giving within and between cluster estimates (Brown and Prescott, 2006; Smith, 1997; Norušis, 2005). Such models

may also control for measurement errors (Breslow, 1996). With relatively few cases within clusters, structural equation latent level-and-difference modeling may be used as an alternative (Newsom, 2002). Both statistical methods are used as an illustration of the analyses of within and between cluster levels and variations of age. In addition, the multilevel relationship between age and mortality is analyzed. Since the total sample consisted of four strata, potential strata effects are accounted for (Muthén and Satorra 1995, Stapleton 2006).

Cox regression is used to analyze survival models with continuous and categorical predictor variables (Bradburn et al., 2003). Age is analyzed as a continuous variable, since categorizing a continuous variable may give biased estimates and is encumbered with reduced statistical power (Royston et al., 2006; Cohen et al., 2003). Interaction models often introduce multicollinearity problems and resulting in instability in estimates. Different solutions exist; centering and incremental significance testing (Hair et al., 1998), or the use of the residualized interaction term (Delacroix and Ragin, 1978). Centering changes the interpretation of the main effects and has implications regarding what level of the main effect that is tested for statistical significance (Hair et al., 1998, Cohen et al., 2003). In the present study, the age variable is centered. Visualization may be a good way to present survival differences between cases and controls at low and high levels of age. These age levels are arbitrary set and entered into the Cox regression equation to give predicted scores for women being 30 and 50 years, illustrating survival at those age levels.

Allowing for group differences in mortality rates over time is done by entering variables as time dependent covariates in Cox regression. This procedure frees up and tests the proportional hazard assumption in ordinary Cox regression (Norusis, 2005; Chen et al., 2010). This is not very often verified in research (Bellera et al., 2010). Based on these results, time-restricted Cox proportional models may be chosen. Covariates may be static or time-varying and may have different magnitude in their predictive associations with mortality over time. If time is divided into several restricted interval variables and discrete-time survival models analyzed, predictors may be directly related to mortality in separate time intervals (Muthén and Masyn, 2005; Abbott, 1985).

Proportional hazard models may still be estimated as latent discrete-time survival analyses (Muthén and Muthén, 2007) and used as an approximation to the Cox regression model as long as the categorization of the time variable is sufficiently detailed (Asparouhov et al., 2006). Equal hazards over the entire range of time intervals is then specified with all factor loadings between the latent variable and the mortality status in each time interval specified as one (Muthén and Muthén, 2007). Here, we used two set of models consisting of two- and four year intervals. Using a four year interval will increase the prediction power due to more deaths within each interval, while a more restricted interval is more suitable when shorter time-dependent associations is in focus. The proportional restriction may be freed up in order to analyze different predictive relations in each interval. This is done by removing the latent part of the model and different time intervals are allowed to be predicted by separate logistic regressions (Muthén and Masyn, 2005).

Another test of a non-proportional hazard model could be done by adding predictors over and beyond the latent factor. We have not seen this last procedure used in the literature, but adding parameters to a basis model is used as a strategy in other structural models (Muthén and Curran, 1997). Dependent on the sample size (Kline, 2010), combinations of survival models and other structural equation models may address very flexible research problems (Muthén and Muthén, 2007; Bollen, 1989; Bollen and Curran, 2006; Duncan et al., 2006; Masyn, 2008). Model fit is evaluated with the measures-LogLikelihood, Akaike information criterion (AIC), and the Bayesian information criterion (BIC) (Kline, 2010).

Statistical Package for Social Sciences (SPSS) 18 was used for multilevel analyses (linear mixed model) and Cox survival analyses. Mplus 5.2 was used for multilevel analyses, level and difference models, multilevel Cox regression and discrete-time survival analyses (Muthén and Muthén 2007, Muthén and Masyn 2005).

RESULTS

The mean age is 42.63 (standard deviation (SD) = 9.81) for patients, for controls 42.54 (SD = 9.77). The parametric 95% confidence intervals (CI) are: addicted women: 41.69 to 43.57 and MGPC subjects: 42.11 to 42.96. The stratified 95% bootstrapping of the MGPC group on cluster within the four sequence strata shows a much smaller CI than the parametric CI: 42.53 to 42.55. Mortality was 33.1% in the alcoholic group and 14.6% in the control group ($p < 0.001$; RR = 2.26, OR = 2.89).

Matched data

Multilevel analyses of age in the MGPC group showed the within cluster variation of age to be very small compared to the between cluster variation (SPSS/Mplus: $\sigma_w^2 = 0.09/0.07$, $p < 0.001$); $\sigma_B^2 = 95.05/94.84$, $p < .001$; ICC = 0.999). The standard error of mean (SEM) level-and-difference model confirms between cluster variations in age with equal estimates in an intercept model. A nested model with the age variable constrained to be equal for all within controls and patients shows a better fit than the unrestricted model ($\chi^2 = 33.39$, $df = 24$, $p = 0.096$, root mean square error of approximation (RMSEA) = 0.031, $RMSEA_{close\ fit} = 0.91$; $\Delta\chi^2 = 3.48$, $\Delta df = 5$, $p = 0.63$). This model with control for the statistical stratification effect was only marginally different ($RMSEA_{close\ fit} = 0.92$). Both Mplus and SPSS Cox regression analysis gives identical estimates of the relation between age and time to death (0.09, $p < 0.001$). Mplus multilevel Cox regression analysis show no such within cluster relation between age and mortality (0.08, $p > 0.05$).

