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ORIGINAL ARTICLE

Metabolic syndrome among Ghanaian patients presenting with chronic kidney disease

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Metabolic syndrome (MetS) is a general risk factor for cardiovascular and chronic kidney disease (CKD) in Western populations. This study assessed the relationship between MetS and its components in Ghanaian patients presenting with CKD. The study population comprised of 146 non-dialysed individuals with CKD with mean age of 50.2±1.1 years. Eighty (80) age and sex matched healthy participants without kidney pathology were used as controls. Estimated GFR (eGFR) was calculated using the 4-variable Modification of Diet in Renal Disease (*4v*-MDRD) and CKD was defined as eGFR<60 ml/min/1.73m². MetS was defined as the presence of three or more of the following risk factors according to the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) criteria: elevated blood pressure (BP), low high density lipoprotein cholesterol (HDL-C), high triglycerides (TG), elevated plasma glucose and abdominal obesity. The prevalence of MetS in this study was 30.1% and a significant relationship was observed between the number of MetS components and the presence CKD. The CKD group are about 3 times at risk of developing MetS as compared to the control group (95% CI=0.9-8.8). Female participants with CKD are 9 fold at risk of developing MetS as compared to the male counterparts (95% CI=1.7-47.9). The CKD patients were about 2 fold at risk of developing hypertension (95% CI=1.7-3.3) and diabetes (95% CI=1.2-2.6), about 3 times at risk of developing hypertriglyceridaemia (95% CI=1.1-5.5) and approximately 4 times at risk of developing proteinuria (95% CI=2.7-7.0). Increased WC, TG and SBP are components of the metabolic syndrome which contribute to the initiation and progression of CKD.
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Keywords*:* Metabolic syndrome, diabetes, dyslipidaemia, obesity, chronic kidney disease

INTRODUCTION

Chronic kidney disease (CKD) has become a global public health concern due to its increasing prevalence (Coresh *et al.*, 2003) and the associated increase in risk of end-stage kidney disease (ESKD), cardiovascular disease (CVD) and untimely deaths (Muntner *et al.*, 2002; National Kidney Foundation, 2002). Identifying and treating risk factors for development of CKD may therefore be the best approach to preventing and/or delaying adverse outcomes (National Kidney Foundation, 2002).

MetS, characterized by a clustering of abdominal obesity, hypertriglyceridaemia, low high-density lipoprotein cholesterol (HDL-C), elevated blood pressure (BP), and high fasting blood glucose (FBG), has been associated with an increased risk for the development of diabetes and CVD as well as an increased mortality from CVD and all causes (Ford, 2005; Reynolds and He, 2005). The National Cholesterol Education Program Adult Treatment

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Panel (NCEP-ATP III) criteria defines MetS as having at least three of the following: abdominal obesity; high triglyceride levels; low high-density lipoprotein (HDL) cholesterol; hyperglycaemia; and hypertension (NCEP, 2001).

MetS is important for several reasons: (a) it is one of the causes of CKD (Kambham *et al.*, 2001), (b) it can be treated at lower cost if detected early and (c) it is a predictor of CVD (Iseki *et al.*, 2004). A few epidemiological studies among the global adult population especially in the United States of America have reported that MetS is associated with CKD and microalbuminuria (Chen *et al.*, 2004; Kurella *et al.*, 2005). Growing economic development over the years has led to changes in lifestyle and diet, and consequently an increased prevalence of obesity in Ghana. Thus, MetS with its association to obesity is expected to be even more prevalent now and in the future. However, there is paucity of data on the relationship between MetS and CKD. The aim of the present study therefore was to establish the relationship between MetS and CKD in the Ghanaian population.

MATERIALS AND METHODS

Study area and subjects

This study was carried out at the Komfo Anokye Teaching Hospital (KATH), Kumasi and the Tamale Teaching Hospital (TTH) between August 2007 and September 2009. One hundred and forty six (146) patients comprising eighty (80) females and sixty-six (66) males within the age range of 20-80 years were recruited into the study after the objectives of the study had been clearly explained to them in English and/or the local dialect. Patients with clinically diagnosed CKD who were yet to commence dialysis were randomly selected for the studies with patients on any form of dialysis being excluded from the study.

The aetiology of the CKD ranged from diabetic nephropathy, 90(61.6%) patients; chronic glomerulonephritis, 12(8.2%) patients; adult polycystic kidney disease, 1(0.7%) patient; hypertensive nephropathy, 10(6.8%) patients and chronic kidney disease of unknown aetiology, 33(22.6%) patients. Eighty (80) healthy volunteers of similar age and sex distribution were studied as controls. The participation of the respondents who are all indigenes of Ghana was voluntary and informed consent was obtained from each of them. The study was approved by the School of Medical Sciences and the Komfo Anokye Teaching Hospital Committee on Human Research, Publication and Ethics (SMS/KATH/CHRPE).

Sample collection

Venous blood samples were collected after an overnight fast (12–14 hours), between 7 am and 10 am. About 5 ml of venous blood was collected out of which three 3 ml was dispensed into vacutainer® plain tubes and 2 ml into fluoride oxalate tubes. After centrifugation at 500 g for 15 min, the serum and plasma were stored at - 80°C until assayed.

Biochemical assays

Serum Biochemistry was performed with ATAC® 8000 Random Access Chemistry System (Elan Diagnostics, Smithfield, RI, USA). Parameters that were determined include; fasting blood glucose (FBG), serum creatinine (CRT), total cholesterol (TC), triglycerides (TG) and high density lipoprotein cholesterol (HDL-C). Serum low density lipoprotein cholesterol (LDL-C) was calculated using the Frederickson-Friedewald's formula (Friedewald *et al.,* 1972). The methods adopted by the automated instrument for the estimation of the above parameters was according to the instructions provided by the reagent manufacturer-JAS™ diagnostics, Inc. (JAS Diagnostics, Inc. Miami Florida, USA). TC determination was according to the method described by Trinder (Trinder, 1969). TG determination employed a modified Trinder method (Trinder, 1969; Barham and Trinder, 1972). LDL-C determination: LDL-C (mmol/l) was calculated according to Friedwald's formula in accordance with the manufacturer's instructions i.e. LDL_C=TC - TG⁄2-HDL_C.

Urine protein estimation

Early morning urine was collected in plastic containers from the respondents and urine protein was determined using the dip-stick qualitative method

(CYBOW™ DFI Co Ltd, Gimhae-City, Republic of Korea).

Anthropometric variables

Anthropometric measurements included height to the nearest 0.5 cm without shoes and weight to nearest 0.1 kg in light clothing. Subjects were weighed on a bathroom scale (Zhongshan Camry Electronic Co. Ltd, Guangdong, China) and their height measured with a wall-mounted ruler. Body mass index (BMI) was calculated by dividing weight (kg) by height squared (m^2). Waist circumference (WC) (to the nearest centimetre) was measured with a Gulick II spring-loaded measuring tape (Gay Mills, WI) midway between the inferior angle of the ribs and the suprailiac crest. Blood pressure was measured by trained personnel using a mercury sphygmomanometer and a stethoscope. Measurements were taken from the left upper arm after subjects had been sitting for >5 minute in accordance with the recommendations of the American Heart Association (Kirkendall et al., 1967). Duplicate measurements were taken with a 5 minute rest interval between measurements and the mean value was recorded to the nearest 2.0 mmHg.

Estimation of GFR

The 4-variable Modification of Diet in Renal Disease (4v-MDRD) equation was used to estimate the GFR of both participants with CKD and controls using serum CRT. This equation has been found to be the most accurate among the renal function equations in CKD applicable to Ghanaians (Owiredu et al., 2008). The eGFR result from the equations was used to stratify the study population into five categories corresponding with the five stages of CKD in the Kidney Disease Outcome Quality Initiative (K/DOQI) classification (NKF/KDOQI™, 2002). The staging classified GFR ≥ 90 ml/min/1.73 m^2 as stage 1; 60-89 ml/min/1.73 m^2 as stage 2; 30-59 ml/min/1.73 m^2 as stage 3; 15-29 ml/min/1.73 m^2 as stage 4; and < 15 ml/min/1.73 m^2 as stage 5.

Definitions

CKD defined as eGFR<60 ml/min/1.73m2.

MetS was defined according to the criteria of the National cholesterol education program, adult treatment panel III (NCEP ATP III) to include individuals with three or more of the following five components: (1) abdominal obesity- (waist circumference > 102 cm for men, or > 88 cm for women); (2) high TG ≥ 1.7 mmol/L (150 mg/dl); (3) low HDL-C : men < 0.9 mmol/L (< 40 mg/dl) or women < 1.0 mmol/L (< 50 mg/dl); and (4) High BP (systolic BP ≥ 130 mmHg or diastolic BP ≥ 85 mmHg or treatment of hypertension); and (5) high fasting glucose ≥ 6.1 mmol/l (NCEP, 2001).

Statistical analysis

The results are expressed as Means ± SEM. Unpaired t-test was used to compare mean values of continuous variables and χ^2 was used to compare discontinuous variables. A level of $p<0.05$ was considered as statistically significant. MetS (or its components) and other known risk factors for CKD were included in the model. Odds ratio (OR) (with 95% CI) of CKD by the number of metabolic risk factors were calculated. GraphPad Prism version 5.00 for windows was used for statistical analysis (GraphPad software, San Diego California USA, www.graphpad.com).

RESULTS

General characteristics of the study population

Table 1 represents the general characteristics of the study population. Participants with CKD had significantly higher levels of urine protein, serum creatinine and lower levels of estimated GFR as compared to the control subjects; however there was no significant difference between the ages of the cases and controls. The mean values of most components of the metabolic syndrome were significantly higher when the CKD group were compared to the control group i.e. the CKD group had significantly higher WC, had higher blood pressure [systolic blood pressure (SBP) and diastolic blood pressure (DBP)], higher fasting blood glucose (FBG) and had higher lipid levels (i.e. TG and TC) than the control group (Table 1). When CKD patients were stratified according to the presence or absence of the MetS, those with MetS were significantly older, had higher SBP, and higher levels of TG compared to those without MetS. The mean value of HDL-C was significantly lower among those with MetS

Table 1: General characteristics of study population with and without metabolic syndrome

Parameters	Control (n=80)	CKD (n=146)	MetS		Gender	
			MetS+CKD (n=44)	MetS-CKD (n=102)	CKD-Female (n=80)	CKD-Male (n=66)
Age (yrs)	46.3 ± 1.9	50.2 ± 1.1	61.0 ± 2.6	44.0 ± 1.6††	46.2 ± 2.3	48.1 ± 1.7
BMI (kg/m²)	24.6 ± 0.8	24.4 ± 0.4	27.6 ± 1.3	24.8 ± 0.5†	26.2 ± 0.9	24.3 ± 0.6
WC (cm)	74.1 ± 1.7	85.0 ± 1.4*	89.4 ± 3.1	82.3 ± 1.6†	84.6 ± 2.2	84.0 ± 1.9
SBP (mmHg)	120.7 ± 1.8	140.4 ± 3.8***	154.5 ± 4.3	135.6 ± 2.4†	144.7 ± 3.5	136.5 ± 2.8
DBP (mmHg)	70.4 ± 1.2	90.3 ± 2.6***	98.2 ± 2.7	87.3 ± 1.7†	93.4 ± 2.5	87.7 ± 1.8
PRT (g/l)	0.04 ± 0.02	1.2 ± 0.2***	0.7 ± 0.2	1.1 ± 0.2	1.2 ± 0.4	1.2 ± 0.3
CRT (µmol/l)	105.9 ± 3.9	268.0 ± 25.6***	371.2 ± 82.6	353.9 ± 47.5	221.8 ± 25.0	325.3 ± 47.4
FBG (mmol/l)	5.3 ± 0.2	8.7 ± 0.3***	7.8 ± 0.5	6.9 ± 0.3	6.8 ± 0.5	7.2 ± 0.6
HDL-C (mmol/l)	1.3 ± 0.05	1.6 ± 0.2	1.1 ± 0.1	1.4 ± 0.1††	1.4 ± 0.1	1.3 ± 0.1
TG (mmol/l)	1.5 ± 0.1	1.8 ± 0.1*	2.7 ± 0.1	1.9 ± 0.1†	1.8 ± 0.2	2.2 ± 0.3
TC (mmol/l)	4.5 ± 0.1	5.3 ± 0.3*	5.6 ± 0.2	5.3 ± 0.2	5.4 ± 0.4	5.3 ± 0.4
eGFR (ml/min/1.73 m²)	92.4 ± 5.7	57.6 ± 4.1***	99.7 ± 13.4	89.3 ± 6.9	50.2 ± 4.1	66.8 ± 7.6§
Prevalence of MetS	3 (3.75%)	44 (30.1%)			29(36.2%)	15 (22.7%)

BMI = Body mass index, WC= Waist circumference, SBP = Systolic blood pressure, DBP = Diastolic blood pressure, PRT = Proteinuria, CRT = Creatinine, TC = Cholesterol, HDL-C = High density lipoprotein, TG = Triglyceride, FBG = Fasting blood glucose, eGFR = estimated glomerular filtration rate, MetS = Metabolic syndrome. *p<0.05, **p<0.01, ***p<0.001; †p<0.05, ††p<0.01; §p<0.05 when the groups were compared.

compared to those without MetS. Furthermore, when the CKD patients were classified by gender, the female subjects had significantly lower estimated GFR compared to the control group. The risk of developing MetS is similar among both sexes (Table 1).

Relative risk of developing MetS risk factors

Table 2 represents the odds ratios of MetS risk factors in CKD stratified by the presence or absence of MetS and gender. When compared with the control subjects, the CKD patients were about 9 fold at risk of developing hypertension (95% CI = 3.1- 25.1) and diabetes (95% CI = 4.7-18.2), about 2 times at risk of developing hypertriglyceridaemia (95% CI = 1.3-4.2) and approximately 4 times at risk of developing low HDL (95% CI= 1.5-13.4). The risk of developing proteinuria is several folds in the CKD patients compared to the controls (OR=409; 95% CI = 24.7-6759).

When the CKD patients were stratified based on the presence or absence of metabolic syndrome, those with MetS were about 7 times at risk of developing hypertension (95% CI = 2.9-16.8), obesity (95% CI = 2.8-16.0) and proteinuria (95% CI = 3.0-16.4) and 3 times at risk of developing diabetes (95% CI = 1.2-6.4) (Table 2). Furthermore, the risk of developing hypertriglyceridaemia is several folds among those with MetS compared to those without MetS (OR = 18.2; 95% CI = 5.2-63.6). The risk of developing obesity (OR = 0.2; 95% CI = 0.1-0.6) and proteinuria (OR = 0.4; CI = 0.2-0.8) is less pronounced in the males compared to the females (Table 2).

Comparison between patients with increasing number of comorbidities

The comparison between patients with increasing comorbidities is shown in Figure 1. Comorbidity was defined as the presence of one or more risk factors of MetS. Participants with greater number of comorbidities (≥3) also had higher WC ($F_{3,46}$ = 2.878; p =0.046), BMI ($F_{3,46}$ = 4.112; p=0.010) and SBP levels ($F_{3,43}$ =2.546; p=0.048). For those having zero, one or two comorbidities, the WC levels were 68.1±4.7 m, 86.4±2.5 m and 86.6±5.3 m respectively. The BMI levels were 19.2±1.0 kgm^{-2}, 27.3±1.2

Table 2: Odds Ratios of MetS risk factors in CKD stratified by presence/absence of MetS or gender

Variables	Raised BP	Raised FG	Obesity	Raised TG	Reduced HDL-C	Proteinuria
Control (n=80)	4/80(5.5%)	14/80(17.5%)	13/80(16.2%)	22/80(27.5%)	4/80(5.0%)	0/80(0.0%)
CKD (n=146)	45/146(30.8%)	97/146(66.4%)	36/146(24.6%)	69/146(47.2%)	28/146(19.2%)	105/146(72.0%)
OR(95% CI)	8.9(3.1- 25.1)***	9.3(4.7-18.2)***	1.7(0.8-3.4)ns	2.3(1.3-4.2)**	4.5(1.5-13.4)**	409(24.7-6759)***
Stratified based on metabolic syndrome						
CKD-MetS (n=102)	31/113(27.4%)	43/113(38.0%)	17/113(15.0%)	40/113(35.3%)	30/113(26.5%)	28/113(24.8%)
CKD+MetS (n=44)	24/33(72.8%)	21/33(63.6%)	18/33(54.5%)	30/33(90.9%)	12/33(36.3%)	23/33(69.7%)
OR(95% CI)	7.0(2.9-16.8)***	2.8(1.2-6.4)*	6.8(2.8-16.0)***	18.2(5.2-63.6)***	1.6(0.7-3.6)	7.0(3.0-16.4)***
Stratified by gender						
CKD+Female (n=80)	25/80(31.2%)	52/80(65.0%)	28/80(35.0%)	45/80(56.2%)	16/80(20.0%)	64/80(80.0%)
CKD-Male (n=66)	20/66(30.3%)	45/66(68.2%)	8/66(12.1%)	34/66(51.5%)	16/66(24.2%)	40/66(60.6%)
OR(95% CI)	0.9(0.5-1.9)ns	1.1(0.6-2.3)ns	0.2(0.1-0.6)**	0.8(0.4-1.6)	1.6(0.7-3.5)	0.4(0.2-0.8)*

HDL-C = High density lipoprotein cholesterol, CKD = Chronic kidney disease, OR = Odds ratio, CI = Confidence interval, BP = Blood pressure, FG = Fasting glucose, TG = triglyceride, CKD+MetS=CKD patients with metabolic syndrome, CKD-MetS=CKD patients without metabolic syndrome and ns=not significant *p<0.05, **p<0.01, ***p<0.001.

kgm-2, 25.3±1.6 kgm-2 for those with zero, one or two comorbidities respectively. The SBP levels for those with zero, one, two comorbidities were 124.0±4.0 mmHg, 131.4±5.7 mmHg and 143.4±7.7 mmHg respectively. However, DBP showed no significant difference (p=0.128).

From figure 2, serum creatinine (CRT) ($F_{3,44}$ = 0.7791; p = 0.512) and eGFR ($F_{3,42}$ = 0.1953; p = 0.899) showed no significant difference for trend. For those having zero, one, two or at least three or more comorbidities, the eGFR levels were 108.3±28.4 ml/min/ 1.73 m2, 87.5±20.6 ml/ min/1.73 m2, 86.4±17.7 ml/min/1.73 m2 and 99.7±24.2 ml/min/1.73 m2 respectively. The serum CRT levels were 216.6±8.1 μmolL-1, 311.6±103.7 μmolL-1, 485.8±159.9 μmolL-1 and 263.3±122.3 μmolL-1 for those with zero, one, two and at least three or more comorbidities respectively.

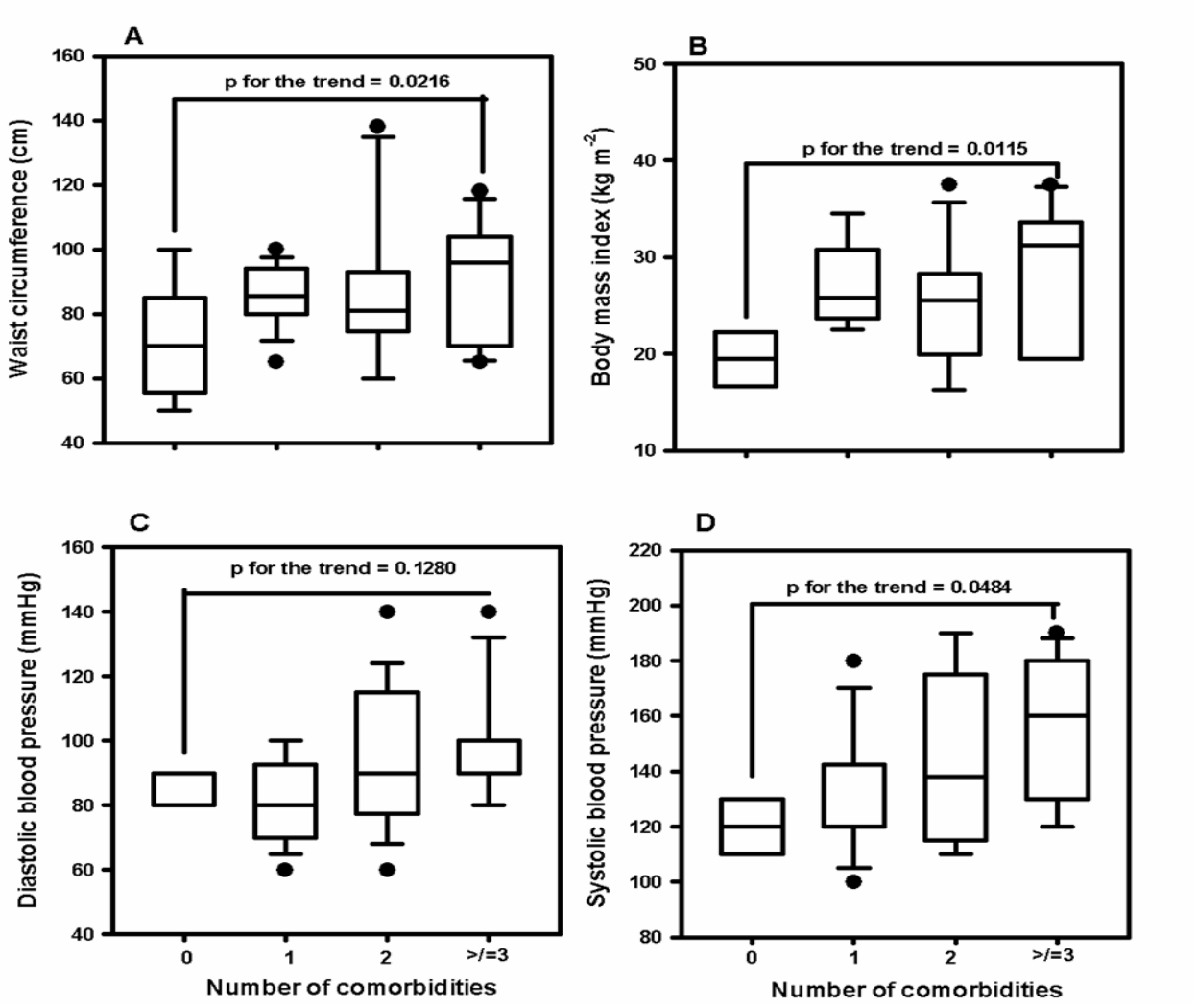

Figure 1: Comparisons of BMI, DBP, SBP and WC between participants with a different number of comorbidities of the MetS in CKD. The lower and upper margins of the box represent the 25th and 75th percentiles, with the extended arms representing the 10th and 90th percentiles, respectively. The median is shown as the horizontal line within the box. Outlying points are shown individually.