Group difference in mortality dependent on age: The interaction effect

A Cox regression analysis shows the mortality risk among patients relative to controls to be 2.61. After accounting for the variable age, this estimate is 2.67 (Exp(B), $p < 0.001$). When the interaction term with centered age variable is included, this group estimate is 3.31 ($p < 0.001$), which indicate the group mortality difference at mean age level. The hazard ratio of the interaction term was 0.96 ($p < 0.001$). Figure 1 and Appendix 1 illustrates how the interaction effect influences the survival plot, with stronger mortality difference for younger than older patients.

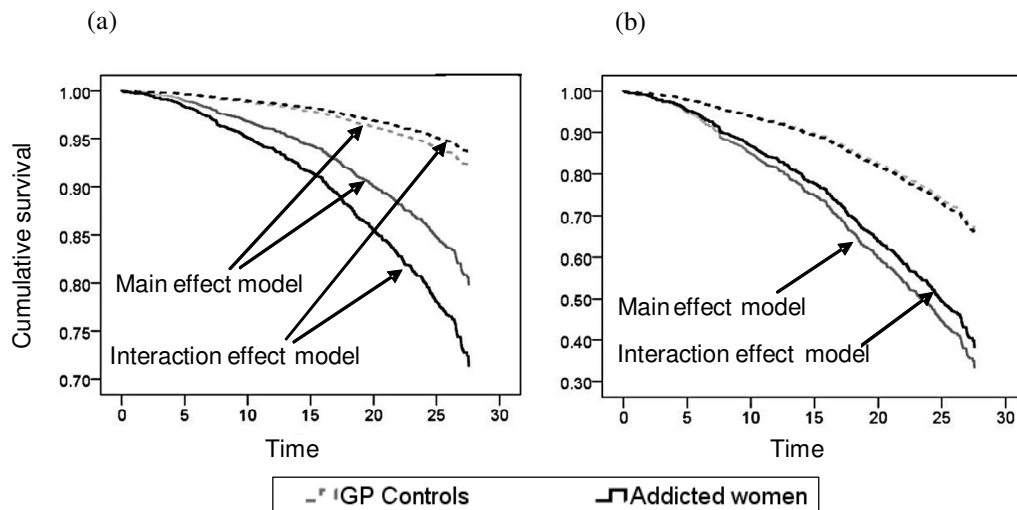


Figure 1. Age adjusted survival plots for addicted women and matched controls (MGPC). The plots are based on one standard Cox regression without interaction terms between group and age and one interaction model within this effect included. The expected survival is illustrated for younger and older women, set to (a) 30 or (b) 50 years.

Table 1. Survival analyses results for addicted women (ALC) and matched general population controls (MGPC) with age and group as time varying covariates. The variable age is centered.

Variable	b	Exp(B)	95% CI (lower)	95% CI (upper)	p
Group (ALC - MGPC)	1.161	3.19	2.56	3.98	***
Age	0.065	1.07	1.04	1.09	***
Age × Time	0.002	1.00	1.00	1.00	*
Age × Group × Time	-0.002	1.00	1.00	1.00	***

*p < 0.05 **p < 0.01, ***p < 0.001, b = unstandardized regression weight.

Analyzing group differences over a long follow-up time

Inspection of the log minus log plots of patients and controls as strata effects show parallel lines and confirm the assumption of proportional hazard. Further exploration of this assumption was done by entering the variables group and age (centered) as time dependent covariates. Table 1 shows age and the interaction between age and group to be statistically significant.

Since the coding for the patient group is one, the last time interaction effect in the table will even out the interaction effect of age and time. An increasing relative mortality risk for patients compared to controls is found among younger females over time, while relative mortality risk is decreasing among older women.

Latent discrete-time survival models based on these two-year intervals show identical results compared to the SPSS Cox regression analysis (Group = 1.21, age = 0.09, and Group × age = -0.04, all p-values < 0.001) (Model fit: LogLikelihood = -3150.41, akaike information criterion (AIC) = 6336.82, Bayesian information criterion

(BIC) = 6441.33). A multilevel latent discrete-time analysis, with the cluster variation in relationship between age and mortality accounted for, gives almost identical results. Another discrete-time survival model allows for direct group predictions of mortality within separate time intervals in addition to the already specified proportional hazard model accounted for by the latent factor. This shows the time interval 2 to 4 years to be statistically significantly predicted (b = 1.12, Exp(B) = 3.06, p < 0.05). This adds more evidence of non-proportionality in mortality between the groups over time. After accounting for educational level associations, the mortality ratio between cases and controls is found to be 3.65 (Exp(B)) for women at average age level and over all education levels. Education levels are found to be statistically significant related to mortality (Mplus results: Group = 1.30, age = 0.10, Group × age = -0.04, Edu_H1 = 0.32, and Edu_H2 = 0.28, all p-values < 0.01; Model fit: LogLikelihood = -2545.12, AIC = 5130.24, BIC = 5243.12).