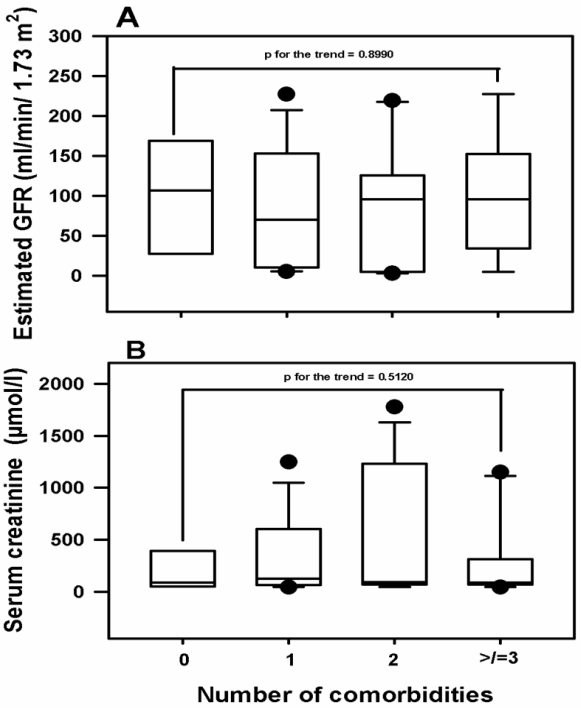

Figure 2: Comparisons of eGFR and serum Creatinine between participants with different number of comorbidities of MetS in CKD. The lower and upper margins of the box represent the 25th and 75th percentiles, with the extended arms representing the 10th and 90th percentiles, respectively. The median is shown as the horizontal line within the box. Outlying points are shown individually.

Many of the participants had multiple comorbidities; and those with a greater number of comorbidities also had higher TG ($F_{3,45}$ = 3.593; p = 0.027) and lower HDL-C ($F_{3,46}$ = 5.573; p = 0.002). However, FBG ($F_{3,44}$= 1.533; p = 0.219) and TC ($F_{3,46}$ = 0.403; p = 0.751) showed no significant difference for trend. The TG levels were 1.2±0.5 $mmolL^{-1}$, 1.4±0.2 $mmolL^{-1}$, 2.4±0.4 $mmolL^{-1}$ or 2.7±0.3 $mmolL^{-1}$ for those with zero, one, two, and at least three or more comorbidities respectively. The low HDL-C levels for those with zero, one, two or and least three or more comorbidities were 1.6±0.3 $mmolL^{-1}$, 1.8±0.2 $mmolL^{-1}$, 1.1±0.1 $mmolL^{-1}$ or 1.0±0.1 $mmolL^{-1}$ respectively (Figure 3).

Risk factors of developing MetS among the various CKD group

Table 3 represents the odds ratios of MetS risk factors at various stages of CKD. When participants with CKD were classified into the various stages, the risk of developing hypertension decreased from about 10 times in stage 1 to about 7 times in stage 2 before increasing to about 9 times for stage 3, decreased to 6 times in stage 4 and increased to about 14 times in stage 5. The risk of having hyperglycaemia also increased from stage 1 to stage 3, and then decreased in stage 4 and 5, whereas the risk of developing obesity remained fairly stable throughout the various stages (1-5). The risk of developing low HDL-C decreased from stage 1 to stage 2 before increasing in stage 3, with a further decrease in stage 4, and finally increasing again at stage 5. The risks of developing hypertriglyceridaemia slightly increased progressively reaching the highest value at stage 5. MetS risk increased and reached a peak at stage 3, and decreased at stage 4 before finally increasing again at stage 5. The risk of developing proteinuria from this study fluctuated through the stages reaching a value greater than the initial value at stage 5 (Table 3).

DISCUSSION

This randomized case-controlled study sought to determine the prevalence of MetS and the relationship between the components of MetS and CKD in a Ghanaian population presenting with various stages of CKD. This study indicated the prevalence of MetS as defined by the NCEP ATP III criteria to be 30.1% of the participants. This finding is consistent with studies done in Australia (31%), Thailand (30.1%) and 34.1% in over 40 year olds in China but slightly lower than what was reported in Bangladesh (37%) (Johnson *et al.*, 2007; Zhang *et al.*, 2007; Satirapoj *et al.*, 2011; Nath *et al.*, 2012). This could be attributed to differences in the selection of participants, the MetS definition used and also the fact that MetS is an independent factor for CKD development. The current study also observed a high prevalence of MetS in female CKD participants compared to male CKD participants. This is consistent with observations made in numerous studies including the Virgem das Graças

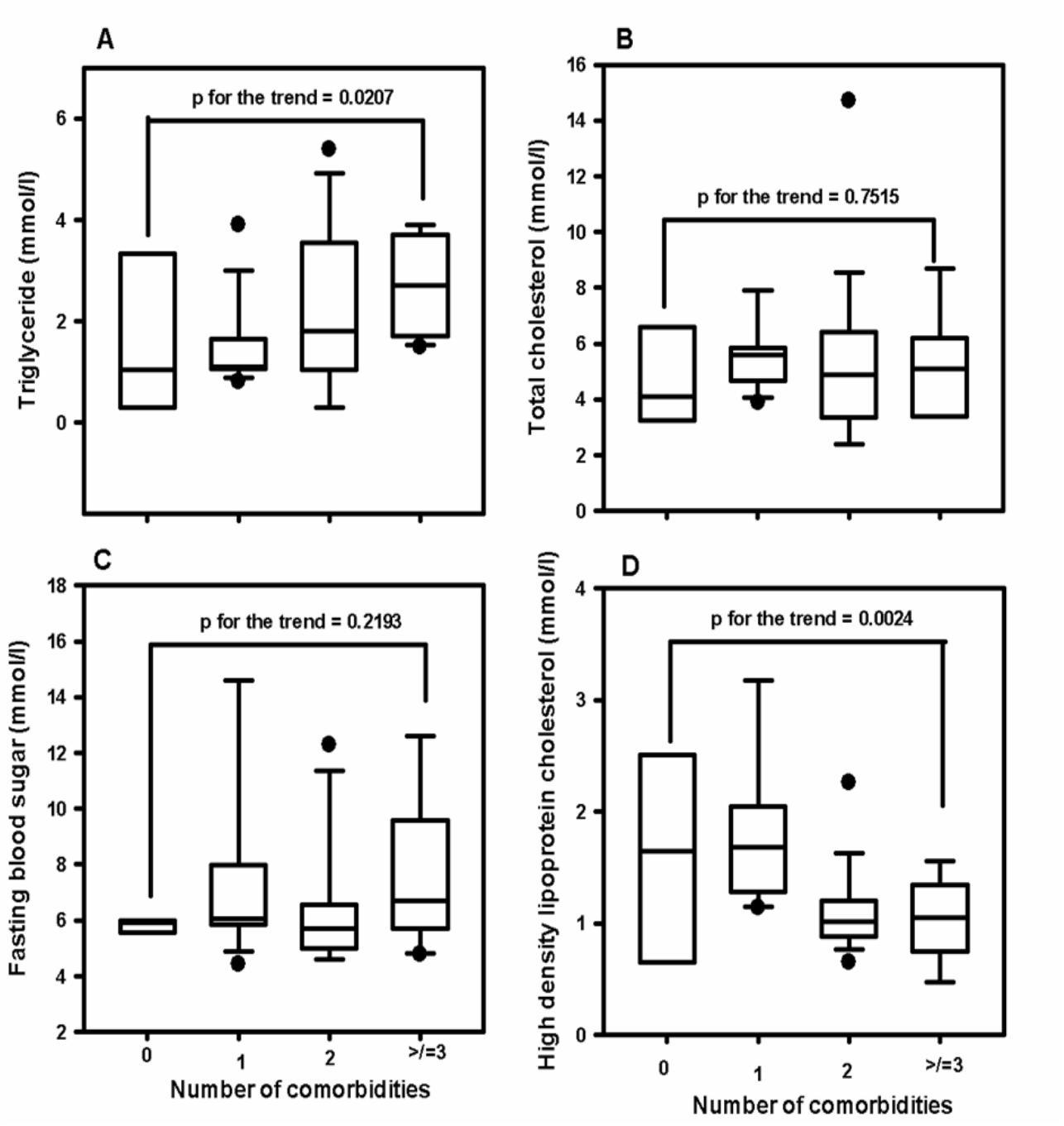

Figure 3: Comparisons of FBG, TG, TC and HDL-C between participants with different number of comorbidities of MetS in CKD. The lower and upper margins of the box represent the 25th and 75th percentiles, with the extended arms representing the 10th and 90th percentiles, respectively. The median is shown as the horizontal line within the box. Outlying points are shown individually.

Table 3: Odds ratios of MetS risk factors at various stages of CKD

Parameter	Stage 1 (n=24)	OR (95% CI)	Stage 2 (n=35)	OR (95% CI)	Stage 3 (n=37)	OR (95% CI)	Stage 4 (n=25)	OR (95% CI)	Stage 5 (n=24)	OR (95% CI)
Hypertension	8(33.3%)	9.5(2.5-35.4)	9(25.7%)	6.6(1.8-23.2)	12(32.4%)	9.1(2.7-30.8)	6(24.0%)	6.0(1.5-23.4)	10(41.6%)	13.6(3.7-49.4)
FGB	13(54.1%)	5.5(2.1-15.0)	26(74.3%)	13.6(5.2-35.3)	28(75.6%)	14.6(5.7-37.8)	18(72.0%)	12.1(4.2-34.5)	12(50.0%)	4.7(1.7-12.6)
Obesity	5(20.8%)	1.3(0.4-4.3)	8(22.8%)	1.5(0.5-4.1)	9(24.3%)	1.6(0.6-4.3)	10(40.0%)	3.4(1.2-9.3)	4(16.7%)	1.0(0.3-3.5)
TG	10(41.6%)	1.8(0.7-4.8)	18(51.4%)	2.8(1.2-6.4)	18(48.6%)	2.5(1.1-5.6)	10(40.0%)	1.7(0.7-4.5)	13(54.1%)	3.1(1.2-8.0)
Low HDL	4(16.7%)	3.8(0.8-16.5)	5(14.3%)	1.9(0.8-12.6)	11(29.7%)	8.0(2.3-27.4)	5(20.0%)	4.7(1.2-19.3)	7(29.1%)	7.8(2.0-29.8)
Proteinuria	5(20.8%)	45.0(2.4-857)	12(48.0%)	149(8.3-2671)	12(32.4%)	79(4.5-1381)	10(40%)	109(6.0-1961)	7(29.1%)	69(3.7-1266)
MetS	6(25.0%)	8.5(1.9-37.5)	13(37.1%)	15.1(3.9-58.0)	13(35.1%)	14.0(3.6-52.9)	4(16.0%)	4.8(1.0-23.5)	8(33.3%)	12.8(3.0-53.7)

Stage 1=eGFR ≥90 mL/min/1.73m²; stage 2 = eGFR 60-89 mL/min/1.73m²; stage 3 = eGFR 30-59 mL/min/1.73m²; stage 4 =eGFR 16-29 mL/min/1.73m²; stage 5 = eGFR<15 mL/min/1.73m² TG=triglycerides; TC=total cholesterol; HDL=high density lipoprotein; FGB=fasting blood glucose; OR=odds ratio.

community study (Dallongeville *et al.*, 2004) and that of Nath *et al.*, (2012) who reported prevalence rates of 32.35 and 42.5% for males and females respectively in a cross-sectional study involving 300 CKD patients in Bangladesh.

High TG and low HDL cholesterol have been identified as independent risk factors for initiation and progression of CKD (Fried *et al.*, 2001). However, in this study increased TG but not low HDL-C was predictive of CKD development as observed in earlier studies by Luk *et al.*, (2008). The processes underlying the role of lipids in the initiation of renal injury have not been fully elucidated.

In the current study, obesity was defined using the NCEP ATP III criteria for diagnosis of MetS and measured WC to determine abdominal obesity. Participants with MetS and CKD also had significantly higher WC a finding consistent with observations made in other studies (Kwan *et al.*, 2007; Chou *et al.*, 2008). The strong association between MetS and renal damage can be explained in the light of the role played by obesity related glomerulopathy. Even though the mechanism by which waist circumference increase the risk of CKD has not been well explained, it has been linked with the production of inflammatory cytokines like leptin, interlukin-6 (IL-6) tumour necrotic factor-alpha (TNF-alpha) and adiponectin (Satirapoj and Supasyndh, 2007). These cytokines, mostly produced by the adipose tissue, play a role in kidney damage in patients with MetS by activating sympathetic nervous activity, aggravating renal haemodynamics, in addition to increasing inflammatory and oxidative states (Iseki, 2008).

High systolic blood pressure is prevalent in CKD as observed among the CKD subjects with MetS in this study. High systolic blood pressure is a determinant of CKD progression and should therefore be the focus of control of antihypertensive therapy (Young *et al.*, 2002). The association of CKD with isolated systolic hypertension (and wide pulse pressure) may be explained by increased vascular stiffness. Wide pulse pressure appears to be a marker of vascular stiffness and cardiovascular calcification, a predictor of cardiovascular risk in the elderly (Bielak

et al., 2004) and it is associated with increased mortality in patients with renal disease (Klassen *et al.*, 2002).

The relationship between the MetS and the incidence of CKD is that of MetS components directly causing harm to the kidneys through systemic atherosclerosis. Individual components of MetS, including glucose intolerance, hypertension and dyslipidaemia, could act directly as risk factors for renal injury through renal or systemic atherosclerosis according to previous epidemiological studies (Humphrey *et al.*, 1989; Whelton *et al.*, 1996; Hunsicker *et al.*, 1997). In the present study, it was found that clusters of these risk factors had a stronger impact on the development of CKD than individual risk factors. Additionally, the accumulation of three or more of the metabolic disorders outlined by the NCEP ATP III criteria promoted the development of CKD or progression of GFR decline. These findings support the hypothesis that clusters of atherogenic metabolic disorders induce renal vessel injury, resulting in deterioration of renal function (Ninomiya *et al.*, 2006).

CONCLUSION

The prevalence of MetS in CKD patients was 30.1% using the NCEP ATP III criteria and increased WC, TG and SBP are components of the metabolic syndrome which contribute to the initiation and progression of CKD. A critical assessment of Met S and its components should be included in the monitoring and management scheme of CKD patients in order to reduce its prevalence and thus control the progression of CKD.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Anti-secretory effects of a dichloromethane fraction of the stem bark of *Piliostigma reticulatum* (Cesalpiniaceae)

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This study reports the effect of a dichloromethane fraction of the stem bark of *Piliostigma reticulatum,* a plant with anti-diarrhoeal properties, on the concentrations of electrolytes and the weight of water in castor oil-induced diarrhoea model in rats. The concentrations of ions in the supernatant of the small intestine content, obtained after centrifugation of the intraluminal fluid, were measured by flame photometry. The fraction showed a dose-dependent decrease of electrolytes concentration of [Na^+], [K^+], [Cl^-] and [Ca^{2+}], compared to the vehicle control. The ion concentrations were significantly reduced by the fraction at 125, 250 and 500 mg/kg, in the same range of inhibition obtained in rats treated by loperamide (5mg/kg), used as the reference anti-diarrhoeal drug. Quantity of water in faeces was also significantly reduced by the dichloromethane fraction at 250 and 500 mg/kg, and by loperamide. Results from the study showed that the dichloromethane fraction obtained from a crude extract of the stem bark of *P. reticulatum* possesses anti-secretory activity. These results suggest that the anti-diarrhoeal properties of the plant could partly be mediated by its anti-secretory activity and could therefore justify its use in traditional medicine to treat diarrhoea.

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Keywords*:* Castor oil induced-diarrhoea; electrolytes*;* loperamide, plant extract

INTRODUCTION

Diarrhoea is characterised by a discharge of semi-solid or watery faecal matter from the bowels three or more times per day (Hirchhorn, 1980; Snyder and Merson, 1982). It involves an increase in the fluidity and the number of faeces associated to an increased secretion of water and electrolytes (Field *et al.,* 1989; Longe and Dipiro, 1992; Dosso *et al.,* 2012). Diarrhoea is a public health problem especially for children under the age of five years. It is the second most common cause of infant deaths worldwide claiming over 2.6 million deaths in 2009 alone (UNICEF/WHO, 2009). It is estimated that 2.2 million children will die from diarrhoea and related diseases this year; 80% of them in the first two years of their life; 42,000 a week, 6,000 a day (Rehydration Project, 2012).

A report also indicates that up to 17% of children on admission in the paediatric ward die of diarrhoea (Mabeku *et al.,* 2006). In Côte d'Ivoire, the prevalence of diarrhoea in the population is 26.2%, and in Abidjan, the country's main city, it is evaluated to be 27.9% for diarrhoeas provoked by rotavirus in infants of 0-5 years old (Akoua-Koffi *et al,* 2007). Herbal medicine is a safe and economical source of bioactive compounds including substances of synergistic and/or side effects neutralizing potential (Gilani and Atta-ur-Rahman, 2005). It

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is now important to identify and evaluate available natural drugs as alternatives to currently used anti-diarrhoeal drugs, which are not always free from adverse effects (Harman *et al.,* 1992).

Piliostigma reticulatum (DC.) Horscht (Caesalpiniaceae) which is generally found in the west of Africa and particularly in the north of Côte d'Ivoire is traditionally used in treating many disorders, including diarrhoea (Yelemou *et al.,* 2007; Dosso *et al.,* 2012). Some of its vernacular names are niama (Malinké, Bambara), niamairi (Dioula) in Cote d'Ivoire (Kerharo and Bouquet, 1950), and abafe (Yoruba), kalga, kalgo (Hausa), thoingii pilostigma (local English) in Nigeria (Ainslie, 1937; Etuk *et al.,* 2009).

In a previous study, results showed that an ethanol extract of the stem bark of *Piliostigma reticulatum* significantly reduced the gastrointestinal transit, the number, volume and weight of faeces in rats (Dosso *et al.,* 2012). A preliminary investigation of various fractions obtained from the ethanolic extract of the stem bark of *Piliostigma reticulatum* suggests that the dichloromethane fraction bears highest anti-diarrhoeal properties (*unpublished data*). In the present study, we sought to investigate the anti-secretory activity, as a possible mechanism of action, of the dichloromethane fraction obtained from a crude ethanolic extract of the stem bark of *Piliostigma reticulatum* in a castor oil-induced diarrhoea model in rats.

MATERIALS AND METHODS

Plant collection

Stem barks of *Piliostigma reticulatum* (DC.) Horscht (Ceasalpiniaceae) were collected in Abidjan (South region of Côte d'Ivoire) in October 2007. The plant was identified and authenticated by Pr AKE-ASSI Laurent. A voucher specimen (N° 18033) of the plant was deposited in the herbarium of the National Centre of Floristic, University of Cocody-Abidjan.