In order to explore different mortality ratios in different time intervals, the latent variable is removed from the model. Table 2 shows no group differences for 3 intervals

Table 2. Prediction of mortality within discrete-time intervals (2 year). Predictors are group (addicted women versus matched general population control women - MGPC), age, and interaction between group and age. Fit statistics are given for full (M_0) and restricted (M_1) models with difference between these models.

Variable	Group		Age		G × A	
	b	OR	b	OR	b	OR
Time interval						
0-2	1.36*	3.91	0	1	-	
2-4	2.01***	7.45	0.04	1.05	-	
4-6	0.46	1.58	0.08**	1.08	-	
6-8	0.74*	2.1	0.09***	1.09	-	
8-10	1.87***	6.5	0.14***	1.15	-0.08*	0.92
10-12	0.85*	2.35	0.07**	1.07	-	
12-14	0.72	2.06	0.06**	1.06	-	
14-16	0.90*	2.46	0.11***	1.12	-0.09*	0.92
16-18	1.32***	3.76	0.08***	1.09	-	
18-20	1.17**	3.23	0.10***	1.1	-	
20-22	1.03**	2.79	0.08***	1.09	-	
22-24	0.62	1.86	0.10***	1.11	-	
24-26	1.41***	4.1	0.11***	1.12	-	
26-28	1.35**	3.84	0.02	1.02	-	
LogLikelihood	M_0	-3126.64	M_1	-3134.84	$\Delta M_1 M_0$	8.2
AIC		6367.28		6359.68		-7.6
BIC		6698.24		6477.98		-220.26

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. M_0 = full model, M_1 = restricted model without non-significant interaction effects, OR = odd ratio based on logistic regression, AIC: Akaike information criterion, BIC: Bayesian information criterion, b = unstandardized regression weight.

intervals and relatively large variation in the other time-dependent group estimates. Two interaction effects between group and age are found. The results from the four year interval model confirm the group mortality difference to be quite different in different periods (odds ratio: 1.82 to 5.88). Educational level contrast variables were added to the analyses of four year intervals. The difference between patients and matched controls is now statistically significant in all intervals except the second (4 to 8 years) and the interval 12 to 16 years (mortality estimates: 5.54, 1.00, 4.04, 1.00, 5.33, 3.09, and 4.64). The education level variables (Edu_H1 and H2) are statistically significant, related to mortality in the intervals 4 to 8 (Edu_H2 = 1.52) and in the interval 8 to 12 (Edu_H1 = 1.90 and Edu_H2 = 1.76).

DISCUSSION

The mortality ratio between patients and controls is found to be 2.3/2.9. The estimate is 2.67 when time to death and age is included in the analyses. The interaction result between group and age shows the risk estimate to be 3.31 at the average age level, while it is 3.65 when accounting for education levels. Higher educational level

is associated with lower mortality risk. Thus, educational level is a factor to include when analyzing patient and control difference in mortality (Rogers et al., 2010; Thygesen et al., 2008; Saarni et al., 2008). Discrete two and four year intervals show varying mortality ratios between the groups, with estimates up to 7.45. These findings illustrate how mortality estimates depend on how time and event related variables are treated and analyzed.

Clustered data

The small within cluster variation of age does not contribute statistically significantly regarding mortality. Finding equal results when accounting for the multilevel data structure are not obvious in all studies and such statistical models are well suited for checking how the matching procedure turned out. Significant within cluster variation in predictor levels and their relations with the outcome variable would indicate problems with this sampling procedure of matched controls. Multilevel analyses, controlling for cluster and stratification variations, give additional information about data (Muthén and Satorra, 1995).

Interaction effects

Mortality differences between patients and controls are found to be stronger for younger than older women. This finding illustrates the importance of considering the inclusion of interaction terms in time-to-event analyses and thereby account for important within group heterogeneity. A model including interaction effects may be misinterpreted, as the interpretation of main effects is changed in contrast to the model with main effects only. In interaction models, one main effect is tested when the other main effect is zero, while a model without interaction terms is testing one main effect over all levels in the other variable (Hair et al., 1998). Centering reduces the multicollinearity problem and makes the interpretation easier. In our case, the uncentered interaction model tested the group effect when age was zero, while in the centered interaction model the group difference was tested when age was at the average level. The last model is of course most relevant. However, the total model will in both cases give identical pictures, as main effects or lack of such effects only should be interpreted in relation to the interaction effect (Pedhazur and Schmelkin, 1991; Hair et al., 1998).

In the present study, age is important to include both as a main effect and in the interaction term with group membership. We have elsewhere documented reduced mortality for addicted women who received a specialized treatment relative to mortality among women who received "treatment as usual" (Gjestad et al., 2011). In that study, no effects were found without including interaction terms into the analyses. Then, a stronger mortality difference was found among younger than older women and early in the follow-up period than later on. This illustrates how an exclusive focus on the main effects not always gives the complete picture.

Time-dependent relations in long term follow-up intervals

Results from Cox regression analyses with time dependent covariates and discrete-time survival analyses show that the mortality difference between the groups is varying over time. These findings illustrate how non-proportional hazard models may give other results than proportional hazard models. The non-proportional hazard discrete-time survival analyses based on two-year intervals reveal that patients in our study do not differ from controls regarding mortality in the two-year interval after treatment, which could imply the possibility of a time limited treatment effect (Cuijpers et al., 2004). In this way, to specify a latent discrete-time survival analysis gives the possibility of analyzing the effect of a set of predictors directly on mortality in all time-intervals, the non-proportional

hazard model, in addition to the predictive relationship through the latent factor, giving the proportional hazard part of the model. This method increases the flexibility in model specification.

Conclusions

This paper illustrates how results obtained from mortality data are affected by the statistical procedures used. Differences in follow-up time, the selection of control samples, and the handling of variables contribute independently and together to reported mortality differences. Mortality estimates reported in epidemiological and clinical studies may be affected by factors that may be accounted for when groups are being made equal by matching variables (for example age, gender, and geographic location). However, other left out variables from the matching procedure may still contribute to some biases in the estimated risks. Applying different statistical models showed varying risk estimates, higher for the younger than for older women, and higher estimates early than later in the follow-up period. Thus, the overall estimate is only one way of reporting this group difference. Other studies have found mortality risks among women alcoholics to be about 6 (Dahlgren and Myrhed, 1977), 5 (Lindberg and Ågren, 1988), 5 (Berglund, 1984), 4 (Smith et al., 1994), and 3 (Schmidt and Popham, 1980). These studies did not use matched controls or control for confounding factors, they were of very different follow-up duration, and different statistical models were used. Such differences between studies come in addition to differences related to the samples involved as explanations for the findings and are relevant methodological aspects for other time-to-event analyses as well, for example treatment termination, relapse, drop-out, and hospital readmission.

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APPENDIX

Analysis syntax

Appendix 1. Age adjusted survival plots for addicted women and matched controls (MGPC). The plots are based on one standard Cox regression without interaction terms between group and age and one interaction model within this effect included. The expected survival is illustrated for younger and older women, set to 30 or 50 years.

A1: SPSS syntax: COX regression with interaction effects plotting survival for 30 and 50 years old subjects. Age is treated as a continuous variable.

```
COXREG TIME
/STATUS=DEAD(1)
/PATTERN age(30) BY Group
/PATTERN age(50) BY Group
/CONTRAST (Group)=Indicator(1)
/METHOD=ENTER Age Group Age*Group
/PLOT SURVIVAL
/PRINT=CI(95) CORR BASELINE
/CRITERIA=PIN(.05) POUT(.10) ITERATE(20).
```

A2: COX regression with time-dependent covariates

```
TIME PROGRAM.
COMPUTE T_COV = T_.
COXREG TIME
/STATUS=DEAD(1)
/METHOD=ENTER AGEc Group AGEc*Group
/method=enter AGEc*T_COV Group*T_COV
/method= enter AGEc*Group*T_COV
/PRINT=CI(95) CORR
/CRITERIA=PIN(.05) POUT(.10) ITERATE(20).
```

A3: Mplus Multilevel Cox regression

```
TITLE: Multilevel Cox regression
DATA: FILE = alc_mgpc_survival.dat;
VARIABLE:
NAMES = Case EWAnr Ewanr2 Sequence
Seq_2 Group Group8 Age G_x_A Age_L2
Age_L1 Dead Time ;
USEVARIABLES = Age_L1 Age_L2 Group
Dead Time EWAnr2 G_x_A ;

Cluster = EWAnr2 ;
Categorical = Group ;
within = Age_L1 ;
between = Age_L2 Group G_x_A ;
Survival = Time (ALL);
Timecensored = Dead (1 = NOT 0 = Right) ;
ANALYSIS:
Type = twolevel ;
Basehazard = off ;
MODEL:
%within%
Time on Age_L1 ;
%between%
Time on Age_L2 ;
Time on Group ;
Time on G_x_A ;
Output:
Sampstat ;
cinterval ;
```

A4: Mplus Time-discrete survival analysis

```
TITLE: Latent time-discrete survival model
DATA: FILE = survival.dat ;
VARIABLE:
NAMES = Case EWAnr Ewanr2 Sequence Seq_2 Group Group8 Age G_x_A
Age_L2 Age_L1 Dead Time D1-D14 DB1-DB7 ;
USEVARIABLES = D1-D14 Age ;
Categorical = D1-D14 ;
Missing = all (999) ;
```

ANALYSIS:
Estimator = MLR ;

MODEL:
f by D1-D14@1 ;
f on Age ;
f@0 ;

Output:
Sampstat ;
cinterval ;

The two-year intervals D1-D14 is coded 0 if subject is alive and 1 if a person dies in that actual period. After that point of time, intervals are coded missing data (999). D1-D14 is declared as categorical variables. The latent factor f with factor loadings pre-specified as 1 on all periods constitutes a proportional hazard time-discrete model. In this case, the survival function is regressed on the variable *age*.

The model may be expanded in order to include a multilevel time-discrete survival model including group, age and the interaction term group x age

```
MODEL:
%within%
D1-D14 on Age_L1 ;
%between%
F by D1-D14@1 ;
F@0 ;
F on Age_L2 Group G_x_A ;
D1-D14 on Group ;
```

Full Length Research Paper

Maternal and neonatal seroprevalence of hepatitis B surface antigen in a hospital based population in South-South, Nigeria

J. O. Alegbeleye, T. K. Nyengidiki* and J. I. Ikimalo

Department of Obstetrics and Gynaecology, College of Health Sciences, University of Port Harcourt, P. M. B. 5323 Choba, Rivers State, Nigeria.