Preparation of dichloromethane fraction

Stem barks of *Piliostigma reticulatum* were washed with water, cleaned, cut into smaller pieces and kept at room temperature for two weeks. They were then ground into a fine powder using a cutting mill (Retsch SM 100-1390 rev/min, Labo and Co, France). The powder (100 g) was extracted with 2 litres of a solution of ethanol (96%) / water (80:20, yielding a final ethanol concentration of 76.8%) for 24 hours with constant stirring using a shaking water bath (Kottermann, Germany) (this operation was repeated twice). The extract was filtered twice through cotton wool, then through a filter paper (Whatman grade 1, Sigma-Aldrich, France). The filtrate was concentrated using a rotavapor (Buchi, Switzerland) at 45°C, and dried on a water bath (Kottermann, Germany). The percentage yield was found to be 13.6%.

After successive liquid–liquid fractionations, five fractions (heptane, dichloromethane, ethyle acetate, butanol and water) were obtained from the crude ethanol extract (Harborn, 1984; Samsam-Shariat, 1992). From dried ethanol extract (starting with 10 g dissolved in 100 mL of water), heptane (800 mg = 8%), dichloromethane (900 mg = 9%), ethyl acetate (1700 mg = 17%), n-butanol (3200 mg = 32%) and aqueous (2100 mg = 21%) fractions were obtained respectively. The dichloromethane fraction was further selected for this study because in a previous preliminary study, it was the most potent anti -diarrhoeal agent (*unpublished data*). This was subsequently referred to as dicloromethane fraction or fraction.

Animals

Healthy, young adult albino rats of Wistar *strain* (age 5-6 weeks, weighing 150-200 g) of both sexes were obtained from UFR Biosciences (University of Cocody-Abidjan, Côte d'Ivoire). They were housed in stainless steel cages (34 cm ´ 47 cm ´ 18 cm) with soft wood shavings as bedding, fed with normal commercial pellet diet (Ivograin®, Abidjan, Côte d'Ivoire) and given water *ad libitum*. They were allowed to acclimatize to standard laboratory temperature conditions (temperature 24-28 °C, relative humidity 60-70%, and 12 hour light-dark cycle) for one week before the experiments. They were deprived of food for at least 18 hours prior to experiments but allowed free access to drinking water. The equipment usage, handling and sacrificing of the animals were performed in accordance

with the European Council legislation 87/609/EEC for the protection of experimental animals (Mitjans, 2008). The protocols for the study were approved by the Departmental Ethics Committee.

Phytochemical analysis of the fraction

The dichloromethane fraction was screened for the presence of tannins, flavonoids, alkaloids, sterols, saponins, polyphenols, polyterpenes and anthraquinones. Detection of these constituents was performed according to the method described by Bekro *et al.,* (2007).

Castor oil-induced enteropooling and electrolyte secretion

Rats were divided into five groups of six animals each; they were pre-treated with normal saline (0.9% NaCl), loperamide (5 mg kg^{-1}) and dichloromethane fraction (125, 250 and 500 mg kg^{-1}) by oral gavage. After one hour, the rats received 2 ml of castor oil orally, and an hour later they were sacrificed. For each rat, the small intestine was removed and tied with thread at the pyloric end and the ileo-caecal junction. The intestinal content was drained into a graduated tube. The Na^+, K^+, Cl^- and Ca^{2+} concentrations in the supernatant, after centrifugation of the intraluminal fluid, were measured by flame photometry (Azdu *et al.,* 2003; Boominathan *et al.,* 2005).

Determination of the content of water in the faeces of rats

Thirty rats were divided into five groups of six animals each. The groups were pre-treated respectively with normal saline (0.9% NaCl), loperamide (5mg kg^{-1}) and dichloromethane fractions (125, 250 and 500 mg kg^{-1}) by oral administration gavage. After one hour, the rats received 2 ml of castor oil, and were sacrificed 1 h after castor oil administration. The small intestine was removed, tied with thread at the pyloric end and the ileo-caecal junction. The intestinal content was weighed with the electronic balance PM 4600® (Mettlertoledo, Germany) and dried under reduced pressure in a drying oven at 45° C (Memmert U30, Germany). According to the method of Navarro *et al.,* (2006) the difference between the weight of humid faeces (WHF) and the weight of dried faeces (WDF) was calculated to obtain the weight of water (WW). The percentage of intestinal content in water was also calculated.

$$\mathbf{WHF - WDF = WW}$$

$$\mathbf{\%\ of\ intestinal\ content\ in\ water} = \left(\frac{\mathbf{WW}}{\mathbf{WHF}}\right) \times \mathbf{100}$$

Data Analysis

GraphPad Prism Version 5.0 for Windows (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses and IC_{50} determination. $P \leq 0.05$ was considered statistically significant in all analysis. The graphs were plotted using Sigma Plot for Windows Version 11.0 (Systat Software Inc., Germany).

RESULTS

Phytochemical analysis of the fraction

Phytochemical screening tests of dichloromethane fraction revealed the presence of major components such as tannins and flavonoids. Polyphenols and reducing sugars were also present, and anthraquinones, alkaloids, coumarins, polyterpenes and sterols were absent.

Effect of fraction on the concentration of sodium

The dichloromethane fraction dose-dependently and significantly ($P \leq 0.01$-0.001) decreased the concentration of sodium in comparison to the vehicle-treated group. This significant decrease was obtained at fraction doses of 250 and 500 mg mL^{-1} (Figure 1a). In rats treated by loperamide, the concentration of sodium was also significantly decreased by 46.48% ($P \leq 0.001$; Figure 1a; Table 1).

Effect of fraction on the concentration of potassium

The concentration of potassium was significantly reduced by the dichloromethane fraction at 125, 250 and 500 mg mL^{-1} to 0.79 ± 0.04; 0.49 ± 0.02 and 0.35 ± 0.03 mg mL^{-1} compared to the control

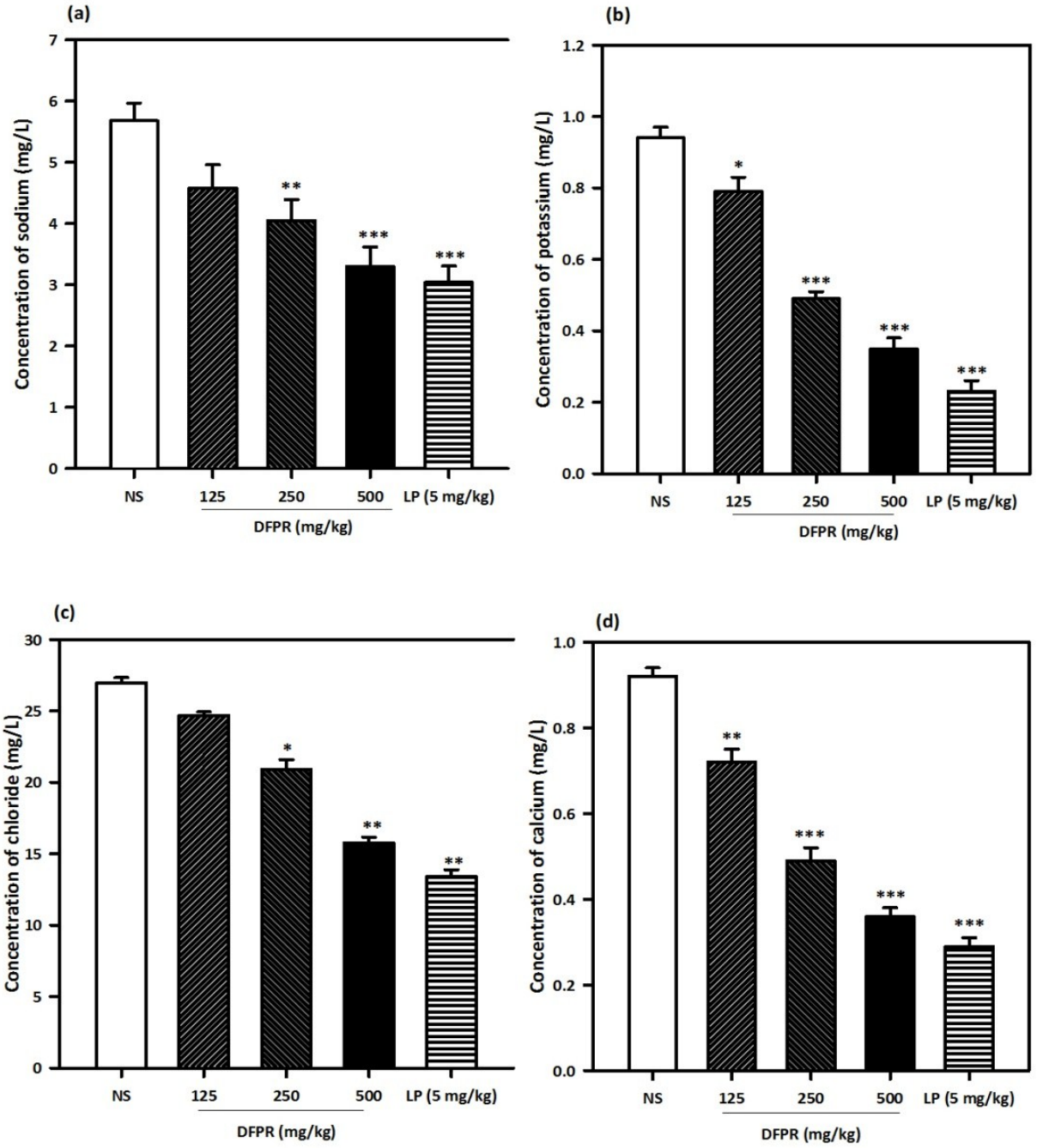

Figure 1: Effects of the dichloromethane fraction of *Piliostigma reticulatum* (DFPR) and loperamide (LP) on faecal concentration of (a) sodium; (b) potassium; (c) chloride and (d) calcium (mg/L). Data are mean ± SEM (n=6). *p < 0.05, **p < 0.01, ***p < 0.001 compared to vehicle treated group (one-way ANOVA followed by a Dunnett's Multiple Comparison Test).

Table 1: The effect of the dichloromethane fraction and loperamide on the percent inhibition of electrolytes and content of water

Samples	Inhibition (%)					Content of water (%)
(mg kg^{-1})	Sodium	Potassium	Chloride	Calcium	Water	
NS	--	--	--	--	--	29.78
LP 5 mg kg^{-1}	46.48	75.53	50.29	68.48	61.90	50.95
DCMf 125	19.37	15.96	8.49	21.74	1.90	46.60
DCMf 250	28.70	47.87	22.40	46.74	29.05	41.73
DCMf 500	41.90	62.77	41.47	60.87	47.62	42.47

NS: Normal Saline; LP: Loperamide; DCMf: dichloromethane fraction

(0.94 ± 0.03 mg mL^{-1}) (P≤0.001) respectively (Figure 1b). The percentage of inhibition of the fraction at 500 mg mL^{-1} was 62.77% (Table 1). Loperamide also significantly reduced the concentration of the potassium to 0.23±0.03 mg mL^{-1} (P≤ 0.001) compared to the control.

Effect of fraction on the concentration of chloride

The decrease of the concentration of chloride was significant (P≤ 0.01; P≤ 0.001) at 250 and 500 mg mL^{-1} of fraction respectively (Figure 1c). The concentration of chloride was also significantly lowered by loperamide to 50.29% (P≤ 0.01) (Figure 1c; Table 1).

Effect of fraction on the concentration of calcium

The fraction significantly (P≤ 0.001) decreased the concentration of calcium to 0.72±0.03; 0.49±0.02 and 0.36 ± 0.02 mg mL^{-1}, at 125, 250 and 500 mg mL^{-1} respectively (Figure 1d). The percentages of inhibition of the fraction were 21.74, 46.74 and 60.87% respectively at 125, 250 and 500 mg mL^{-1} (Table 1). Loperamide also significantly reduced the concentration of calcium to 0.29±0.02 mg mL^{-1} (P≤ 0.001) (Figure 1d).

Effect of fraction on the weight of water

The weight of water in intestinal content was decreased by the dichloromethane fraction. The weight was significantly (P≤ 0.01) reduced at 250 and 500 mg mL^{-1} to 1.49±0.12 and 1.10±0.16 g. with percentage reductions of 29.05 and 47.62% respectively. Loperamide significantly decreased the weight of water to 0.80±0.20 g (P≤ 0.01) respectively (Figure 2).

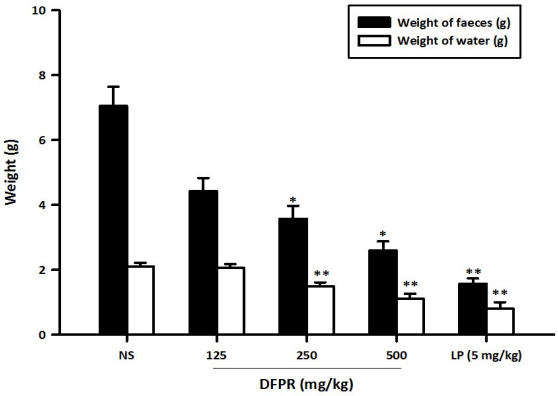

Figure 2: Effects of the dichloromethane fraction of *Piliostigma reticulatum* (DFPR) and loperamide (LP) on the weight of faeces and water contained in the faeces of rats. Data are mean ± SEM (n=6). *p < 0.05, **p < 0.01, ***p < 0.001 compared to vehicle treated group (one-way ANOVA followed by a Dunnett's Multiple Comparison Test)

DISCUSSION

This study intended to demonstrate the anti-secretory activity of *Piliostigma reticulatum* in castor oil-induced diarrhoea in rats. Diarrhoea generally may be characterized as the abnormally frequent

expulsion of faeces of low consistency which may be due to a disturbance in the transport of water and electrolytes in the intestines (George and Lutterodt, 1992; Gabriel *et al.,* 2004). Secretory and osmotic diarrhoea results in excessive loss of electrolytes and water (George and Lutterodt, 1992) leading to dehydration and subsequent death. WHO recommends oral rehydration solution which in many cases is a life saver (WHO, 2005). Castor oil causes diarrhoea due to its active metabolite, ricinolic acid (Ammon, 1974; Watson, 1962), which stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte and water permeability of the intestinal mucosa. Its action also stimulates the release of endogenous prostaglandin (Galvez *et al.,* 1993). A previous study indicates an anti-diarrhoeal property of an ethanolic extract of the stem bark of *P. reticulatum* and that this activity is high in the dichloromethane fraction obtained from the ethanolic extract (Dosso *et al,.* 2012; *unpublished data*). Present results from this study suggest an added property since the fraction significantly decreased the concentration of the electrolytes and water content of faeces obtained from rats pre-treated with castor oil. This will go a long way as an adjunct treatment to oral rehydration therapy in the management of diarrhoea.

Loperamide, the reference agent used, has anti-motility and anti-secretory properties (Couper, 1987). The similarity of the results obtained by the fraction and the reference drug loperamide on the reduction of water quantity and ions concentrations could suggest the same mechanism-based on anti-mobility and anti-secretory properties of *P. reticulatum*.

The phytochemical screening of dichloromethane fraction of the stem bark of *P. reticulatum* revealed that tannins and flavonoids are the major components, whereas polyphenols and reducing sugars were minor components. It is possible that these components observed could be responsible for the anti-secretory activity of dichloromethane fraction of *P. reticulatum*.

CONCLUSION

This study demonstrates the anti-secretory property of dichloromethane fraction from the ethanolic extract of the stem bark of *P. reticulatum*. This may be responsible for its anti-diarrhoeal activity. This attribute provides a useful and additional rationale for the use of *P. reticulatum* in diarrhoea management by traditional healers.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Evaluation of changes in pro-inflammatory cytokines in malnourished children: A Ghanaian case study

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Protein-energy malnutrition (PEM) is a public health problem and is associated with high morbidity and mortality. PEM is linked with changes in biochemical and immunological parameters. This study aimed at determining the level of pro-inflammatory cytokines among healthy (control) children and those with PEM as diagnostic indicators for PEM. The study was conducted between December 2009 and June 2010 comprising a total of 115 children (35 controls and 80 malnourished children) aged between 8 – 36 months attending the Maternal and Child Health Hospital (MCHH), Kumasi. Anthropometric parameters including weight, height and mean-upper arm circumference as well as immunological and biochemical parameters (interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α), albumin, total protein) were assessed among the studied population and the control group. After the analysis, 67.5% had marasmus, 18.8% had marasmic kwashiorkor and 13.8% had kwashiorkor. There were no statistically significant differences ($p>0.05$) in the mean total protein concentration of the subjects before (66.3± 1.6 g L-1) and after (69.6± 1.7 g L-1) nutritional supplement when compared to that of the controls (68.37± 1.4 g L-1) . Serum albumin concentration in the control group (43.2 ± 0.9 g L^{-1}) was significantly higher than the concentration in the subject group before treatment (38.7 ± 0.9 g L^{-1}, $p=0.0027$). The mean concentration of IL-6 in the subjects at baseline (46.1 ± 7.5 pg mL^{-1}, $p=0.0008$) and after treatment (26.3 ± 5.2 pg mL^{-1}, $p=0.0148$) were significantly higher than that in the control group. A 43.8% decrease in the mean concentration of IL-6 was observed after treatment. TNF-α concentration before treatment (82.1 ± 6.0 pg mL^{-1}) was significantly higher when compared to the mean concentration in the control group (55.8 ± 2.2 pg mL^{-1}). The study observed increases in pro-inflammatory response in malnourished children with IL-6 concentration being a significant indicator of PEM in the subjects compared to TNF- α.

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Keywords*:* Protein-energy malnutrition, children, kwashiorkor, haematology, Ghana

INTRODUCTION

Protein–energy malnutrition (PEM) is a problem of public health importance in many developing countries. It is a body depleting disorder that has been identified as an important underlying factor in about 50% of deaths of children <5 years of age in developing countries (Black *et al.*, 2003). Children between the ages of 12 to 36 months who are susceptible to infections are particularly at risk (WHO, 2000). In Ghana, about 40% of all childhood (Under five) deaths are due to malnutrition. It is estimated that about 84% and 68% of children living in the rural and urban areas respectively are affected (GDHS, 2003; GDHS, 2008). Protein-energy malnutrition in surviving children is known to be associated with a significant impairment of cell-mediated immunity, phagocyte function, complement system, secretory immunoglobulin A antibody concentrations, cytokine production and an

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altered immune response as well as susceptibility to infection (Chandra, 1991; Pelletier *et al*., 1995).

Lack of food or presence of infections that increase the body's nutrient requirements and losses are the main cause of PEM (WHO, 2000). It has been suggested that cytokines play an important role in the nutrition-infection complex. Protein-calorie malnutrition, deficiency of fatty acids, vitamins and trace elements impair cytokine production (Muñoz *et al*., 1995). On the other hand, infections increase pro-inflammatory cytokine production interfering with nutritional status by impairing metabolic activity and by inducing anorexia (Muñoz *et al*., 1995). The diagnosis of malnutrition in children has generally been based on measurements of nutritional status, which include assessments of oral intake, weight loss, anthropometric data, and determination of cell-mediated immunity, biochemical parameters, physical examination and body composition analysis (Hulst *et al*., 2004). The aim of the study is to evaluate the changes in pro-inflammatory cytokines in malnourished children, before and after nutritional intervention.

MATRIALS AND METHODS

This hospital-based case control study was conducted at the Maternal and Child Health Hospital (MCHH) in the Subin Sub-Metro in the Kumasi Metropolitan area of the Ashanti Region. All children between the ages of 8 to 36 months attending the child welfare clinic and the malnutrition rehabilitation center of MCHH during the period of December 2009 - June 2010 were recruited after fulfilling the inclusion criteria. Signed informed consent was obtained if parent or guardian demonstrated understanding of the study and was willing to enroll the child. The interview was conducted in Twi which is the local dialect in the region. The study was approved by the Committee on Human Research, Publications and Ethics (CHRPE), School of Medical Sciences, Kwame Nkrumah University of Science & Technology (KNUST), Kumasi, Ghana.

A total of 80 children attending the malnutrition rehabilitation center of MCHH with anthropometric measurements of weight for age <70% (Z-scores) and weight for height <80% (Z-Scores) who were finally put on a starter (F-75) (*for phase 1 treatment with duration of 2 – 7 days*) and catch up (F-100) (*for phase 2 treatment with duration of 1 – 3 days*) formula diet regimen were included in this study. Children who were on either micronutrient supplementation or on other medications were excluded from the study. A total of 35 children attending the child welfare clinic for routine checkups with weight for age >90% (Z-scores) and weight for height >90% (Z-Scores) were recruited as controls.