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Despite the existence of a safe and effective vaccine, Nigeria has remained a hyper-endemic area for hepatitis B virus infection, with estimated 12% of the total population being chronic carriers. Vertical transmission is an important route of transmission for hepatitis B virus infection. Neonates who contact hepatitis B virus infection will have an almost 90% risk of developing chronic hepatitis B surface antigen (HBsAg) carrier state and chronic liver disease. The objectives of this study was to determine the sero-prevalence of hepatitis B Virus among pregnant women, rate of vertical transmission and identifying potential risk factors associated with the infection. This study was an observational cross-sectional study of 250 pregnant women who presented to the labour ward of the University of Port-Harcourt Teaching Hospital (UPTH). Blood samples from all consenting pregnant women and corresponding umbilical cord blood were collected at delivery. A structured proforma designed for this purpose was used to obtain socio-demographic information and the presence of risk factors. Data collated was analysed using Statistical Package for Social Sciences (SPSS) 17.0 for windows[®] statistical software with $P < 0.05$ at 95% confidence interval. The mean age of the pregnant women studied was 33.5 ± 0.8 years, while the mean parity was 1.58 ± 0.5 . HBsAg was detected in 15 women, giving a seroprevalence rate of 6% and a neonatal seroprevalence rate of 3.2%. All HBsAg-positive babies were born to HBsAg-positive mothers with a vertical transmission rate of 53.3%. Hepatitis B virus infection amongst parturients was more in patients with history of termination of pregnancy and multiple sexual partners ($P < 0.05$). An intermediate prevalence of hepatitis B virus infection was identified which justifies the need for routine screening in pregnancy in order to identify and treat the infection, thus reducing the risk of vertical transmission of the virus. Contraceptive options aimed at prevention of pregnancy and sexually transmitted infection (STI) should be encouraged.

Key words: Hepatitis B surface antigen (HBsAg), seroprevalence, University of Port-Harcourt Teaching Hospital (UPTH), Nigeria.

INTRODUCTION

Hepatitis B virus (HBV) infection is a serious global public health problem and is endemic in Africa, including Nigeria with the viral antigen initially called Australian antigen (Weinbaun et al., 2008; Andre, 2004; Dane et al., 1970). HBV is the prototype member of the Hepadnaviridae

(hepatotropic DNA virus) family with virions which are double-stranded particles, measuring 40 to 42 nm in diameter with an outer lipoprotein envelope that contains three related envelope glycoproteins (or surface antigens) (Uyar et al., 2009; Hinnachi et al., 2009). The infection

*Corresponding author. E-mail: tammynyengs@yahoo.com. Tel: +2348037109486.

can be acute or chronic, while adults that acquire acute infection usually recover or can be managed by supportive therapy, the chronic type is ultimately fatal (Shepard et al., 2006). Diagnosis of HBV infection is usually through serological and virological markers. Hepatitis B surface antigen (HBsAg) is the hallmark of HBV infection and is the first serological marker to appear in acute HBV infection, and persistence of HBsAg for more than 6 months suggests chronic HBV infection (Kao, 2008). Globally, over 2 billion people are infected with the virus and over 350 million have chronic infection (Eke et al., 2011). Infection with this virus does not only leads to acute illnesses, but chronic illnesses like liver cirrhosis and hepatocellular carcinoma which accounts for more than 1 million deaths globally (Eke et al., 2011; El-Magrahe et al., 2010). The prevalence of hepatitis B virus infection is relatively high in Africa, having the second highest number of chronically HBV-infected individuals (Mbaawuaga et al., 2008).

Acute HBV infection occurs in 1 to 2 of every 1000 pregnancies with 1 to 5% of pregnant women being chronic carriers of HBV (Ugwuja, 2010). If the acute maternal infection occurs in the first trimester and resolves, the risk of neonatal infection is minimal. However, an infection during the second and third trimester poses a threat of 10 and 90%, respectively for vertical transmission (Obi et al., 1993).

The risk of transmission depends on the degree of maternal infectivity and the genomic type of the virus (Ezegbudo et al., 2004). These babies are at serious risk of developing chronic liver disease, cirrhosis of the liver and hepatocellular carcinoma in later life and up to 25% of them will die as adults due to liver disease. (Baba et al., 1999). There are three mechanisms of HBV transmission from HBsAg-positive mothers: (i) trans-placental intra-uterine transmission; (ii) transmission during delivery by contact with maternal infected fluids in the birth canal; and (iii) post natal transmission from mothers to infants during child care or through breastfeeding (Chen and Chang, 2010). Vertical transmission has been reported in 90% of infants born to HBsAg positive mothers (Pennap et al., 2011; Akani et al., 2005).

Nigeria is classified among the group of countries endemic for HBV infection with a current infected population of 18 million (Ojo and Anibijuwon, 2009). Despite the existence of a safe and effective vaccine, Nigeria has remained a hyper-endemic area for HBV infection, with an estimated 12% of the population being chronic carriers (Jatua and Yabaya, 2009). Sero-prevalence studies on HBsAg in Nigeria have shown that the prevalence of the infection in pregnant women range from 2 to 15.8% (Ojo and Anibijuwon 2009; Ndams et al., 2008; Ducan et al 1995; Candotti et al., 2007). Even though studies have been carried out on HBV infection in different parts of Nigeria, and in different sub-groups of individuals, the prevalence among pregnant women and information regarding the vertical transmission rate is scanty from the South-South region of the country. Since

most transmission occur intra-partum, this study is aimed at determining the sero-prevalence of HBsAg at delivery and the rate of vertical transmission. It will also show the risk factors associated with maternal infection.

Objectives

The aim of this study is to determine the maternal and neonatal seroprevalence of HBsAg amongst parturients. The specific objectives are: (1) to find out the prevalence of HBV in pregnant women presented in labour to the University of Port Harcourt Teaching Hospital; (2) to identify known risk factors for Hepatitis B viral infection in parturients; (3) to determine the sero-prevalence of HBV in babies delivered at UPTH.

METHODOLOGY

Study area

This study was carried out at the labour ward of the University of Port Harcourt Teaching Hospital in River State of Nigeria from 5th September to 5th December 2011.

Study design

This was an observational cross-sectional study involving all pregnant women presented in labour to the University of Port-Harcourt Teaching Hospital. The purpose of the study was duly explained to the participants prior to the study at the antenatal clinic and an informed consent was obtained, this was indicated on their antenatal cards. When they presented in labour, their socio-demographic characteristics and risk factors for HBV infection were documented in a structured proforma for each participant.

Inclusion criterion

The inclusion criteria for the study was any pregnant woman who gave consent to participate in the study.

Exclusion criteria

Those excluded from the study are: patients who withheld their consent for inclusion in the study; those immunized against hepatitis B infection (information obtained by verbal confirmation of previous immunization); and patients with HIV comorbidities were excluded.

Sample size

Sample size calculation was done using the Fish formula with the prevalence of hepatitis B at 15.8% (Baba et al., 1999). with an error margin (d) of 0.05.

$$N = Z^2 P (1-P) / d^2$$

The minimum sample size was thus calculated to be 206 with a allowance of an attrition rate of 20%. However, a total of 250 consecutive women who presented in labour were used for the study. In a

Table 1. Socio-demographic characteristics of the women.

Characteristic	n	%
Age (years)		
20-24	24	9.6
25-29	112	44.8
30-34	81	32.4
35-39	31	12.4
>40	2	0.8
Parity		
Para 0	107	42.8
Para 1-4	141	56.4
Para>5	2	0.8
Occupation		
Civil servant	22	8.8
Public servant	59	23.6
Business woman	68	27.2
Housewife	73	29.2
Student	28	11.2
Educational status		
Primary	6	2.4
Secondary	60	24.4
Tertiary	184	73.6

pilot study conducted, an average of 10 women were admitted in the labour ward on a daily basis. Patients presenting to the labour ward who had given consent for the study were recruited as they presented.

Five milliliters of blood from the pregnant woman (coded M) and same volume of corresponding umbilical cord blood (coded C) were collected at delivery into plain specimen containers (without anticoagulants). The blood samples were then left to stand for about 5 min to obtain serum. The ACON HBsAg *in-vitro* diagnostic test strips were used for the test (ACON Laboratories, USA). With the arrows pointing downwards, the ACON strip was immersed into the serum for 10 to 15 s. The maximum line (MAX) on the strip was observed and the strip was then placed on a non-absorbent surface, the timer was set for 15 min, awaiting the red lines to appear. Two distinct red lines appeared, one on the control region (C) and the other red line on the test (T) region. The intensity of the red line varied depending on the concentration of the HbsAg in the specimen. Therefore, any shade of red line in the test (T) region was considered positive, one red line on the control (C) region and no shade of red colour on the test (T) region was considered as negative. The test was invalid if the control line failed to appear and was discarded.

Data management and analysis

All participants' proforma was collected at the end of every day from the labour ward and data entry errors were checked at the labour ward before collation. The forms were checked for completeness at the end of each day.

Data entry and statistical analysis were done using statistical software (SPSS for windows® version 17.0, SPSS Inc.; Chicago, USA). Chi square test was used for categorical variables. Results

were presented as frequency tables and $P < 0.05$ at 95% confidence interval.

Ethical consideration

Ethical approval for this study was obtained from the ethical review board of the hospital. A written informed consent was also obtained from every participating woman.

RESULTS

Two hundred and fifty women were enrolled into the study during the three month period from September 5 to 5th December, 2011. Of these, 15 tested positive to HBsAg, giving a sero-prevalence rate of 6% in the study population.

The age range of the women studied was 20 to 45 years. The mean age of the pregnant women was 33.5 ± 0.8 years, with majority of the women in the 25 to 29 years age group constituting 44.8% of the total population. 184 (73.6%) of the women had tertiary level of education while 60 (24.4%) and 6 (2.4%) had secondary and primary level of education, respectively. About one-third, 73 (29.2%) of the women in the study population were housewives (Table 1).