Laboratory investigations

Three millilitres (3 ml) of blood sample was collected from both the malnourished and healthy subjects who fulfilled the inclusion criteria of which 2 ml was dispensed into vacutainer® plain tubes and allowed to clot. The clotted samples were centrifuged for 10 minutes at 1250 x g and serum stored at -80^{o}C until analyzed. A portion of the sera was used to determine serum total protein and albumin using the Vitalab Flexor E (Vital Scientific NV Netherland) chemistry analyzer. The remaining portion of the serum was used for the analysis of IL-6 and Tumour Necrosis Factor-alpha (TNF-α) using Enzyme Linked Immunosorbent Assay (Enzyme Linked Immunosorbent Assay D System (Abingdon UK). The remaining 1 ml of the blood sample was dispensed into monovet® ethylene diamine tetraacetic acid (EDTA) tubes and used for the analysis of haemoglobin concentration (Hb) and total white blood cell count (WBC) using Sysmex 2000i xt (Sysmex Corporation, Kobe, Japan). Blood films were also prepared for malaria parasites. Because most of the children were admitted directly as out-patients and received their treatment on a weekly basis, follow up blood samples were taken between the 8th (*for children who were able to complete phase 1 of F-75*) to 16th (*for children who completed phases 1, transition phase and phase 2, F-75 and F-100*) days during the time of nutritional intervention. During this period, the children were stable, gained appetite and fluid and electrolyte imbalances were corrected (Reid *et al*., 2002).

Statistical analysis

Continuous data are expressed as mean ± SD whilst categorical data are expressed as proportions. Statistical comparisons were analyzed using *one-way ANOVA* and corrected with Bonferroni's Multiple Comparison test (*post-hoc)*. Student's *t*-test (paired) was used to compare means in subjects before and after treatment. The chi square test statistics was used to compare the statistical significance of proportions. A *P value* of less than 0.05 was considered significant. All statistical analysis was performed using GraphPad prism version 5.0 for windows.

RESULTS

Percentage changes in the concentration of haematological parameters in the control group compared to that of the subjects at baseline (before treatment) and after treatment are presented in Table 1. The mean haemoglobin concentration in the control group (12.0 ± 0.2 g dL^{-1}) was significantly higher than that in the subjects before (8.1 ± 0.2 g dL^{-1}; p<0.0001) and after treatment (8.5 ± 0.2 g dL^{-1}; p<0.0001). The mean haemoglobin concentration does not only increase by 3.2%, the proportion of subjects with haemoglobin concentration <11.0 g dL^{-1} also decreased by -6.2% after treatment. Conversely, the mean total white blood cell counts (TWBC) of 12.4 ± 0.7 k μL^{-1} and 11.2 ± 0.6 k μL^{-1} in the subjects before and after treatment respectively were significantly higher than the mean TWBC of 8.8 ± 0.4 k μL^{-1} in the control group (p=0.0006 and p=0.0153 respectively). A decrease in TWBC of -9.9% and a -13.7% decrease in the proportion of children with TWBC >12.0 k μL^{-1} was observed in the subjects after treatment. The proportion of children in the control group who tested positive for malaria parasites was significantly higher when compared to the subject group before (p=0.0080) and after (p=0.0486) treatment (Table 1).

The mean concentration of total protein in the control group (68.4 ± 1.4g L^{-1}) compared to that in the subjects before (66.3 ± 1.6 g L^{-1}) and after treatment (69.6 ± 1.7 g L^{-1}) showed no statistically significant differences (p>0.05) (Table 2). However, a percentage increase of 5.8 was seen in the mean concentration of total protein in the subjects after treatment compared to the baseline concentration. Serum albumin concentration in the control group (43.2 ± 0.9 g L^{-1}) was significantly higher than the concentration in the subject group before treatment (38.7 ± 0.9 g L^{-1}) (p=0.0027). A 6.8% increase in the mean concentration of serum albumin concentration was observed in the subjects after treatment (Table 2). The proportion of children in the subject group with a total protein concentration <60 g L^{-1} decreased by -16.3% after treatment whilst the percentage proportional de-

Table 1: Changes in the concentration of the haematological parameters in the study population

		SUBJECTS					
Variable	**CONTROL**	**BEFORE**	**AFTER**	**%Δ**	**p**	**p***	**p****
N	35	80	80				
Haemoglobin	12.0 ± 0.2	8.1 ± 0.2	8.5 ± 0.2	3.2	<0.0001	<0.0001	0.1573
<11.0 g dL^{-1}	5(14.3)	80(100.0)	75(93.8)	-6.2	<0.0001	<0.0001	0.0231
TWBC	8.8 ± 0.4	12.4 ± 0.7	11.2 ± 0.6	-9.9	0.0006	0.0153	0.1831
<4.0k μL^{-1}	0(0.0)	1(1.3)	2(2.5)	1.2	0.5065	0.3453	0.5600
>12.0k μL^{-1}	3(8.6)	36(45.0)	25(31.3)	-13.7	0.0001	0.0091	0.0734
Malaria parasites	3(8.6)	0(0.0)	1(1.3)	1.3	0.0080	0.0486	0.3158

TWBC = total white blood cells, %Δ = percentage change, p = defines the level of significance when control was compared to subjects (before); p* = defines the level of significance when control was compared to subjects (after); p** = defines the level of significance when subjects (before) was compared to subjects (after)

Table 2: Changes in the concentration of biochemical parameters in the study population

Variable	SUBJECTS			%Δ	p	p*	p**
	CONTROL	BEFORE	AFTER				
N	35	80	80				
Total Protein (g L^{-1})	68.4 ± 1.4	66.3 ± 1.6	69.6 ± 1.7	5.8	0.4226	0.6615	0.1612
<60g L^{-1}	3(8.6)	27(33.8)	14(17.5)	-16.3	0.0047	0.2145	0.0186
Albumin (g L^{-1})	43.2 ± 0.9	38.7 ± 0.9	41.1 ± 0.9	6.8	0.0027	0.1476	0.0479
<35g L^{-1}	4(11.4)	26(32.5)	13(16.3)	-16.2	0.0179	0.5027	0.0167

%Δ = percentage change, p = defines the level of significance when control was compared to subjects (before); p* = defines the level of significance when control was compared to subjects (after); p** = defines the level of significance when subjects (before) was compared to subjects (after)

crease in children with albumin concentration <35 g L^{-1} was -16.2% (Table 2).

From table 3, the mean concentration of interleukin -6 (IL-6) in the subjects at baseline (46.1 ± 7.48 pg mL^{-1}) and after treatment (26.3 ± 5.2 pg mL^{-1}) were significantly higher than that in the control (7.0 ± 1.4 pg mL^{-1}) group (p=0.0008 and p=0.0148 respectively) with a -43.8% decrease in the mean concentration of IL-6 being observed after treatment. The proportion of children with IL-6 concentration >14 pg mL^{-1} also decreased by 6.2% in the subject group after treatment. Tumour necrosis factor-alpha (TNF-α) concentration in the subject group before treatment (82.1 ± 6.0 pg mL^{-1}) was significantly higher when compared to the mean concentration (55.8 ± 2.2 pg mL^{-1}) in the control group but no statistically significant difference was observed in the TNF-α concentration in the subject

Table 3: Changes in the concentration of immunological analytes in the study population

Variable	SUBJECTS			%Δ	p	p*	p**
	CONTROL	BEFORE	AFTER				
N	35	80	80				
Cytokines							
IL-6 (pg mL^{-1})	7.0 ± 1.4	46.1 ± 7.5	26.3 ± 5.2	-43.8	0.0008	0.0148	0.0320
IL-6 >14pg mL^{-1}	5(14.3)	42(52.5)	37(46.3)	-6.2	0.0001	0.0011	0.4292
TNF-α (pg mL^{-1})	55.8 ± 2.2	82.1 ± 6.0	72.5 ± 6.9	-11.4	0.0053	0.1110	0.2992
TNF-α >8.1pg mL^{-1}	35(100.0)	80(100.0)	80(100.0)	0.0			

IL-6 = interleukin 6, TNF-α = Tumour necrosis factor-alpha, %Δ = percentage change, p = defines the level of significance when control was compared to subjects (before); p* = defines the level of significance when control was compared to subjects (after); p** = defines the level of significance when subjects (before) was compared to subjects (after)

group before and after (72.5 ± 6.9 pg mL^{-1}) treatment. A percentage decrease of 11.4% was observed in the mean TNF-α concentration of the subjects after treatment (Table 3).

DISCUSSION

Changes in haematological and biochemical parameters are known to provide valuable information and act as sensitive indicators for overall management of PEM (Mishra *et al.*, 2009). The alteration in the level of biochemical parameters were said to be related to food intake and biochemical metabolism mandatory during growth and development of children less than five years of age (Mishra *et al.*, 2009).

The significant reduction in mean haemoglobin concentration (i.e. 100% anaemic) at baseline as well as the 6.2% decrease in the proportion with anaemia after intervention shows the ability of diet intervention to improve upon haemoglobin concentration and this finding compares well with that of Mishra *et al.,* (2009). Gabay and Kushner, (1999) also reported on the effect of infections on erythropoiesis and the general lack of response to haematinics in the presence of active infection in children with PEM. A significant proportion of the subjects (45.0%) had elevated levels of total white blood cells (TWBC) when compared to the controls (8.6%) and this proportion decreased by about 13.7% after nutritional intervention. Bhan *et al.,* (2003) attributed elevated TWBCs in children with severe PEM to asymptomatic infections and severe nutritional deficiency is imminent in the failure of the immune system to respond to chemotaxis, opsonization and phagocytosis of bacteria, viruses or fungi. Children with PEM in this study might therefore have asymptomatic infections as evidenced by the elevated TWBCs which could have had a negative impact on erythropoiesis hence the resultant decreases in haemoglobin concentration observed in the subjects at baseline.

Mishra *et al.,* (2009) further showed a strong association of hypoproteinaemia in their PEM group compared to the control group with the risk of protein energy malnutrition being 3.7. Likewise, significantly higher decline in serum albumin level in the PEM group compared to the control group gave a relative risk of 5.2. A significant proportion of the subjects (33.8%) with PEM in this study developed hypoproteinaemia in comparison to the controls (8.6%) at baseline and this proportion decreased by about 16.3% after nutritional intervention. Also, 32.5% developed hypoalbuminaemia compared to 11.4% of the controls at baseline and this significant proportion decreased by 16.2% after nutritional intervention. These findings confirmed the contribution of hypoproteinaemia and hypoalbuminaemia in PEM and agree well with that of Mishra *et al.,* (2009). Sullivan (2001) in his study on serum proteins related hypoalbuminaemia to increased vascular permeability to albumin probably mediated by cytokines (IL-6 and TNF-α). This study observed increased concentrations of IL-6 in the subjects at baseline which decreased by 6.2% after nutritional intervention and as such could have contributed to the significant decrease in serum albumin at baseline.

Different studies have produced varying reports on pro-inflammatory cytokines in the malnourished. Whilst Muñoz *et al.,* (1994) and Abo-Shousha *et al.*, (2005) indicate that pro-inflammatory cytokine levels in the malnourished are reduced, many researchers in this area have reported increases (Vaisman *et al.,* 1989; Stenvinkel *et al.,* 2002; Azevedo *et al.,* 2005 and Cederholm *et al.,* 1997) Morlese *et al.*, (1996) suggested that increase in the pro-inflammatory cytokines could be due to stimulations either by the presence of endotoxin, bacterial exotoxin, fungi or viruses. This corroborate with a study conducted by Malave *et al.,* (1998), who showed that CRP and IL-6 increased to approximately similar levels in sera from undernourished and control children with overt infections. These cytokines, during acute generalized infections initiate acute-phase reactions which include fever, malaise, myalgia, headaches, cellular hypermetabolism and multiple endocrine and enzyme responses (Beisel, 1995).

The acute-phase reaction and its cytokine-driven hypermetabolism have high nutritional costs (Beisel *et al.*, 1977; Roubenoff *et al.*, 1994; Constans *et al.*, 1995). Cytokine-induced malnutrition is therefore

initiated by hypermetabolism (Beisel *et al.*, 1977; Roubenoff *et al.*, 1994) with its high basal metabolic rates. Body nitrogen and other elements are lost quickly, while body water and sodium are being retained (Beisel *et al.*, 1977). Glucose and urea synthesis are both increased during cytokine-induced malnutrition, but ketone production is slowed (Beisel *et al.*, 1977). Oxidation of branched-chain amino acids is increased and acute-phase plasma glycoproteins are created (Beisel *et al.*, 1977) thereby activating the immune system. Opposite responses to such metabolic instances are typical of uncomplicated starvation (Beisel, 1995). Significantly increased concentration of IL-6 was observed in subjects (52.5%) in this study when compared to controls (14.6%) at baseline and because starvation is rarely uncomplicated in children, the resultant malnutrition observed in subjects in this study could be generally influenced by cytokine-induced (IL-6) components.

Tumour necrosis factor (TNF) plays essential role in the development of the metabolic and pathological consequences of the stress response (Fong *et al.*, 1990). It has been detected in the serum of patients experiencing various diseases, such as parasitic or bacterial infections, tumour-bearing disease, burns and acute hepatic failure (Marano *et al.*, 1990). Giovambattista *et al.,* (2000) observed that basal TNF serum concentrations were significantly higher in malnourished children than in controls. In analyzing TNF-α concentration in the subjects and controls in this study however, no significant differences in TNF-α concentration was observed at baseline and after nutritional intervention. This finding is in agreement with that of Dulger *et al.,* (2002), who reported no significant difference in the concentration of TNF-α in children with PEM compared to controls in their study on pro-inflammatory cytokines in Turkish children with PEM.

CONCLUSION

This study observed increases in inflammatory response in children with PEM with IL-6 concentration being a significant diagnostic indicator of PEM in the subjects compared to TNF-α concentration. The impact of dietary intervention on haematological and biochemical indices assessed in this study shows the ability of nutritional intervention to achieve immunomodulation, promote growth, and improved immunity, general well-being and development of malnourished children less than five years of age.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Metabolic syndrome among garage workers in the automobile industry in Kumasi, Ghana

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Metabolic syndrome (MetS) is associated with cardiovascular diseases and diabetes but little is known about its prevalence among the active healthy population whose occupational activity is mainly manual and energy based. The aim of this study therefore, was to determine the prevalence of MetS and its components among garage workers in the automobile industry using three existing definitions. Two hundred garage workers were recruited from Bantama (86) and Sofoline (114) in Kumasi, Ghana. Anthropometric measurements including body mass index (BMI), waist to hip ratio (WHR) and waist to height ratio (WHtR) were measured. Blood pressure of subjects was also taken. Laboratory analysis included fasting blood sugar (FBS), total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C). The prevalence of MetS among the studied population was 18%, 16% and 13% using NCEP ATP III, WHO and IDF criteria respectively. Reduced HDL-cholesterol was the most prevalent component for ATP III (38.5%); central obesity was the most prevalent component for WHO (53.0%) and raised FBS was the most prevalent component for the IDF definition (54.0%). MetS seems to be on the increase among the manually active population even in the absence of obesity. There is therefore, an urgent need for a health policy shift towards control and prevention of MetS in Ghanaians.

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INTRODUCTION

Metabolic syndrome (MetS) constitutes a cluster of synergistically interacting cardiovascular risk factors which is mainly characterized by insulin resistance measured by the homeostasis model assessment or fasting insulin, abnormal glucose tolerance (fasting blood glucose or 2-hour postprandial blood glucose), atherogenic dyslipidaemia (increased triglycerides, decreased high-density lipoprotein cholesterol), elevated blood pressure and obesity (generalised obesity or central obesity) (Maumus *et al.*, 2005; Owiredu *et al.*, 2008). Other associated pathophysiologic conditions are physical inactivity, aging and polycystic ovarian syndrome (Motala *et al.*, 2009).

There are different definitions of MetS, including the criteria of the World Health Organization (WHO), European Group for the Study of Insulin Resistance, American Association of Clinical Endocrinology, National Cholesterol Education Program Adult Treatment Panel III (ATP III) and the International Diabetes Federation (IDF). Available data suggest that the prevalence of MetS vary according to age, ethnicity, race and criteria used (Cornier *et al.*, 2008). Though different countries and regions are at different stages, sub-Saharan Africa as a whole is at the centre of the most rapid demographic and epidemiologic transitions in world history. The future impact of this on the prevalence of the MetS is unknown, but is a matter of concern.

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None of the sub-Sahara African countries is excluded from the problems of poverty, increasing urbanization and westernization of lifestyle (Kamadjeu *et al.*, 2006).

The fact that physical inactivity and poor nutrient intake associated with modern lifestyle are thought to make a major contribution to the development of the syndrome poses the challenge of the increasing prevalence of MetS among the inactive workforce such as sedentary workers. According to global estimation, an epidemic of chronic diseases of lifestyle will increase and the largest proportional and absolute increases would occur in developing regions of the world, including Africa of which Ghana is no exception (Diabetes Atlas, 2006). This expected increase would be due in part to the projected increase in the urban population and in the aging populations across the world. Recent estimates by IDF for Africa indicates that among adults between 20–79 years, the regional prevalence of diabetes would increase from 3.1% in 2007 to 3.5% in 2025, with a corresponding 80% increase in numbers, from 10.4 million to 18.7 million (Diabetes Atlas, 2006).

Data on MetS are scanty in many African countries. The limited data from sub-Saharan Africa are based on clinical studies in defined high-risk groups of patients, such as those with type 2 diabetes (Titty *et al.*, 2008), pregnancy-induced hypertension (Turpin *et al.*, 2008) and chronic kidney disease (Owiredu *et al.,* under review). CVD risks and related complications are also said to vary very importantly between countries in sub-Sahara Africa (Addo *et al.*, 2007; Mensah, 2008). Besides, most of these studies are limited by the variable criteria used for defining MetS. Although the prevalence of CVD risk factors and of some of the individual components of the MetS have been reported, e.g. type 2 diabetes, obesity, and hypertension (Owiredu *et al.*, 2008), there is little information about the prevalence of the MetS or its components in workers whose occupation exposed them to conditions that might influence the prevalence of MetS. This study therefore seeks to determine the prevalence of MetS using WHO, NCEP ATP III and IDF definitions and the risk factor profile of MetS among garage workers in the automobile industry in Kumasi, Ghana.

MATERIALS AND METHODS

Subjects

This study was conducted between January and March, 2009. The subjects of this study were recruited from an adult population in Kumasi, Ashanti Region, Ghana. Two hundred men who work in the automobile garage industry (sprayers, straightener, auto mechanics, auto electricians, and smelters) in Bantama (n=86) and Sofoline (n=114) were recruited for this study. The study participants were predominantly male because the workforce is mainly men. The participation of the subjects was voluntary and informed consent was obtained from each subject. Subjects who were on medications that are known to modify serum lipid, blood pressure (BP), or carbohydrate metabolism were excluded from the study. The study was approved by the Committee on Human Research Publication and Ethics, KATH/SMS-KNUST, Ghana.

Sample collection and preparation

Five ml of venous blood sample was collected from the antecubital vein between 7 to 9 am after an overnight fast (12-16h). Two ml of the blood was dispensed into fluoride oxalate tubes whilst the rest was dispensed into vacutainer plain tubes. The samples in the fluoride oxalate tube and vacutainer plain tubes were centrifuged at 500 *g* for 15 min within 30 minutes of sample collection and separated into plasma and serum respectively. The plasma was used for the estimation of [glucose] while the serum was used for the estimation of lipid profile. The parameters that were determined include: Fasting Blood Sugar (FBS), Total Cholesterol (TC), Triglycerides (TG), High Density Lipoproteins (HDL-C) and Low Density Lipoproteins (LDL-C) using BT 3000 automated Chemistry analyzer. The methods adopted for the automated instrument for the determination of the above parameters were according to the reagent manufacturer's instructions - JAS™ diagnostics Inc. (JAS Diagnostics, Inc. Miami Florida, USA).

Anthropometric variables

Anthropometric measurements included height to the nearest 0.5 cm without shoes and weight to nearest 0.1 kg in light clothing were measured. Subjects were weighed on a bathroom scale (Zhongshan Camry Electronic Co. Ltd, Guangdong, China) and their height measured with a wall-mounted ruler. Blood pressure was taken by qualified nurses using a mercury sphygmomanometer and stethoscope. Measurements were taken from the left upper arm after subjects had been sitting for more than five (5) minutes in accordance with the recommendation of the American Heart Association (Kirkendall *et al.*, 1967). Duplicate measurements were taken with a 5 minute rest interval between measurements and the mean value was recorded to the nearest 2.0 mmHg.

Metabolic Syndrome Definitions

National Cholesterol Education Program, Adult Treatment Panel III (NCEP ATP III).

MetS was defined according to the criteria of the National Cholesterol Education Program, Adult Treatment Panel III (NCEP ATP III) to include individuals with three or more of the following five components: (1) abdominal obesity (waist circumference > 102 cm for men, or > 88 cm for women); (2) high TG ≥ 1.7 mmol L^{-1} ; (3) low HDL-C : men < 0.9 mmol L^{-1} or women < 1.0 mmol L^{-1} ; and (4) High BP (systolic BP ≥ 130 mm Hg or diastolic BP ≥ 85 mm Hg or treatment of hypertension); and (5) high fasting glucose ≥ 6.1 mmol L^{-1} (NCEP, 2001).