Figure 1 shows the sero-prevalence rate for HBsAg against age in the study population, the prevalence was the highest among the 20 to 24 years age group, where 3 out of the 24 (12.5%) women tested positive to HBsAg and the least prevalence was seen in the 30 to 34 years age group where only 2 (2.5%) out of the 81 women tested positive to HBsAg. Figure 2 shows the relationship between parity and HBV infection in the study population. 141 (56.4%) were multiparous, 107 (42.8%) were nulliparous, while 2 (0.8%) were grand multiparous. Eleven of the 15 women that were sero-positive to HBsAg were nulliparous (73.3%) while the remaining 4 were multiparous (26.7%). The mean parity was 1.58 ± 0.5 . There was an inverse relationship between parity and HBV infectivity, which was statistically significant ($P = 0.04$).

The women's occupation as a risk factor to the acquisition of HBV showed that the highest prevalence was seen in civil servants where 2 (8%) of the 25 women in this group were sero-positive for HBsAg (6.8%) and the least prevalence was seen in public servants (3.4%).

All the participants in the study were married with 243 (97.2%) in monogamous and 7 (2.8%) in polygamous marriages. All 15 women that were positive to HBsAg were in monogamous marriages. 117 (46.8%) of the women gave a history of termination of pregnancy in the past, while 133 (53.2%) had no termination of pregnancy. Among those who had a history of termination of pregnancy, 11 (9.4%) tested positive to HBsAg; this was statistically significant ($P = 0.04$).

Table 2 highlights the relationship between the risk factors and HBsAg positivity. Eight out of the 15 women that were positive to HBsAg had 3 to 4 sexual partners in the past ($P = 0.01$), while the remaining 7 had 1 to 2 sexual

Table 2. Prevalence of HbsAg associated with risk factors.

Variable	Number	HBSAg (+)	HBSAg (-)	P value	Relative risk
Risk factor					
Induced abortion	117	11	106	0.04	3.13
Dental procedure	62	3	59	0.66	0.76
Previous c/s	62	2	60	0.31	0.47
Tattoo/tribal marks	56	4	52	0.68	1.26
Previous surgeries	53	5	48	0.24	1.86
IV drug use	44	1	43	0.28	0.33
Blood transfusion	21	2	19	0.48	1.86
Monogamous marriage	243	15	228	0.99	1.02
Polygamous marriage	7	0	7	0.99	0.98
No. of sexual partners					
1-2	191	7	184	0.01	0.27
3-4	55	8	47	0.01	4.01
>4	4	0	4	0.73	1.59

*Statistically significant; P < 0.05.

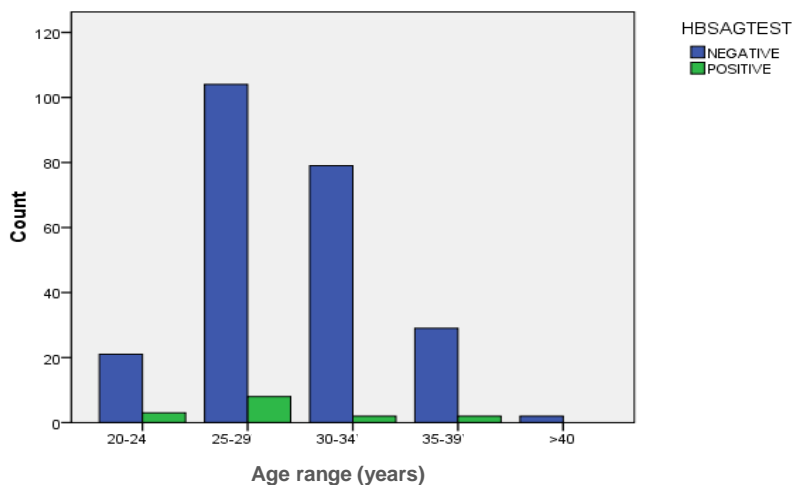


Figure 1. Seroprevalence of HBsAg status according to age group.

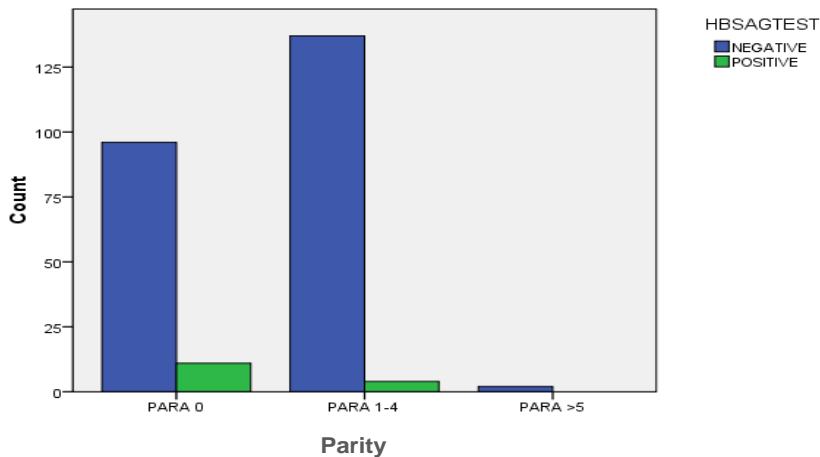


Figure 2. Seroprevalence of HBsAg status according to parity.

sexual partners ($P=0.01$). Of the 21 women with a previous history of blood transfusion, 2 tested positive to HBsAg, this was not statistically significant ($P=0.48$). Forty-four women gave a history of intravenous drug use, of these, one was positive to HBsAg. This was not statistically significant ($P=0.28$). Sixty two of the women gave a history of previous dental procedure; of these 3 were sero-positive to HbsAg ($P=0.66$), while 5 of the 53 women with previous surgical procedures were sero-positive to HBsAg ($P=0.24$). It was noted from this study that previous caesarean section, type of marriage and scarification marks or tattoos were not significantly associated with HBV infection ($P=0.31$ and 0.68 , respectively).