International Diabetes Federation (IDF)

According to the new definition by the International Diabetes Federation (IDF) (Alberti et al., 2006), MetS can be diagnosed if central obesity (waist measurement >90 cm for men or >80 cm for women) is accompanied by any 2 of the following 4 factors: (1) TG levels of 1.7 mmol L^{-1} or greater, (2) an HDL cholesterol lower than 1.03 mmol L^{-1} for men or lower than 1.29 mmol L^{-1} for women, (3) a blood pressure (BP) of 130/85 mm Hg or greater or treatment of previously diagnosed hypertension, and (4) a fasting blood glucose (FBG) of 5.6 mmol L^{-1} or greater or previously diagnosed type 2 diabetes.

World Health Organization (WHO)

WHO criteria (1999) (Alberti *et al.*, 2006) requires the presence of diabetes mellitus, impaired glucose tolerance or insulin resistance and any two of the following: (1) Body mass index (BMI) ≥30 kg m^{-2} and/or waist-to-hip ratio >0.90 (male), >0.85 (female), (2) blood pressure ≥140/≥90 mm Hg or on medication, (3) diabetes ≥6.1 mmol L^{-1} or on medication for diabetes, impaired glucose tolerance or insulin resistance, (4) triglyceride ≥1.7 mmol L^{-1} and/or HDL-C <0.91 mmol L^{-1} (male), <1.01 mmol L^{-1} (female).

Statistical Analysis

The results are expressed as Means ± SEM. Unpaired *t*-test was used to compare mean values of continuous variables and χ^2 was used to compare categorical variables. A level of $p<0.05$ was considered as statistically significant. GraphPad Prism version 5.00 for windows was used for statistical analysis (GraphPad software, San Diego California USA, www.graphpad.com).

RESULTS

The general characteristics of the study population are as shown in Table 1. The mean age of the studied population was 30.2 ± 7.8 years and the mean duration of work was 8.0 ± 6.1 years. Whereas the prevalence of obesity using BMI, WC and WHR were 2.0% 1.0% and 1.0% respectively, the prevalence of hypertension and diabetes were 12.0% and 6.0% respectively (Table 1). Using Pearson's correlation, age and duration of work correlated positively with blood pressure (i.e. SBP and DBP) (data not shown).

The prevalence of MetS among the studied population was 18%, 16% and 13% using NCEP ATP III, WHO and IDF criteria respectively (Table 2). Using NCEP ATP III criteria, the highest prevalence of components of MetS was reduced HDL-C (i.e. 38.5%), followed by raised FBS (34.0%), raised TG (31.5%), raised BP (20.0%) and central obesity

(1.0%). From the WHO criteria, the highest prevalence of components of MetS was central obesity (53.0%), followed by raised FBS and raised TG (i.e. 34.0%), reduced HDL-C (17.0%) and finally raised BP (12.0%). Raised FBS had the highest prevalence rate (54.0%), followed by reduced HDL-C (38.5%), raised TG (31.5%), raised BP (20.0%), and obesity (19.0%) using the IDF criteria (Table 2). Using the IDF criteria, almost half of the study population had a metabolic score of two, whereas about 30% of the study population had a metabolic score of two using the NCEP ATP III and WHO criteria (Table 2).

Table 1: General characteristic of the studied population

Variables	Total (n=200)
Age (yrs)	30.2 ± 7.8
Duration of work (yrs)	8.0 ± 6.1
WC (cm)	82.9 ± 10.6
SBP (mmHg)	122.3 ± 17.5
DBP (mmHg)	75.9 ± 11.6
BMI (kg m^{-2})	24.1 ± 3.1
WHR	0.9 ± 0.1
Hb (mg dl^{-1})	14.2 ± 1.2
FBS (mmol L^{-1})	5.6 ± 0.9
TG (mmol L^{-1})	1.5 ± 0.5
HDL-C (mmol L^{-1})	1.1 ± 0.3
TC (mmol L^{-1})	4.5 ± 1.0
LDL-C (mmol L^{-1})	2.7 ± 0.8
Alcoholics (%)	23.3
Smokers (%)	4.7
Obesity-BMI (%)	2.0
Obesity-WC (%)	1.0
Obesity-WHR (%)	1.0
Hypertension (%)	12.0
Diabetes (%)	6.0

Data are presented as mean ± SD and categorical data presented as percentages. BMI – body mass index; WC –waist circumference; WHR – waist-to-hip ratio; Obesity-BMI = BMI ≥ 30 kg m^{-2}, Hypertension = blood pressure ≥ 140/90 mmHg, Obesity-WC = WC > 102 cm, Obesity-WHR = WHR >1.0, Diabetes = fasting blood sugar greater or equal to 7.0 mmol L^{-1}

Using the NCEP ATP III and WHO criteria, the study participants with MetS were significantly older and had been on the job for a longer period compared to those without MetS. Interestingly, straighteners were more associated with Mets (22.2%) compared to those without MetS (6.1%) as shown in table 3.

DISCUSSION

This study provides data on the prevalence of MetS and its components among occupational group subjects. In an active workforce such as garage workers in the automobile industry, it is presumed that since the nature of their vocation offers them the opportunity to be physically active, one would have expected that the MetS and its components would have been drastically reduced or even absent. However, this study has established that MetS is a major health problem, even among the healthy active population who are physically active with minimal sedentary lifestyle. These findings, thus, call for sector-specific strategies for health promotion and prevention or treatment of MetS and its specific components.

This study shows that about 13% to 18% of garage workers in the automobile industry in Kumasi can be classified as having the MetS depending on the definitive criteria. The prevalence of MetS was 18% and 16% among the studied population using the NCEP ATP III and WHO definition respectively. In contrast, the prevalence of MetS by using IDF definitions was 13%. These MetS prevalence rates observed in this study are similar to the 15.1% rate observed among installation and machinery operators and machine assemblers (Sanchez-Chaparro *et al.*, 2008) and the 12% prevalence rate observed among manual workers in Spain (Alegria *et al.*, 2005).

The main reason why the IDF criteria presented the lowest prevalence rate is due to over reliance of the IDF criteria on central obesity whose prevalence was very low in this study. Thus, when the IDF criteria is used, persons without central obesity who may have other characteristics of the MetS may not be diagnosed, whereas the use of the

Table 2: Prevalence of metabolic syndrome and its components among the studied population

Components of the MetS	NCEP-ATP III definition	WHO definition	IDF definition
Central obesity or obesity (%)	2(1.0)	106(53.0)	38(19.0)
Raised blood Pressure (%)	40(20.0)	24(12.0)	40(20.0)
Raised FBS (%)	68(34.0)	68(34.0)	108(54.0)
Raised TG (%)	64(31.5)	68(34.0)	64(31.5)
Reduced HDL-C (%)	77(38.5)	34(17.0)	77(38.5)
Prevalence of MetS (%)	36(18.0)	32(16.0)	26(13.0)
Metabolic score			
0	50(25.0)	40(20.0)	29(14.5)
1	87(43.5)	94(47.0)	65(32.5)
≥ 2	63(31.5)	66(33.0)	106(53.0)

Table 3: Prevalence of socio-demographic characteristic and work type among the studied population stratified by MetS

	NCEP ATP III		WHO		IDF	
Parameters	Present (n=36)	Absent (n=164)	Present (n=32)	Absent (n=168)	Present (n=26)	Absent (n=174)
Age (yrs)	33.8±10.0	29.4±7.1*	34.4±9.5	29.4±7.3*	30.5±6.9	30.1±8.0
Duration of work (yrs)	10.4±6.6	7.4±5.8*	10.0±6.4	7.6±6.0*	7.8±4.5	8.0±6.3
Alcoholics (%)	21.4	23.6	26.7	22.5	30	22.4
Smokers (%)	0.0	5.6	0.0	5.6	0.0	5.3
BMI ≥ 30 (kg m^{-2})	5.6	1.2	0.0	1.2	0.0	1.1
Work type (%):						
Welder	11.1	4.9	12.5	4.8	0.0	6.9
Vulganizer	0.0	4.9	0.0	4.8	0.0	4.6
Straiter	22.2	6.1*	12.5	8.3	7.7	9.2
Sprayer	5.6	6.1	12.5	4.8	0.0	6.9
Spare part	0.0	1.2	0.0	1.2	7.7	0.0
Scrap dealer	0.0	1.2	0.0	1.2	0.0	1.1
Mechanics	22.2	42.7	25.0	41.7	38.5	39.1
Liner	0.0	2.4	0.0	2.4	0.0	2.3
Automechanic	0.0	7.3	12.5	4.8	7.7	5.7
Autoelectrician	16.7	11.0	18.8	10.7	15.4	11.5

Data are presented as mean ± SD and categorical data presented as percentages, *p < 0.05 when those with metabolic syndrome were compared with those without metabolic syndrome using unpaired t-test or Fischer's exact test.

WHO definition may underestimate MetS in non-diabetic subjects because it is primarily based on the presence of diabetes or impaired glucose tolerance or insulin resistance. The NCEP ATP III definition however, seems to be more convenient because of its flexibility in terms of the criteria used to diagnose MetS.

Hypertension is a 'silent killer' in many countries including Ghana (Amoah, 2003a; Cappuccio *et al.*, 2004; Owiredu *et al.*, 2008) and an important component of the burden of cardiovascular disease in all medical care services (Lim *et al.*, 2000). The overall prevalence of hypertension in this study was 12.0% and remarkably this is close to about a third of the prevalence of 29.9% found among the general male population in Kumasi (Cappuccio *et al.*, 2004) and 28.3% reported in Accra (Amoah, 2003a). The 12.0% is also lower than the 19% to 48% reported by Bosu, (2010) in a systemic review of an epidemic of hypertension in Ghana. The nature of the work of these studied populations (more physically active) may be protective as demonstrated in the lower prevalence of both generalised and central obesity rates. Current knowledge also suggests the importance of increased body mass index especially visceral fat in the pathophysiology of hypertension (Fujita, 2007).

The 2.0% prevalence rate of obesity among the garage workers in the automobile industry who are physically active at work compares favourably with the national prevalence rate of 2.8% reported by Biritwum *et al.*, (2005). This indicates that lifestyle factors play an important role in the aetiology of obesity among the Ghanaian population. This prevalence rate is however, lower than the 4.2% found among the artistic professionals among Dutch workers (Proper *et al.*, 2010). Since this study focused on an active population whose occupational activity is mainly energy and manual based, it stands to reason that this could be a contributory factor to the low percentage levels of obesity compared to other published data (Amoah, 2003b; Amoah, 2003c; Owiredu *et al.*, 2008). Since the type of work is associated with the total daily physical activity (Proper *et al.*, 2006), it was assumed that those working in occupations that require a certain amount of physical activity, would have a low prevalence of obesity. Besides, there is increasing evidence for the association of sedentary behaviour and obesity (Brown *et al.*, 2005; Proper *et al.*, 2007).

Using the revised diagnostic criteria for diabetes by an Expert Committee of the American Diabetes Association (ADA, 1997) and World Health Organization (WHO) (Alberti *et al.*, 1998) (i.e. a threshold of fasting plasma glucose of 7.0 mmol L^{-1}), the prevalence of diabetes among this population was 6.0%, confirming that diabetes is on the ascendency among adult Ghanaians. The relatively high rate of diabetes among active artisan garage workers has important public health implications for health planners. There is an urgent need for a health policy shift towards control and prevention of diabetes in Ghanaians considering the expected rise in the rate of diabetes (King *et al.*, 1998) that is likely to accompany cultural modification and increasing urbanization. Though this study reports a lower prevalence of diabetes as compared to a previous study from Accra (7.7%) (Amoah *et al.*, 2002), it is higher than the 4.0% prevalence reported by Abubakari *et al.*, (2009) among urban adults in West Africa. Studies conducted in Cameroon, South Africa and Tanzania have also reported diabetes prevalence rates ranging from 0.7 to 10.6% (Levitt *et al.*, 2000).

CONCLUSION

The prevalence of metabolic syndrome among the studied population was 18%, 16% and 13% using NCEP ATP III, WHO and IDF criteria respectively. The main contributors to MetS in the study population are reduced HDL-cholesterol for ATP III; central obesity for WHO and raised FBS for the IDF definition which are all reported to be on the increase in prevalence in the general population of Ghana. There is therefore an urgent need for health policy makers to shift their attention towards control and prevention of MetS in Ghanaians considering the expected rise in the rate of MetS components that is likely to accompany cultural modification and increasing urbanization.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Self–reported eye disorders and visual hazards among Ghanaian mine workers

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Because mining is an important industrial sector in many parts of the world, substantial progress has been made in the control of occupational health hazards associated with it. However, there are possibilities for further risk reduction. A cross-sectional study was conducted at Goldfields (Gh.) Ltd., Tarkwa to find out the prevalence of self reported eye diseases among the miners and visual hazards in the mine using standardized questionnaires. Four hundred and six (406) workers engaged in mining activity were conveniently sampled for the study. They all answered a questionnaire that solicited information on their socio-demographic data, health history, vital eye safety information and eye screening. Tests performed included visual acuity and pinhole examination. Overall, 117 (28.8%) confirmed previous diagnosis of an eye disease with presbyopia as the most reported eye condition in 5.2% of the subjects. While visual impairment was found in 28.1% of the study population only 1.4% reported previous history of refractive errors. Flying dust was named as the potential eye hazard in the mine by 39.7% of the workers. Only 10% of the workers had had some form of eye injuries. Chemical usage was 41.1% among the respondents while 7.9 % complained about intensity of light at the workplace. Eye diseases and visual impairments were reported among miners. Visual hazards were also found in the mine. Eye protection controls should be strengthened and an occupational eye safety and health programmes integrated into the general safety programme of the mine.

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Keywords*:* Visual hazards, Occupational health, Goldfields, Ghana, Ultraviolet

INTRODUCTION

Occupational health is a cross-disciplinary area concerned with protecting the health, safety and welfare of people engaged in a work (ILO/WHO, 2010). Such a programme aims at the promotion and maintenance of the highest degree of physical, mental and social well-being of workers, the protection of workers from risks adverse to health, prevention of ill health caused by working conditions, placement and maintenance of the worker in an environment adapted to his physiological and psychological capabilities (ILO/WHO, 2010). To achieve the above goal among mine workers calls for a multifaceted approach to the job hazard assessment, risk evaluation and health surveillance, including eye examinations.

Occupational vision which is part of the general health assessment is concerned with the efficient and safe visual functioning of an individual within the work environment. It encompasses more than just the prevention of occupational eye injuries, but includes vision assessment of workers taking into account their specific vision requirements and the demand these requirements place on them (Gregory, 1996). Underlining this assertion is the fact that vision is a critical aspect of many jobs (Occupational Vision Requirements, 1994). In Ghana, the legal framework for ensuring health and safety at work places is contained in the Factories, Offices and Shops Act (328) of Ghana, 1970 (Employment and relations centre, Ghana; 2010). The main provisions concern improvements neces-

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sary to attain internationally accepted standard for safety, health and welfare of workers. To prevent the occurrence of eye diseases, mine hazards that are a threat to the eye, needs to be identified. Identification of potential hazards to the eye could lead to the prevention of accidents at the workplace (Cornford, 1970). Although it is impossible to prevent all workplace accidents, the institutional anticipation of hazards is a major step in securing the safety and health of workers. The major hazards against which eye protection will be needed in the workplace are projectiles, chemicals (splashes and fumes), radiation (especially visible light, ultraviolet (UV) and infrared (IR)) and heat (Gregory, 1996). Common projectiles in the mining industry include pieces of screwdriver blade, drill bits, grinding wheel, metal debris, rock, steel rod and dust (Donoghue, 2004). Projectiles cause injuries like corneal or conjunctival abrasion and foreign body sensation penetrating eye injury, blunt trauma and some being toxic to the eye.

The mining activity also often makes use of hazardous and carcinogenic chemicals which can be acidic, alkaline, organic solvent and surfactants. For instance, sodium cyanide used in mining is thought to cause eye redness and pain, mercury poisoning causes damage to the central nervous system, endocrine system and kidney with its attendant effects on the visual system (International Chemical Safety Cards; 2008; Canadian centre of occupational health and safety, Canada; 2009). Again acrylamide exposure causes slight injury to the eye, irritation, sleepiness, and dizziness (The Minerals Council of Australia, 2002). Long term (chronic) exposure to radiation for example has been associated with the development of cataract (Pitts, 1993). Poor vision has been related to improper or inefficient lighting, contrast, working distance, viewing time and poor design of the work environment (Gregory, 1996) which potentially causes occupational injury due to eye fatigue and limits productivity. For example, close precision work has been associated with loss of far visual acuity.

Axial elongation of the eyeball, extraocular muscle tension, increased intraocular pressure (IOP) and increased lens power are all interrelated mechanisms that can affect visual acuity in long-term close work like mining (Browler *et al.,* 1991). Prolonged near work can also stress accommodation such that workers with low accommodative amplitude or flexibility will complain of asthenopia, fatigue, diplopia, watering and blurred vision due to convergence insufficiency (Gong *et al.,* 2003). The use of eye protection devices is required to prevent exposure to the eye at the workplace. The eye protection chosen for specific work situation should depend upon the nature and extent of the hazard present, the circumstances of exposure, other protective equipment used, and personal visual needs (National Institute for Occupational Safety and Health, 2010). The eye protection should also be adjustable to fit the individual, allow peripheral vision, be comfortable and possibly correct for the worker's refractive error. Personal eyewear recommended for miners include goggles, face shields, safety glasses and full-face respirators (Goldfields International Ltd, 2008).

The role of poor vision in causing accidents at mine sites, the occurrence of eye diseases either caused or exacerbated by the exposure to mine hazards like chemicals, heat and dust, and the ramification that mining related systemic diseases have on the visual system have not well been investigated. This present study was therefore an attempt to document reported eye diseases and visual impairment among miners and potential hazards to the eye.

MATERIALS AND METHODS

Sampling

A cross-sectional descriptive study was conducted. A section of the study population actively involved in mining and processing were conveniently sampled to represent the mine workers at Goldfields Ghana Limited, Tarkwa. Using the expression $n = Z^2 (1 - p)(p)/b^2$, (where n = minimum sample size, p = anticipated prevalence [assumed to be 50%], b = desired error bound taken as 5% and Z = the standard score at 95%), a minimum sample size of 314 was calculated, this was however increased to 406 to account for attrition rate.

Procedure for data collection
A structured questionnaire was used to solicit respondents socio demographic data (age, sex, etc), previous and current work history, workstation, medical history, current use and type of mediation, the use of industrial grade protective eye wear, any eye injury sustained, nature of work and associated hazards, and their general impression and opinion about safety at their workstations. Participants who had low educational background or for some other reason could not fill the questionnaires were assisted by reading and explaining it to them whiles those who could read and understand were given the opportunity to take the questionnaires home and return next day with it for the eye examination. Out of a total of 500 questionnaires distributed, 406 were recovered and evaluated for onward analysis.

All the 406 respondents had their visual acuity (VA) taken. Information obtained from observing events at the mine was used to supplement and corroborate data from the questionnaires and interviews. A critical evaluation and examination of the mining site was undertaken by the researchers in the form of a tour at the mine to ascertain the presence of potential hazards to the eye. Conscious attempts were made to observe precautionary measures that were in place to prevent accidents and protect the eyes. Informal interviews were held with some key persons who by reason of their official position could respond to some of the information collected during the questionnaire administration. The results of the visual acuity test, data collected from respondents and observations and interviews formed the basis for analysis.

Ethical Consideration
Institutional approval to carry out the study was obtained from the Management of the mines and the Department of Optometry, University of Cape Coast and individual workers before the commencement of the study. A research consent form was given to each participant for completion and those who required assistance were offered the necessary help by the researchers. The workers were adequately informed that participation in the study was voluntary and that they were free to withdraw from participation at any stage.

Data management and analysis
The data obtained from the questionnaires was crosschecked from different sources within the mine and per interview with officials to establish their veracity and authenticity or otherwise. This helped minimize bias on the part of the respondents. Entry visual acuity (VA) of 6/9 or worse in the better seeing eye was recorded as a visual impairment (WHO, 2004). The cut off VA (6/9) was used because mining is a visually demanding job and workers need an accurate vision to prevent accidents. In all comparisons, a p value less than 0.05 was considered statistically significant. Data obtained were analyzed using the Statistical Package for Social Sciences (SPSS v 15).

RESULTS

General characteristic of the studied population
Out of the 500 questionnaires a total of 406 (81.2%), responded representing an attrition rate of 94 (18.8%). Of the 406 respondents, 374 (92.1%) were males and 32 (7.9%) females. The mean age of the respondents was 41.1 years (range = 20 – 61, SD = 8.9). Majority of respondents fell within the age range of 41-50 (40.6%), followed by 31-40 (29.3%), then 21-30 (14.8%), 51-60 (14.0%) and 61 -70 (0.7%). The least recorded age range was 'less than 21' (0.5%), an indication that most of the workers were middle aged.

Visual acuity assessment
All 406 subjects had their visual acuities measured (Table 1). This was recorded as habitual visual acuity taken with or without their spectacle prescriptions. Twenty three individuals representing 5.7% were wearing glasses and therefore had their VA taken with their spectacles on. Three hundred and eighty three (383) of the 406 (94.3%), however had their VA taken without any aid. Significant visual impairment (VA ≤ 6/18) was observed in 12 (3.0%) individuals and moderate impairment (VA ≤ 6/9 - 6/12) in 102 (25.1%) (WHO, 2004). The results showed that the majority (292/406; 71.9%)

of the workers had normal visual acuity of 6/6 or better. There were 142 (35.0%) individuals who came under fairly poor distant vision range of 6/9 to 6/12. Eleven (2.7%) workers came under category one (VA ≤ 6/18 - 6/60) of the World Health Organization (WHO, 2004) grades of visual impairment. There were however 5 (1.2 %) persons who qualified as blind in at least one eye. Notably, one worker had a vision of counting fingers at 2 metres (CF@ 2M) in the better eye and interestingly had no spectacle correction for his impairment. Visual impairment was found in 114 (28.1%) of the respondents.

Reported eye diseases and disorders

Table 1: Distribution of Visual acuities in the worse and better Seeing Eye

Visual acuity	Worse eye	Better eye
6/5	156 (38.4)	231 (56.9)
6/6	57 (14.0)	61 (15.0)
6/9	121 (29.8)	83 (20.5)
6/12	21 (5.2)	19 (4.7)
6/18	29 (7.1)	10 (2.5)
6/24	8 (2.0)	0 (0.0)
6/36	3 (0.7)	1 (0.2)
6/60	5 (1.2)	0 (0.0)
3/36	1 (0.2)	0 (0.0)
CF@ 1M	1 (0.2)	0 (0.0)
CF@ 2M	2 (0.5)	1 (0.2)
CF@ 3M	1 (0.2)	0 (0.0)
Total	406(100)	406 (100)

CF = Counting fingers

Previously diagnosed eye diseases were reported by 117 (28.8%) compared to 289 (71.2%) workers who did not report of any previously confirmed diagnosis. The relationship between the reported eye diseases and sections of work was not statistically significant (χ^2= 16.1, p= 0.64). However, the majority (81.2%) of the cases came from the, mining, engineering, mineral resources and the metallurgy departments. Presbyopia (reading difficulty), 21(5.2%), was the most reported condition among the workers with only about half of them having spectacle correction. Though there was also no statistical significance (χ^2 = 37.9, p= 0.66) between the total mining experience of workers and the eye conditions reported, majority of the cases were reported among workers who had mining experience between 1-15 years. Table 2 shows the types of eye conditions reported. For 76 (65.0%) individuals, the confirmed date of diagnosis of the eye conditions was within the last five years, 19 (16.2%) between 6 -10 years, 4 (3.4%) in 11-15 years and 2(1.7%) cases had occurred in the last 16 -20 years.

Diagnosed medical conditions were also reported by the workers. One hundred and twelve (27.5%) confirmed a previous history of systemic disease whilst 289 representing 71.1% did not. Overall, hypertension was the most reported in 51 (12.7%) subjects. Others were musculoskeletal pain 7 (1.7), malaria 14(3.4), diabetes 5 (1.2%), respiratory tract infections 5 (1.2%), asthma 4 (1.0%) and hearing problem 2 (0.5%), HIV/AIDS 1(0.2%) and diarrhea 4 (1.0%). There was no statistically significant difference (p= 0.525) between the reported systemic conditions and the number of years the respondents have worked in a mining industry.

The use of eye protection and reported eye injuries

The main form of eye protection used on the mine was plastic goggles. These goggles were provided in plain and dark tinted colours, for day and night use. When inquired about their use of eye protection, 276 (68.0%) responded in the affirmative whilst 130 (32.0 %) did not use any eye protection in their work. Out of the total number of respondents who used eye protection, 199 workers representing 72.1% said the eye protection they use protected them adequately, 74 workers accounting for 26.8% said they did not receive adequate eye protection from the eye protective device they wear. The chunk of the workers who use eye protective devices varied greatly among the sections (χ2= 120, p < 0.01). The mining, engineering, metallurgy and mineral resources departments had 111 (40.2%), 70 (25.4%), 36 (13.0%) and 26 (9.4%) individuals using goggles respectively. The remain-

ing six sections together had only 33 (12.0%) workers using eye protection.

On whether the eye protection devices provided by the company was appropriate for the kind of work they do, 231 (81.1%) out of the total of 285 workers responded affirmatively while 54 (18.9%) responded in the negative. Quite a number of those who agreed on its appropriateness maintained that protective devices are provided by the company and they did not have any requisite knowledge to evaluate them. Eye safety and precautionary measures seemed to be very effective in preventing eye injuries as only 40 (10.0%) of 402 individuals had had any form of eye injuries as compared to 362 (90.0%) who had not suffered any eye injury at the mining site. Notably, only 5 (12.5%) of those who reported eye accidents were using eye protection when their eye injury occurred. The remaining 35 (87.5%) were not wearing eye protection. The relationship between the use of eye protection and the occurrence of eye injuries was significant ($\chi 2 = 7.77$, $p = 0.05$).

Visual hazards

The workers reported the use of one or more chemicals at their workstations. While 166 respondents, representing 41.1% of the workers either work with or were exposed to chemicals in the mine, 238 representing 58.9% of the sample population were not exposed to any chemicals. The use of chemical was significant among workers of different sections ($\chi^2 = 92.4$, $p \leq 0.01$). This was more prominent in the mining, metallurgy and engineering (process) sections with 142 individuals (85.5%). Overall, cyanide (15.8%) came up top as the chemical most individuals were exposed to followed by acids (14.3%),ammonium nitrate (11.1%). Caustic soda (5.4%), carbon (2.5%), anfull (6.4%), emulsion (4.7%) and other explosive chemicals were fairly used. Other chemicals mentioned were silica, borax, lime, flux, lead, electrical solvents and degreasers, xylene, hydrogen peroxide, reagents and other alkaline. Other chemicals that were not indicated represented 10.2%.

The mine scored high marks when workers impres-

Table 2: Distribution of eye diseases reported by the studied population

Eye condition	Distribution (%)
Normal	201(49.5%)
Cataract	6(1.6%)
Presbyopia	21(5.2%)
Refractive error	19(4.7%)
Conjunctivitis	18(4.4%)
Trauma	9(2.2%)
Glaucoma	7(1.7%)
Photophobia	8(2.0%)
Other(s)	26(6.4%)
Pterygium	3(0.7%)
Undetermined	88(21.7%)

Table 3: Distribution of the potential visual hazards among the studied population

Visual hazard	Distribution (%)
Dust	161(39.7)
Heat	8(2.0)
Intense light	32(7.9)
Chemicals	93(22.9)
Dim/ dark environment	4(1.0)
Don't know	50(12.3)
Wielding light & sparks	11(2.7)
Computer rays	8(2.0)
Smoke	4(1.0)
Other(s)	15(3.7)
Machinery	5(1.2)
Falling objects	15(3.7)

sion about the lighting condition on the mine at night and in their offices and stores were ascertained. Apart from five respondents who abstained from that assessment, 316(78.8%) workers gave thumps up to the lighting conditions at the mine. However, a significant group of 85 representing 21.2% were not happy with the lighting conditions mainly because of tower light intensity at night, complaining that it either affected their vision or they could not tolerate it.

There were a number of identified hazards in the mine that were of potential threat to the eye (Table 3). Dust was named as the most potential threat to the eye by the miners for which eye protection was needed. One hundred and sixty one who took part in the study representing 39.7% named dust as the most present visual hazard in the mine. Chemicals were named second by 93 respondents with a percentage score of 22.9%. Fifty (12.3%) workers did not know or were not aware of the presence of any visual hazards in the environment while other potential visual hazards recorded 4.9%. When quizzed about their general impression of eye safety in terms of enforcement by management and adherence by workers, overall, 219 of 399 (54.9%) rated it as good, 61 (15.3%) rated it very good and 54 (13.5%) as excellent. Forty five individual representing 11.1% rated the mine eye safety standards as fair, and the least rated was poor by 20 individuals with a percentage of 5.0%.

DISCUSSION

Prevention of the eye from exposure to hazards and injuries is part of the field of occupational safety which can be carried out in the most clear-cut manner. Eye injuries in the workplace however continue to be major cause of morbidity and disability, despite well publicized standards for industrial eye protection. The research sought to undertake eye risk assessment and map up solution patterns by recommending appropriate remedies which when applied will help control preventable occupational eye injuries and disorders; the driving force behind this present study.

Visual examinations in this study were carried out by adopting recommended standardized test procedures, similar to those seen in other studies. The results must however be carefully be extrapolated since the background and hazards in other mining and industrial settings might differ from what pertains at Goldfields, Tarkwa (Desai *et al.,* 1990; Yoruk *et al.,* 2008; Okoye and Umeh, 2002; Abiose and Umeh, 1980; Davies *et al.,* 2007). The mean age of workers in the study was higher than that found in other industrial establishments in Saharanpur, Turkey and Germany (Yoruk *et al.,*2008; Titiya and Murthy,1998; Nicaeus *et al.,*1996). The much older workforce in the mining industry may be due to the ageing population of the country and the fact that the main occupation in the Wassa-West District (Tarkwa) is mining so most of them stay on the job till they retire (Mba, 2010). Majority of the workers (mine workers) had essentially normal visual acuities (Table 1) indicating that the hazards of the working environment have had little impact on the visual status of the workers. However, future visual implications can be far reaching as disorders such as pterygium, cataract and chronic conjunctivitis could potentially obstruct vision at a later stage resulting in needless impairment of vision or blindness (Shields and Sloane, 1991; Akabzaa and Darimani, 2001; ARICANEWS, 2000).

The eye disorders reported in this study (Table 2) were common to those found in studies in other industrial establishments in the world since the risk factors of exposure to ultraviolet radiation and dust were common. (Desai *et al.,* 1990; Yoruk *et al.,* 2008; Okoye and Umeh, 2002; Abiose and Umeh, 1980; Davies *et al.,* 2007). More prominent were diseases caused by carcinogenic and irritant substances and exposure radiations such as cataract, pterygium and chronic conjunctivitis. The mine workers come into direct contact with the visual hazards comprising projectiles and falling objects, dust, chemicals, machinery, heat, intense illumination, smoke, heavy computer usage, and dim/dark room (Table 3). This could have contributed to the prevalence of ocular injury and foreign body sensation recorded in this study especially among technical sections namely mining, engineering, metallurgy and mineral resources departments. There is therefore the need

to wear protective eye devices at all times. The workers should understand the need for safety as the majority has attained at least an intermediate level of education.

Although, an appreciable 67.7% of respondents wore eye protection, some inadequacies regarding the use of ocular protective wears were identified which serves as barrier to ocular protection (Lombardi *et al.,* 2009). Some protective goggles did not fit well allowing fumes, dust and smoke to enter their eyes. Some miners also did not use their eye wear frequently while others complained that their damaged or loss wears were not replaced on time, forcing them to work without protection. The substantial use of protective eye wears reflected considerably, low prevalence of injury recorded in this study. The use of eye protection has been found to contribute substantially to the prevention of eye injuries. (Okoye and Umeh, 2002; Nicaeus *et al.,*1996; Lye, 1995; De la Hunty and Sprivulis, 1994;.Frobose and Gruntzig, 1984; Vasu *et al.,*1990 (Occupational Vision Requirements, 1994).

Nevertheless, there is a need for a strong advocacy and worker education to record a reduced or zero eye accidents at the mine. It was noted that the mine had a standby emergency and first aid team, but it was found that there is no eye first aid personnel among the team. The safety and health training of the mine safety officers were also devoid of eye safety educations. This is against the background that providing eye first aid for injuries involving sensitive parts of the eye such as the cornea ensures re-epitheliazation and comfort in corneal abrasions and prevents visual impairments as a result of injuries sustained (Peate, 2007). A well equipped eye safety tray should contain topical anaesthetic, fluorescein dye, foreign removal spud, a short acting mydriatic agent and antibiotics.

Potential visual hazards identified in the mine were projectiles and falling objects, dust, chemicals, machinery, heat, intense illumination, smoke, heavy computer usage, and dim/dark room. Goldfields (Gh.), Tarkwa was recertified to the new occupational health and safety advisory services (OHSAS) 18001:2007 standard by the Bureau Veritas in 2007 due to its commitment to the safety of its workers (Goldfields International Ltd, 2008). The mine also operates an occupational health and safety policy which provides strategic guidelines as to the intent and action required by each miner throughout the organization. The low scale of injuries affirms the assertion that Ghanaian large scale mines are among the safest in Africa and the world at large (Agbesinyale, 2003).

CONCLUSION

From the study we concluded that there appears to be occupationally related eye diseases and disorders among the mine workers at Goldfields (Gh.) and also confirmed that there are visual hazards in the mine against which eye protection is needed. Generally however, the mine has a good rating as far as eye safety is concerned as expressed by the miners overall impression of management attitude and their adherence to eye safety and precautionary measures. Based on the findings, we recommend the education of managers, workers and purchasing officers on eye safety and its tenets the vigorous enforcement and use of effective eye protective wear in high-risk areas in the mine.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Hypoglycaemic activity of ethanolic leaf extract and fractions of *Holarrhena floribunda* (Apocynaceae)

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Holarrhena floribunda **is a common plant that has traditionally been used in Africa to treat many diseases such as fever, dysentery, sterility and diabetes. This study was set out to evaluate the hypoglycaemic properties of ethanolic leaf extract of *Holarrhena floribunda* and various fractions of this extract in normal fasted and fed-hyperglycaemic rats. Blood glucose levels (g L^{-1}) were determined at the following times: 1) after a 12 hours period prior to drug administration, 2) An hour after the oral administration of the extract (250-1000 mg kg^{-1}), its fractions (1000 mg kg^{-1}), Glibenclamide (10 mg kg^{-1}) or the vehicle and 3) one and four hours after the oral overload of anhydrous glucose (4 g kg^{-1} body weight). The extract showed a remarkable dose-dependent down-regulation of blood glucose in fasted rats at 1000 mg kg^{-1} ($p<0.05$) and significantly reduced or totally prevented the induction of hyperglycaemia at 500 and 1000 mg kg^{-1} respectively. This Glibenclamide-like hypoglycaemic activity of the extract was found to be present in the dichloromethane and ethyl acetate fractions of the plant. Our results show that the leaves of *H. floribunda* possess hypoglycaemic properties and strongly suggest that its usage in traditional and to a larger extent orthodox medicine may be fully explored.**
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Keywords*:* Herbal medicine, bio-guided fractionation, hypoglycaemia, diabetes mellitus, Glibenclamide

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterised by hyperglycaemia resulting from a deficit or malfunction in insulin secretion and/or insulin action, both of which cause the impaired metabolism of glucose, lipids and proteins (Gao *et al.,* 2010). The chronic hyperglycaemia of diabetes is associated with the long term damage, dysfunction and failure of various organs (Lyra *et al.,* 2006). It is the leading cause of kidney failure, heart attack, blindness and lower limb amputation and the fourth main cause of death in most developed countries (Eseyin, 2010).

DM, an epidemic occurring in adults throughout the world and affecting more than 4% of the population worldwide (Kim *et al.,* 2006, Eseyin, 2010), is a major public health problem. Its prevalence in Africa was predicted to increase by 93% in the last 15 years (IDF, 2003). There are no recent data on the prevalence of diabetes in Côte d'Ivoire, but the prevalence was estimated to be 3 - 7% in Abidjan, the main city of the country (Lokrou et *al.* 1986; Sobngwi *et al.*, 2002).

One of the strategies in the treatment of DM is based on the use of oral hypoglycaemic agents such as biguanides and sulfonylureas, or insulin to lower blood glucose levels to normal ranges (Teves

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et al., 2004; Kennan *et al.,* 2005). The search for new agents with lower cost and better efficiency has therefore become a matter of major priority. The great number of plants used to manage diabetic patients in Africa might provide a useful source for the discovery of new compounds that can be used as pharmaceutical entities or simple dietary adjuncts to existing therapies (Hostettmann *et al.,* 2000; Tra Bi *et al.,* 2008). *Holarrhena floribunda* (G. Don) Dur. et Schinz (Apocynaceae), commonly called false rubber tree, is a tropical tree that grows to 17 meters high and 1 meter in girth in the deciduous forest and savannah woodland. It is widely distributed in Côte d'Ivoire, Burkina Faso, Mali and some other parts of West Africa. In these regions, the stem-bark and leaves are used to treat various afflictions such as malaria, fever, dysentery, amoebic diseases, diarrhoea, sterility, amenorrhea and diabetes (Arbonnier, 2002; Fotie *et al.,* 2006; Bayala *et al.,* 2006).

To date, there is paucity of data from studies on anti -diabetic properties of *Holarrhena floribunda.* From the World Health Organization recommendation for the evaluation of the real potential of medicinal plants (WHO, 1980) due to their poor scientific scrutiny and justification for traditional use, the present investigation sought to determine the hypoglycaemic activity of crude ethanolic leaf extract of *Holarrhena floribunda* and its various fractions in normoglycaemic rats.

MATERIALS AND METHODS

Plant collection

The leaves of *Holarrhena floribunda* (G. Don) Dur. et Schinz (Apocynaceae) were collected in Abidjan (the southern region of Côte d'Ivoire) in October 2007. The plant was identified and authenticated by Professor Aké-Assi Laurent, National Floristic Centre of the University of Cocody, Abidjan. A voucher specimen (n°13240) of the plant was deposited in the herbarium of the National Floristic Centre of the University of Cocody, Abidjan.

Preparation of extract and fractions

The leaves of *H. floribunda* were cleaned, washed with water, sliced into small pieces, air dried at ambient temperature for two weeks and then ground into powder using a cutting mill (Retsch SM 100-1390 rev/min, Labo and Co, France). The powder (100 g) was extracted with 2 litres of a solution of ethanol (96%)/water (80:20, yielding a final concentration of ethanol of 76.8%) for 24 hours with constant stirring using a shaking water bath (Kottermann, Germany) (this operation was repeated twice). The extract was filtered twice through cotton wool, then through a filter paper (Whatman grade 1, Sigma-Aldrich, France). The filtrate was concentrated using a rotavapor (Flawil, Switzerland) at 45°C, and dried on a water bath (Kottermann, Germany) to obtain 12.86 g of extract, corresponding to a yielded percentage of 12.86% of the starting material. The dried extract (10 g) was suspended in water (100 mL) and the clear supernatant was successively partitioned (1:1, v/v) by hexane, dichloromethane, ethyl acetate and *n*-butanol to obtain fractions of hexane (HF), dichloromethane (DMF), ethyl acetate (EAF), n-butanol (BuF) respectively. The remaining fraction was designated as aqueous fraction (AF). Each fraction was concentrated and freeze-dried, to yield about 640 mg of HF (6.4%), 720 mg of DMF (7.2%), 1800 mg of EAF (18%), 1080 mg of BuF (10%) and 2500 mg of AF (25%).

Animals

Healthy adult albino Wistar rats (age 4 to 5 weeks, weighing 100 to150 g) of both sexes were provided by UFR Biosciences (University of Cocody-Abidjan, Côte d'Ivoire) and were housed in stainless steel cages (34 cm ´ 47 cm ´ 18 cm) with soft wood shavings as bedding, fed with normal commercial pellet diet (Ivograin®, Abidjan, Côte d'Ivoire) and were given water *ad libitum*. They were allowed to acclimate to standard laboratory temperature conditions (temperature 24-28 °C, relative humidity 60-70%, and 12 hour light-dark cycle) for a week before the experiments. They were deprived of food for at least 18 hours prior to experiments but allowed free access to drinking water. The equipment usage and handling of the animals were performed in accordance with the European Council legislation 87/609/EEC for the protection of experimental animals (Mitjans, 2008). The pro-

tocols for the study were approved by the Departmental Ethics Committee.

Phytochemical analysis
The extract of *H. floribunda* and its fractions were screened for the presence of terpenes, flavonoids, sterols, alkaloids, tannins, coumarins and polyphenols. The detection of these constituents was performed according to the methods described in Bekro et al. (2007).

Acute toxicity
Thirty five rats were divided in seven groups of five animals. The ethanolic leaf extract of *Holarrhena floribunda* (HFE), was administrated orally at doses of 250, 500, 1000, 2000, 4000 and 6000 mg kg^{-1} body weight to the animal groups (one dose per group). The control group received distilled water, at 10 ml kg^{-1}. The animals were observed continuously for 2 h under the following profiles (Barik et al., 2008): (I) Behavioural profile (alertness, restlessness, irritability, and fearfulness), (II) Neurological profile (spontaneous activities, reactivity, touch response, pain response and gait, postural abnormalities), and (III) Autonomic profile (defecation and urination). After a period of 1, 3 and 14 days they were observed for any lethality or death.