Eight out of the 15 babies born to HBsAg-positive mothers were positive to HBsAg, giving a neonatal seroprevalence rate of 3.2% (8/250) and a vertical transmission rate of 53.3% (8/15).

DISCUSSION

The study showed that pregnant women in labour have an intermediate endemicity of 6% for HBV infection according to the world Health organization (WHO) classification for HBV infection (Chen and Chang 2010). This is similar to results obtained in Keffi, Nigeria (Ndam et al., 2008), but higher than some previous studies in Nigeria (Pennap et al., 2011; Akani et al., 2005 Ojo and Anibijuwon 2009). The highest prevalence of HBV infection was found among pregnant women of 20 to 24 years of age and the lowest among those of 30 to 34 years of age. These could be explained by the relationship between hepatitis infection and high risk sexual practices which is noted to be higher amongst the younger age group. Similar results were obtained in Zaria and Ethiopia (Jatau and Yabaya, 2009; Awole and Gebre-Selassie 2005).

Inverse relationship between educational status and HbsAg positivity with less educated women showing the highest positivity had been noted (Eke et al., 2011; Pennap et al., 2011; Ndams et al., 2008). However, in this study, it was noted that educational attainment did not reduce the risk factors for transmission of the infection. This brings to light the need for focused education on prevention of high risk behaviors amongst the educated. One would have expected HBsAg prevalence to be higher in multiparous women, because of repeated risk of exposure to contaminated surfaces and instruments during delivery (Pennap et al., 2011). However, the reverse was the case as the prevalence was significantly higher in nulliparous women. The reason for this may be because most of the nulliparous women (65.4%) had had a termination of pregnancy and had more number of sexual partners in the past which could have exposed them to the virus as compared to the multiparous women in monogamous relationships.

This study showed an intermediate prevalence of

HBsAg among the pregnant women, yet, only few of the known predisposing factors to HBV infection showed statistically significant association. Some of the predisposing factors in previous studies in this environment are no longer applicable because tribal marks, tatoos, reusable needles and surgical blades are no more common practice, particularly in highly educated population in the urban centre like in this study. Abortion is related unprotected to sexual intercourse which results in unplanned pregnancies and also increases the risk of HBV infection if such partners are infected (Duncan et al., 1995). Also, instrumentation during abortion and related activities may serve as sources of exposure since most terminations are done by unskilled persons using contaminated instruments and surfaces (Awole and Gebre-Selassie 2005). This study found that HBsAg positivity was significantly higher in patients who had previous termination of pregnancy ($RR=3.13$; $P<0.05$). This finding is similar to the report from Ethiopia (Awole and Gebre-Selassie 2005).

Policies aimed at reducing the incidence of unsafe abortions and promotion of barrier contraception in the environment may assist in reducing the incidence of this condition. An obvious observation of the four fold risk of acquiring hepatitis B infection amongst women having 3 to 4 sexual partners was noted in this study ($RR=4.05$; $P<0.05$). This brings to light the need to advice strongly against such high risk behaviours amongst parturients. Several studies have shown that vertical transmission of HBV can occur. (Candotti et al., 2007; Chakravati et al., 2005; Wiseman et al., 2009).

In this study, all the babies that tested positive to HBsAg were born to HBsAg positive mothers. The reported variable vertical transmission rates from HBsAg-mothers to their infants depend on several factors, namely viral load, HBV variants, HBV DNA levels, HBsAg-positivity, HBV genotype, sensitivity and accuracy of diagnostic tests (Rumi et al., 1998; Wiseman et al., 2009). These factors correlate with the level of infectivity of the mother in transmitting the virus to the fetus which may explain why some of the babies born to the HBsAg-positive mothers tested negative.

This study has some limitations and issues attracting criticisms. The tool of laboratory analysis, serology by rapid test kit, is less sensitive than amplification assays (liquid phase hybridization, antibody capture approach, branched DNA) and DNA amplification tests based on the polymerase chain reaction which are now considered the gold standard in the diagnosis of HBV infection. These tests are expensive and are not available in most centers. Nevertheless, rapid tests can be used as a screening tool in order to identify those women that would require confirmation of their status and further management.

Conclusion

In order to determine the extent of perinatal transmission

of HBV, the prevalence of HBsAg which is a serum marker for active viral replication among the HBsAg carrier mothers should be determined in a further study. Preventive measures should be taken against unwanted pregnancies, sexually transmitted infections and multiple sexual partners as these are all routes of transmission of the virus. Sensitization on risk factors and routine screening for HBV in pregnancy is strongly advocated to reduce morbidity and treatment of HBV infection in pregnancy to reduce mother-to-child transmission.

Availability and accessibility of hepatitis B immunoglobulin to babies born to HBsAg-positive mothers by Government and Non-governmental organizations is also suggested.

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