Experimental design and induction of hyperglycaemia
For the hypoglycaemic studies, rats were randomly divided into five groups of six rats per group. Group 1 received distilled water orally, group 2 received Glibenclamide (10 mg/kg) (Sanofi-Aventis Pharmaceuticals, NJ, USA) and groups 3, 4 and 5 received 250, 500 or 1000 mg of extract/kg body weight, respectively. These groups were named HFE 250, HFE 500, and HFE 1000. The experimental design of the study consisted of a 12 hours fasting period followed by drug pre-treatments and finally the induction of hyperglycaemia by the oral administration of 4 g kg^{-1} of anhydrous glucose (Oral Glucose Tolerance test). Glibenclamide was used in the study as an anti-diabetic reference drug. Blood glucose levels were measured after the fasting period (T_0), an hour after pre-treatment (T_1) and one and four hours after induction of hyperglycaemia (T_2 and T_3, respectively). Blood samples were obtained by nicking the tails with a sharp razor (Aydin et *al.*, 1995), and glucose concentrations were determined using a one-touch glucometer (Accu-Chek Go®, Roche Diagnostics, Mannheim, Germany). The percentage of glycaemic variation was calculated as a time function by applying the following formula:

$$\% \text{ of blood glucose change} = \left(\frac{Gx - G0}{G0}\right) \times 100$$

Where G_0 = initial blood glucose values and G_x = blood glucose values at x hours time interval.

In a subsequent experiment, rats (n=5 in each group) in the same conditions as described above were pre-treated with a single dose (1000 mg kg^{-1}) of the various fractions (HF, DMF, EAF, BuF and AF), distilled water (Vehicle-control) and Glibenclamide (10 mg kg^{-1}, the reference drug). The hypoglycaemic effects were measured at T_0 (during fasting period), T_1 (an hour after glucose load) and T_2 (two hours after glucose load) and the percentage of blood glucose variation calculated as described above.

Data Analysis
Results are expressed as the mean ± SEM. Data were analysed for statistical significance with a one or two-way ANOVA followed by the Fisher-Snedecor test in the SAS statistical program (SAS, 1999) or by a Bonferroni's post hoc test or a Dunnett's Multiple Comparison Test. At a 95% confidence interval, a p value ≤ 0.05 was considered statistically significant. The graphs were plotted using Sigma Plot for Windows Version 11.0 (Systat Software Inc., Germany).

RESULTS

Phytochemical analysis
Screening the *H. floribunda* ethanolic leaf extract for various phytochemical constituents revealed the presence of components such as terpenes, flavonoids, sterols, alkaloids, tannins, coumarins and polyphenols, but the absence of quinones

Acute toxicity study

The behaviour and faeces of the animals were normal. No observations of any other signs of weakness or mortality in rats receiving orally up to 6000 mg kg^{-1} body weight of the extract. This finding suggests that ethanolic leaf extract of *H. floribunda* leaf is safe or non-toxic to rats.

Effects of the ethanolic extract and its fractions on fasted and fed-hyperglycaemic rats

The effects of increasing doses of the extract on blood glucose levels in fasting and fed-hyperglycaemia in normal rats are shown in Table 1 and Figure 1. In fasted rats (T_0), the measures of blood glucose did not show any significant changes between groups, with a mean blood glucose value of 0.93 ± 0.02 g L^{-1}. An hour after administration of extract and the reference drug in fasted rats (T_1), the results showed that blood glucose levels decreased significantly in rats treated with the Glibenclamide (from 0.89 ± 0.05 to 0.62 ± 0.05 g L^{-1}, $p<0.05$; Table 1, Figure 1) and the extract at 1000 mg kg^{-1} (from 1.0 ± 0.06 to 0.78 ± 0.03 g L^{-1}, $p<0.05$), exhibiting blood glucose decreases of 30 and 22%, respectively.

An hour after the administration of anhydrous glucose (T_2), an important rise ($p<0.001$) in blood glucose level was observed in the vehicle-treated group (corresponding to an increase of 66.66%) (Table 1, Figure 1(A)). This significant increase in blood glucose level at T_2 was also observed in the Glibenclamide group, although to a lesser extent (only 3.37% of increase). The fed-hyperglycaemic animals treated with the ethanolic leaf extract of *H. floribunda* also showed a significant increase in blood glucose levels, although this increase was limited to 25% in the HFE 250 group and 20.43% in the HFE 500 group. Results further showed that the 1000 mg kg^{-1} dose of the extract totally prevent the induction of hyperglycaemia in rats (Table 1, Figure 1(A)).

Four hours after the administration of anhydrous glucose (T_3), it was observed that blood glucose levels were maintained at significantly higher levels in the vehicle-treated group ($p<0.005$) and the HFE 250 group ($p<0.05$) when compared to the blood glucose levels obtained at T_0. However, in the Glibenclamide, EHF 500 and HFE 1000 groups, the blood glucose levels at T_3 had returned

Table 1: Effects of the ethanolic leaf extract of *H. floribunda* (HFE) on normal rats (n =6)

Groups	Blood glucose (g/l)			
	T_0 (initial time)	T_1 (1 hour)	T_2 (2 hours)	T_3 (6 hours)
Control	0.81 ± 0.03	0.85 ± 0.01	1.35 ± 0.16 ###§§§	1.18 ± 0.06## §
Glibenclamide	0.89 ± 0.05	0.62 ± 0.05* #	0.92 ± 0.14*** §	0.74 ± 0.09***
HFE 250	1.0 ± 0.03	1.0 ± 0.05††	1.25 ± 0.12† # §	1.26 ± 0.07††† #§
HFE 500	0.93 ± 0.03	0.85 ± 0.05†	1.12 ± 0.15* §	0.94 ± 0.08* £
HFE 1000	1.0 ± 0.06	0.78 ± 0.03$ #	0.97 ± 0.07** $	0.95 ± 0.06 * $

Values are expressed as mean ± SEM (n = 6). Statistical comparison: # T_0 vs (T_1, T_2 or T_3); § T_1 vs (T_2 or T_3); * Control vs (Glibenclamide, HFE250, HFE500 or HFE1000); † Glibenclamide vs (HFE250, HFE500 or HFE1000); £ HFE250 vs HFE500; $ HFE250 vs HFE1000. **For *, #, †, §, £ or $, p<0.05; for **, ##, ††, p<0.01 and for ***, ###, †††, §§§, p<0.001; one-way ANOVA followed by a Fisher-Snedecor multiple comparison test.**

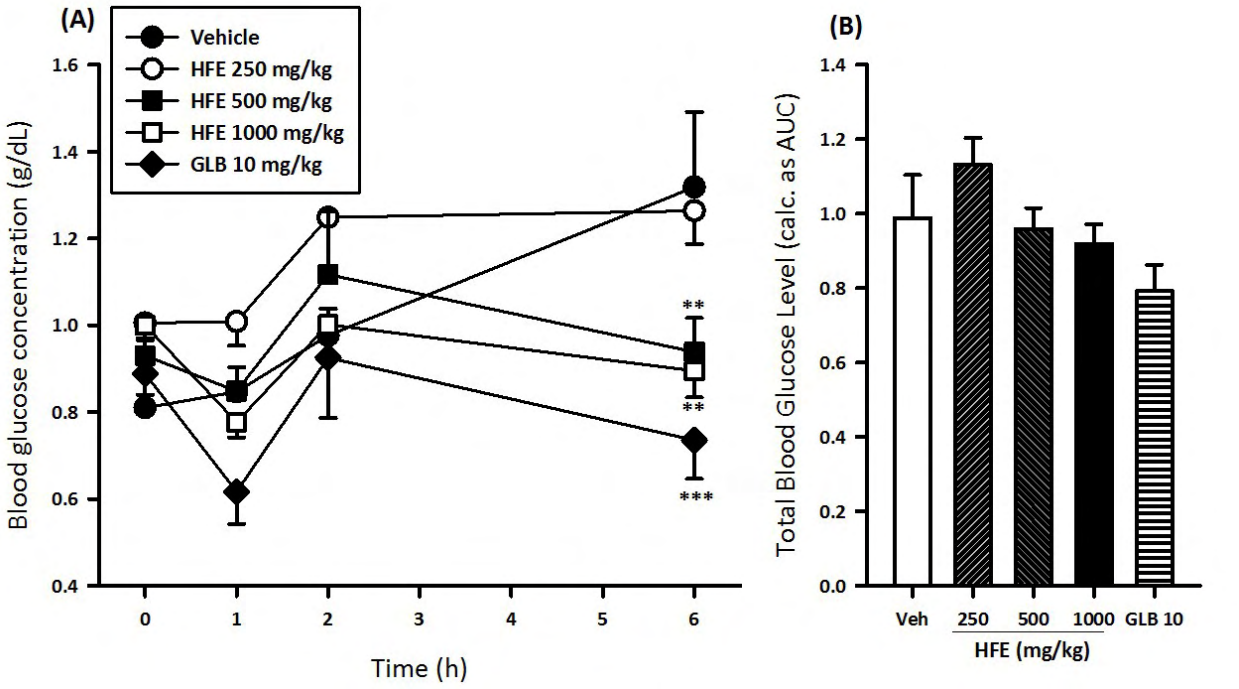

Figure 1: The dose-response effect of HFE 250-1000 mg/kg and glibenclamide (GLB) 10 mg/kg on blood glucose concentration of rats. Left panel (A) show the time-course effects over a six-hour period and the right panel (B) show the total blood glucose level calculated from AUC's over the six-hour period. Data are Means ± SEM (n = 6). $^{**}p < 0.01$, $^{***}p < 0.001$ compared to vehicle treated group (two-way ANOVA followed by a Bonferroni's post hoc test), data in panel (B) were compared to vehicle treated group (Veh) (one-way ANOVA followed by a Dunnett's Multiple Comparison Test).

to their initial values (Table 1, Figure 1(A)). The results of the total blood glucose level (as calculated from the area under the curve, AUC) showed a non-significant dose-dependent decrease in blood glucose concentration by the extract (250-1000 mg kg^{-1}) (Figure 1(B)).

The results obtained for the effect of the various fractions are presented in Figure 2. In the vehicle-treated rats a rise in blood glucose level was observed at one (T_1) and two hours (T_2) after the oral glucose load. In the opposite, Glibenclamide-treated rats showed a significant decrease of blood glucose before at T_0 ($p<0.05$) and after glucose load at T_1 and T_2 ($p<0.001$). Only the dichloromethane and ethyl acetate fractions-treated groups exhibited a significant decrease of blood glucose when compared to vehicle-treated rats. An increase of blood glucose was observed with the rest of the fractions (Figure 2(A)). Among the fractions, though not significant, the total effect on the blood glucose levels were reduced most in the dichloromethane and ethyl acetate fraction-treated groups yet these reductions were inferior to that produced in the Glibenclamide-treated group (Figure 2(B)).

DISCUSSION

The aim of the present study was to evaluate the hypoglycaemic activities of a crude ethanol extract of *Holarrhena floribunda* leaves and the fractions from this extract. This plant was ethno-botanically selected during a 2005 survey in the City of Abidjan

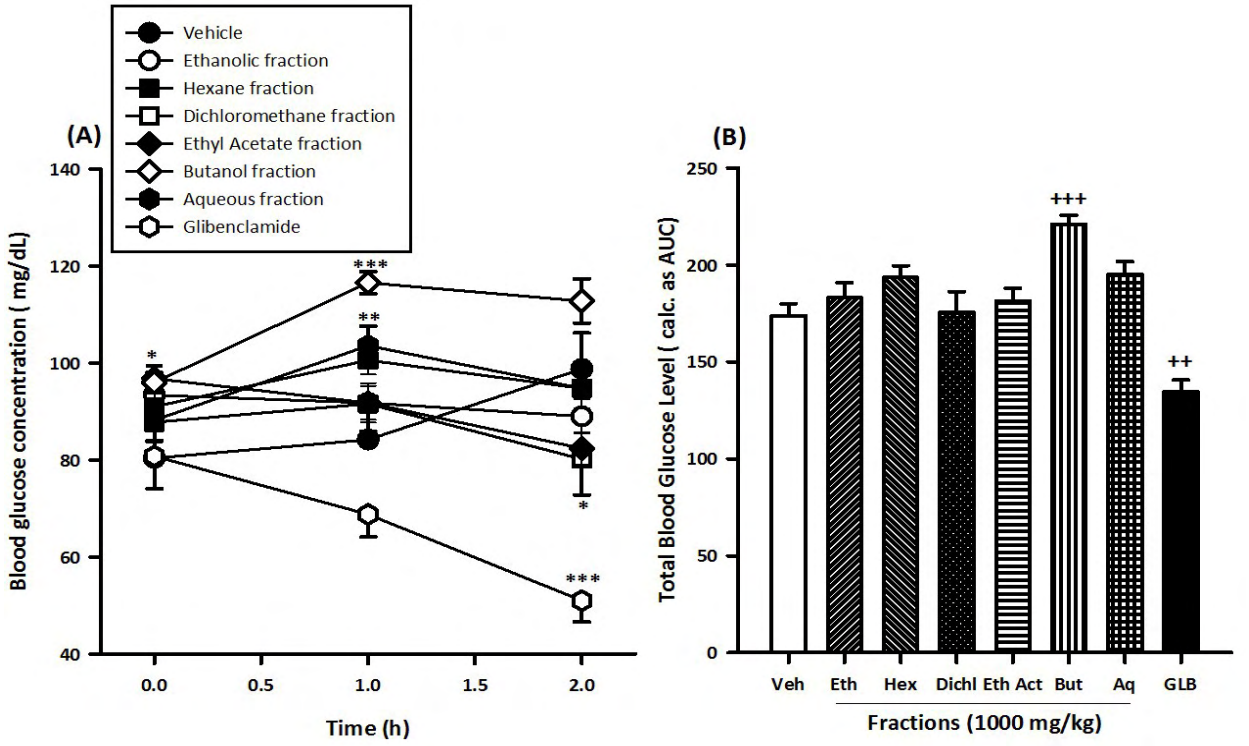

Figure 2: The hypoglycaemic effect of the various fractions of HFE (1000 mg/kg), HFE (1000 mg/kg) and glibenclamide (GLB) 10 mg/kg on blood glucose concentration of rats. Left panel (A) show the time course effects over a two-hour period and the right panel (B) show the total blood glucose level calculated from AUC's over the two-hour period. Data are Means ± SEM (n = 5). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to vehicle treated group (two-way ANOVA followed by a Bonferroni's post hoc test), $^{++}p < 0.01$, $^{+++}p < 0.001$ compared to vehicle treated group (one-way ANOVA followed by a Dunnett's Multiple Comparison Test).

(Côte d'Ivoire) and confirmed by providers of Ivorian traditional medicine, including 9 herbalists and 2 healers. The results of the survey (*data not shown*) have allowed us to collect 21 anti-diabetic plant species, among which *Holarrhena floribunda* was the most frequently quoted. Moreover, to our knowledge this plant has never been studied for its anti-diabetic properties.

The oral glucose tolerance test in normal rats is a simple and appropriate test used by many authors in similar studies and has been used as a routine tool for the preliminary screening of the hypoglycaemic properties of many medicinal plants (Prakasam *et al.,* 2003; Somani *et al.,* 2008; Yasodha *et al.,* 2008). In human, the glucose tolerance test is a standard procedure that is used to diagnose diabetes. One to five percent of people with impaired glucose tolerance (IGT) actually develop diabetes each year. Since impaired oral glucose tolerance (IGT) is indicative of a diabetic predisposition, agents that are capable of bringing blood glucose concentrations within normal limits will help to arrest the progression of impaired glucose tolerance to diabetes (Eseyin, 2010).

The blood glucose values measured in the fasted rats during this study showed that they were normoglycaemic.. These blood glucose values were similar to literature reports describing blood glucose levels in normal rats of equal weight (Dimo *et al.,* 2007; Somani *et al.,* 2008; Yasodha *et al.,* 2008).

The present study shows that the ethanolic leaf extract of *H. floribunda* dose-dependently decreases blood glucose levels in fasted rats and prevents fed-hyperglycaemia induction. In fasted rats, the extract only caused a significant decrease in blood glucose level at the 1000 mg kg^{-1}, which is similar to the hypoglycaemic effect observed with Glibenclamide. The hypoglycaemic effect of Glibenclamide, a second generation sulphonylurea, is explained by both an increase in the endogenous insulin release from pancreatic β-cells and the promotion and facilitation of peripheral glucose uptake and utilisation (Moller, 2001). The *H. floribunda* leaf extract may possibly exert its hypoglycaemic action by similar mechanisms, but further studies should be performed to confirm this hypothesis.

The oral administration of the ethanolic leaf extract of *H. floribunda* also dose-dependently reduced or suppressed the increase in blood glucose level induced by anhydrous glucose, as shown by the limited hyperglycaemic increases of 25% and 20.43% in the HFE 250 and HFE 500 groups, respectively. Moreover, results showed that the 1000 mg kg^{-1} dose of the ethanolic extract totally prevented induction fed-hyperglycaemia in rats. This effect could be explained at least in part by a decrease in intestinal glucose absorption achieved by an extra-pancreatic action that includes the stimulation of peripheral glucose utilization or the enhancement of glycolytic and glycogenic processes with a concomitant decrease in glycogenolysis and gluconeogenesis (Yasodha *et al.,* 2008). Indeed, ethanol extracts from the genus *Holarrhena* have been shown to exert inhibitory activity toward α-glucosidase (Prashanth *et al.,* 2001).

The effect of the various fractions of the ethanolic extract of *H. floribunda* on the blood glucose kinetics in the oral glucose tolerance tests in rats suggest that the hypoglycaemic activity of the ethanolic extract is found in the dichloromethane and ethyl acetate fractions. Thus further work on the effect of these fractions in a diabetic model will produce useful information in the development of a newer therapy for diabetes from this plant source.

The significant increase in the blood glucose level by the butanolic fraction could possibly be due to the presence of an important amount of reducing sugar. This information, if authenticated by a further phytochemical analysis of this fraction, would suggest and recommend to traditional practitioners to avoid preparations from this plant that may be enriched in very polar components in the management of diabetes. The phytochemical screening performed in this study revealed the presence of important components like terpenes, flavonoids, sterols, alkaloids, tannins, coumarins and polyphenols in *H. floribunda* leaves, but also indicated the absence of quinones. This result is in agreement with data reported by other investigators (Hodek *et al.,* 2002; Cemeli *et al.,* 2004; Valachovicova *et al.,* 2004; Zieran *et al.,* 2004; Bogne *et al.,* 2007). The hypoglycaemic property of *H. floribunda* could be mediated by some of these active chemical constituents. Indeed, flavonoids, terpenes, tannins and coumarins have been shown to possess hypoglycaemic activity (Marles and Farnsworth, 1995, Ojewole, 2002). The hypoglycaemic action of flavonoids was reported to be caused by the stimulation of insulin secretion from pancreatic ß-cells or by an insulin-like effect (Marles and Farnsworth, 1995). It has also been shown that alkaloids possess anti-hyperglycaemic activity that is mediated by the inhibition of α-glucosidase (Prashanth *et al.*, 2001).

CONCLUSION

The present study suggests that the leaf extract of *H. floribunda* possesses hypoglycaemic properties in normal rats and this activity lies within the dichloromethane and ethyl acetate fractions of the extract. This study provides some scientific justification for the traditional use of *H. floribunda* in the management of diabetic patients in Côte d'Ivoire.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Ocular discomforts following eyelash extension

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Eyelash extension has become common practice for enhancing beauty among Ghanaian women on occasions such as weddings, festivities, and other social gatherings including funerals. This study was therefore conducted to ascertain the effect of eyelash extension on the eyelid and on vision. One hundred and twenty (120) females who had had prior experience with eyelash extension were interviewed on; reasons for extension, frequency of extension, and various problems encountered after extension of their eyelashes. Majorities (94.2%) of the respondents were aged 10-39 years and were literate (91.5%). About 70% (i.e. 69.2%) have undergone eyelash extension more than once. The major purpose (81.6%) for extending their eyelashes was to enhance their beauty, with eyelash replacement accounting for only 1.7% of respondents. One hundred and seventeen (97.5%) had one or more of these problems; dry eyes, itchy eyelids, tearing, burning sensation, lid swelling and pain, casting of shadows in vision, misdirected eyelashes, purulent discharge and eyelashes falling into the eye. Thirty nine (32.5%) had difficulty removing the eyelash extensions. Irrespective of the various difficulties they encountered 65% still intend fixing their eyelashes again because they thought it was fashionable. Eyelash extension could have unfavourable effect on the eyelid and vision. Beauticians should be well trained to ensure safety of the procedure while females should be educated on the potential ocular health hazards of eyelash extension.
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Keywords*:* Itchy eyelids, dry eyes, lagophthalmos, misdirected eyelashes, false eyelashes, eyelash baldness

INTRODUCTION

The eyelids are modified folds of skin closing the front of the orbit which act to protect the anterior surface of the globe from local injury (Agarwal, 2002). Additionally, they aid in regulation of light reaching the eye; in tear film maintenance, by distributing the protective and optically important tear film over the cornea during blinking and in tear flow, by their pumping action on the conjunctival sac and lacrimal sac (Patel and Meyers, 2011). Eyelashes, which are found on the margins of eyelids, serve to protect the eye from foreign objects such as sand and dust among others (Catania, 1996; Edwards, 2011). Eyelashes are shed, like other types of hair, from the follicle. Each eyelash has its own growth cycle (anaphase) that lasts six to eight weeks so that most eyelashes are present to maintain their collective protective mechanisms.

Eyelash extension has become the latest cosmetic trend all over the world. Long eyelashes are considered a sign of femininity in many cultures, as a result some women seek to enhance their eyelash length artificially, using eyelash extensions (Hadza, 2003). False eyelashes can be used for individuals who have thin or short eyelashes along with mascara to thicken the look of eyelashes which creates a bold look (Champion, 2009). According to Julia, (2011), an Arizona based beautician, eyelash exten-

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sion is a revolutionary way to extend the length and thickness of your eyelashes. The false eyelashes used in the extension are synthetic and tapered from thick to thin to resemble a real eyelash. It is applied on a hair by hair basis to one's lashes for a complete natural look.

To the beautician, the procedure may seem simple. However, from the health care point of view, it is still important to pay close attention to its effect on vision and how safe the procedure is as the intermittent fixing of artificial lashes may interfere with the normal periodic shedding and growth of new lashes and cause some discomforts. Eyelash extension could irritate the eyelid or clog follicles in the eyelid as well as pull out eyelashes during removal (Sachdeva and Prasher, 2008). The procedure does not allow frequent washing of the eyelids which could result in bacterial and/or fungal infection seen as ocular discharge with conjunctivitis and eyelid diseases (Sanford-Smith, 1986). The eyelashes may also be displaced when wet with water or sweat and serve as foreign body on the eye. The aim of the study therefore was to establish the comfort and safety or otherwise of eyelash extension. This will involve ascertaining the frequency of the procedure, identifying reasons why some female extend their eyelashes, finding the class of females involved in the procedure, and identifying some problems they face as a result eyelash extension.

MATERIALS AND METHODS

Study Area

The study was conducted in Kumasi Metropolis of the Ashanti Region of Ghana. Kumasi (Area: 254 km^2) is the second-largest city in the country (after the country's capital city Accra) where tradition is held very high and blends very well with modernity. Kumasi is approximately 300 miles (480 km) north of the Equator and 100 miles (160 km) north of the Gulf of Guinea. It is popularly known as "The Garden City" or "Heart Beat" of Ghana because of its many beautiful species of flowers and plants. In the city, data was collected from some of the well-developed suburbs like Adum, Patasi, Asokwa, Atonsu, Ashanti New town, Santasi and Bantama.

Study design

Questionnaire, which consisted of open and closed-ended questions on the subject, were administered purposively to 120 females in saloons, shops and homes. Information sought for included; demography, reasons for eyelash extension, frequency of extension and various problems encountered after eyelash extension. Interpretation and/or translation were given to those who could not understand clearly the questionnaire.

Ethical considerations

The study was approved by the ethics committee of the College of Health Sciences, the Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. Consent of the respondents was also sought through formal notification. Participants were alerted that participation in the study was voluntary and that they were free to withdraw from participation at any time.

Limitations

Clients' visual acuities were not known before they underwent eyelash extension. The effect on visual acuity therefore cannot be deduced as there was no reference point. Furthermore, males were excluded because eyelash extension is notable among women who are much particular about their eyelashes, showing sign of femininity.

Data Analysis

Data obtained after administration of the questionnaires was analyzed using SPSS 17 for windows. Graph was plotted using GraphPad prism Version 5 for Windows.

RESULTS

Among the 120 females interviewed, 94.2% were between the ages of 10-39 years with the highest number of respondents (68.4%) between the ages 20-29 (Figure 1A). Majority (69.2%) had extended their eyelashes more than once with approximately 50% of them having done it more than three times (Figure 1B). Forty two percent (42%) of the females had secondary education, 33% tertiary education, 18% primary education, with only 7% having no form of education (Figure 1C). The purpose for

the majority (81.6%) extending their eyelashes was to enhance their beauty with eyelash replacement accounting for only 1.7% (Figure 1D).

All participants identified with one adverse event or the other with the majority (n=58) reporting of three to four adverse effects after the procedure (Figure 2A). The adverse events reported after the procedure were dry eyes, itchy eyelids, tearing, burning sensation, lid swelling and pain, casting of shadows on vision, misdirected eyelashes, heavy eyelids, pus release and eyelashes falling into the eye. Figure 2B is a descriptive statistics of the number of participants affected by each of the adverse events (Figure 2A). Asked whether the participants would want to go through the procedure again, 65% responded in the affirmative, whilst 35% said they had no intention of extending their eyelashes again.

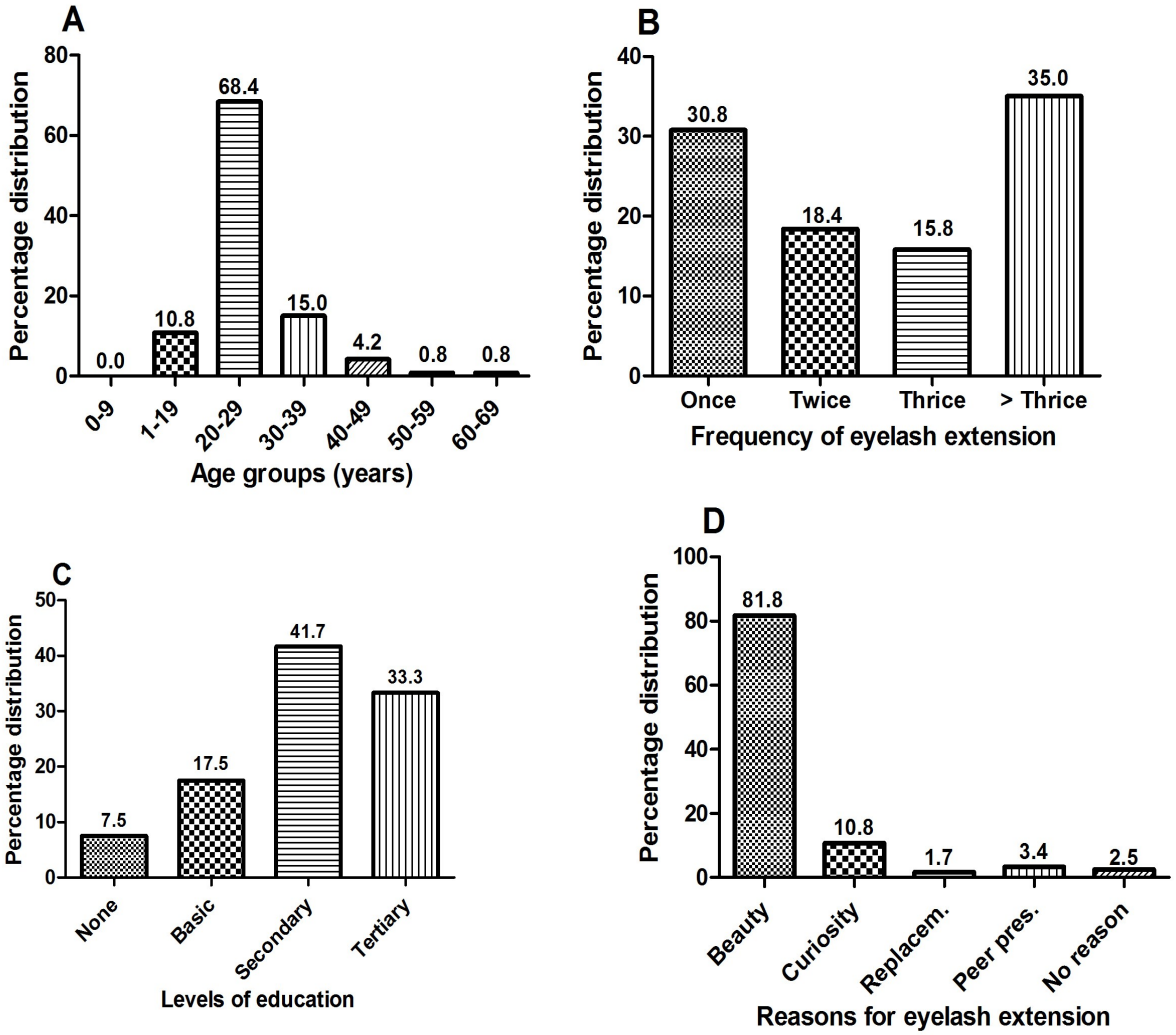

Figure 1: Percentage distribution of the studied population according to age (A), frequency of eyelash extension (B), the level of education attained (c) and the reasons for eyelash extension (D)

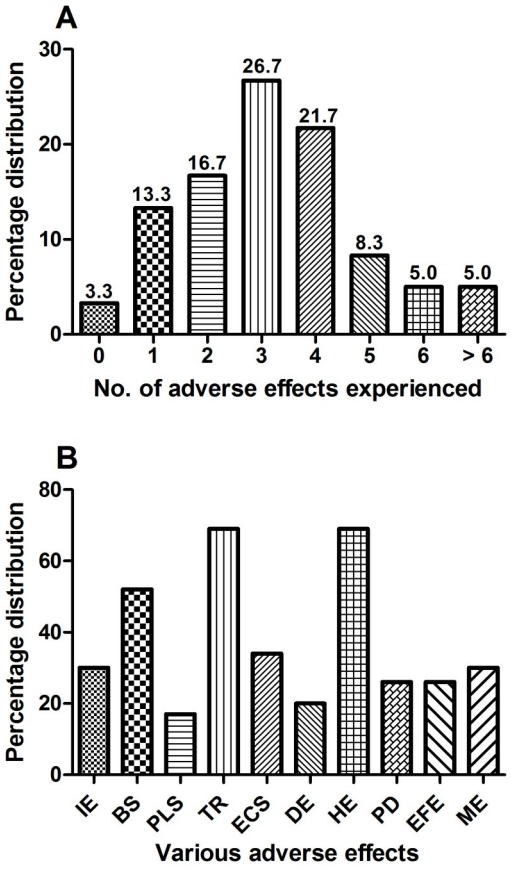

Figure 2: Percentage distribution of the studied population according to the number of adverse effects experienced (A) and the various adverse effects (B). Itchy eyelids (IE), burning sensation (BS), pain and lid swelling (PLS), tearing (TR), eyelashes casting a shadow on vision (ECS), dry eyes (DE), heavy eyelids (HE), purulent discharge (PD), eyelashes falling into the eye (EFE), and misdirected eyelashes (ME).

DISCUSSION

Eyelash extensions involve a number of enhancements designed to add length, thickness and fullness to natural eyelashes (EEC, 2012). Eyelash extension is gradually becoming common practice in Ghanaian cities. Findings from this study showed that 94.2% of the respondents were aged 10-39 years and 91.5% were literates' giving an indication that eyelash extension has caught on with the literate population and the youth. Although these females extend their eyelashes to appear more attractive and to keep abreast with post-modernism, the 10-19 year old females encountered in this study did this to look beautiful for the occasion of being the bride's maid, or flower girl for a wedding, or attempting to enhance ones beauty after graduating from Junior High School.

The age distribution suggests that the economically active age-group of the population (20-29 years) are the majority involved in eyelash extension. It can also be said that this age group represents school going youth groups. Detrimental effects of this procedure on vision therefore could be a risk factor in socioeconomic development (Frick and Foster, 2003). Visual disruptions can and often do interfere with reading and learning. The majority (50%) of the respondents had attended at least a second cycle institution which shows that the procedure is done by the literate population who want to explore other avenues of looking beautiful as this was the main reason for undergoing the procedure. This is quite interesting because one would have thought that, literates should have exempted themselves from eyelash extension for beauty as they are expected to learn more about the procedure before engaging in it. The good news, however, is that findings of the negative implications of eyelash extension could be communicated easily to the literates with better understanding.

Almost all the participants had various problems such as dry eyes, itchy eyelids, tearing, and burning sensation. Research has shown that dry eyes result when there is lack of sufficient lubrication and moisture in the eye (Loft, 2011). Persistent dryness results in itching, burning sensation, as well as foreign body sensation and tearing (Lee *et al.,* 2011). During the entire 90-150 minute procedure, blinking which moisten the ocular surface is drastically reduced (Mouselli, 2010). This could result in dry eyes and its resultant tearing and burning sensation (Bedinghaus, 2007). Occasionally, after eye lash extension, there could be incomplete closure of the eyelids (lagophthalmos) during sleep exposing the

corneal surface to air, dust and microbes among other. This may promote dry eyes syndrome and ocular bacterial or fungal infection (Alfonso, 2008; Amer *et al.,* 2011, Jernigan, 2011).

Some individuals are allergic to the false eyelashes and materials (e.g. glue) used to attach the eyelashes (Jernigan, 2011; The Beauty Insiders, 2012). The glue also causes burning sensation (Dale, 2006; Champion, 2009). A participant said "when the glue melted and mixed with tears, it caused burning sensation in my eyes". Another said "if they change the glue, we can continue doing it", and then one also said "research should be conducted on the composition of the glue to see if it can be modified in order to minimize, if not eliminate totally, the burning sensation it causes". Glue fumes could account for the watery eye and burning sensation. Salons should be using medical or pharmaceutical grade glue which is free of formaldehyde. Non-medical grade glue and glue with formaldehyde can irritate the eyes (GAA, 2012). An experienced and highly regarded professional beautician should perform a patch test in order to determine whether or not an individual is allergic to the materials used (The Beauty Insiders, 2012). According to the participants, most beauticians have not received formal training and therefore are not experts in eyelash extension fixation and for that matter, could end up getting a lot of glue on the eye which can block the puncta if allowed to get to the lower lids. All these contribute to eye irritation and watering (GAA, 2012).

Purulent discharges experienced by 14 of the participants may be due to microbial infection of the eyelid or blepharitis (Grimms and Graham, 2012). Wearing false eyelashes to bed or for more than one day can cause bacteria to collect under the eyelash glue and on the false eyelash, causing eye infections (Champion, 2009). After the procedure, one cannot wash thoroughly her face, let alone the lids and lashes. This reduction in hygienic condition may lead to microbial infection and dirt entering the eye. Other problems encountered as a result of eyelash extension are misdirection of lashes and lashes falling on their eyes of clients. One respondent commented "it is funny because sometimes as you walk, the lashes fall themselves. Some respondents complained that, the artificial lashes fall on their eyes during the procedure when they or the beautician fidget. Others also admitted that, the artificial lashes were not properly fixed (misdirection) and this made some fall on their eyes. One perceived danger is that, the artificial lashes, especially, if the base is calcified with the glue, may scratch the cornea and cause pain as well. The extended eyelashes cast a shadow on vision as some of the lashes were too many and/or too long which might affect the quality of vision.

Another area of concern is the difficulty encountered removing the eyelash extensions. Five individuals experienced pain during the removal and swelling on the upper lid after the removal. Repeatedly pulling off extensions, injure the eyelash hair follicles. The extra weight added to the lashes increasing follicle tension and solvents used to dissolve the sealant glue are potentially harmful to the follicles and irritating to the eyelids (Bauman, 2007). This could explain why those individuals experienced pain during the removal of the synthetic eyelashes and post-removal upper lid swelling.

The lashes get stuck so hard to the lids or the natural lashes that the more they tried to pull it, the more the pain (which eventually leads to some eyelid disorders) and the natural lashes being pulled off (The Beauty Insiders, 2012). Three participants had no option than to leave it permanently on with one participant claiming to have cut the eyelashes short with a pair of scissors. There were comments like "it can pluck your natural lashes if care is not taken", "having done it many times, it has spoilt my natural lashes". According to Trygve Saude (2003), the eyelashes grow and are renewed two or three times a year. This natural phenomenon, if interfered with frequent fixing of false lashes, may pose a risk of eyelash loss or "eyelash baldness" (Bauman, 2007; Jernigan, 2011).

One would have thought that, all these problems encountered, would reduce individual interest in eyelash extension but as much as 67.2% of the clients said they would fix eyelashes again. Thus, so far as eyelash extension enhances one's appearance

irrespective of the aforementioned problems, they may want to have it done again. For the minority who do not want to fix and extend their lashes again only 23 (19%) linked their reason to the associated problems. The others gave reasons such as financial constraints and peer pressure from friends to discontinue this fashion. This therefore indicates that even though there were attending problems associated with the procedure, people do not realize the imminent potentially associated hazards the procedure has on the eye and on vision. Females who patronize this procedure need to be educated on how with eyelash extension one's vision could be affected so as to limit the frequency of involvement with the procedure.

CONCLUSION

Eyelash extensions can seriously predispose patronizing females to eye injury. The eye is a very important but delicate organ of the body; therefore it needs to be well protected to maintain one's sight. Eyelash extension is inimical to the eyelid and could be detrimental to vision and therefore the obsession on eyelash extension should be curbed.

RECOMMENDATIONS

A national survey on eyelash extension and its effect should be conducted in Ghana. If possible, baseline vision of prospective participants could be compared with vision post eyelash extension to ascertain its effect on vision in general. The Ghana Health Service and the Ministry of Health should be alerted on the upsurge of eyelash extension and its inimical effect on the eye and vision so that these organizations can educate eye care practitioners and the general public.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Putative risk factors of pregnancy-induced hypertension among Ghanaian pregnant women

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Hypertensive pregnancy is an important cause of maternal mortality with several risk factors which can be related to regional and ethnic factors. Although there have been many studies worldwide on preeclampsia, not many have come from black Africa and for that matter Ghana. This study sought to identify some putative risk factors of Pregnancy-Induced Hypertension among Ghanaian pregnant women. A case-control study was conducted among pregnant women visiting Komfo Anokye Teaching Hospital (KATH), Kumasi, Ghana between November, 2006 and December, 2007 to determine the risk factors for Pregnancy-Induced Hypertension (PIH). Information on socio-demographic characteristics, medical history and previous obstetric history were obtained by face-to-face interviews and assessed through medical records. One hundred PIH women (thirty with preeclampsia (PE) and seventy with gestational hypertension (GH) and fifty normotensive pregnant women (controls) in the second half of pregnancy were recruited for the study. Advanced maternal age was a significant risk for developing PIH (PE+GH). Obesity increased the risk of PIH. Family history of hypertension increased the risk of developing PIH (*a*OR 6.8; 95% CI 2.3-19.6). Nulliparity was not a risk factor for PE (*c*OR 0.0; 95% CI 0.0–0.2) but was a risk factor for GH (*c*OR 3.0; 95% CI 1.2-7.4) from this study. Condom use in the male partner, contraceptive use in females, change of partner as well as placental hormonal imbalance were also associated with PIH. The findings of this study suggest that, besides maternal aberrations posing risk for PIH, change of partner and placental roles could also be linked to the aetiology of PIH. Furthermore, some risk factors for PIH are similar for both non-African populations as well as black Africans.
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Keywords*:* Hypertension, risk factors, ante-natal, pregnancy, Ghana

INTRODUCTION

Over half a million women die each year of pregnancy-related causes. Ninety-nine (99%) percent of these deaths occur in the developing world (Verwoerd *et al*., 2002) which includes Ghana. The most common cause of these maternal deaths are complications of pregnancy and child birth such as haemorrhage, sepsis, complications of unsafe abortions, hypertensive disorders of pregnancy and obstructed labour (WHO, 1994). Maternal and perinatal morbidity and mortality are also major public health problems in developing countries like Ghana. A study conducted by Osei-Nketiah, (2001) in Ghana established that forty percent (40%) of maternal deaths are as a result of hypertensive pregnancy, antepartum haemorrhage and post partum haemorrhage. Hypertensive disorders of pregnancy remain a major cause of maternal and foetal morbidity and are of grave concern to healthcare professionals not only in Ghana but the world at large. Pregnancy-Induced Hypertension continues to be a major obstetric problem in present-day healthcare practice. It presents a great medical dilemma because it af-

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fects not only maternal health but also puts foetal development at risk (Ahenkorah *et al.*, 2008).

Pregnancy-Induced Hypertension is hypertension that develops as a consequence of pregnancy and regresses after delivery. Pregnancy-Induced Hypertension can be differentiated from chronic hypertension, which appears before 20 weeks gestation or usually continues for more than six weeks after delivery (ACOG, 1996). Gestational hypertension and PE (together referred to as PIH) are conditions of pregnancy characterized by increased blood pressure. Preeclampsia, according to the National High Blood Pressure Education Program of the USA, (National High Blood Pressure Education Group, 2000) is defined as hypertension developing after 20 weeks' gestation with proteinuria and/or oedema. Gestational hypertension on the other hand, is hypertension developing after 20 weeks' gestation without other signs of preeclampsia.

Pregnancy-Induced Hypertension is a known cause of premature delivery, intrauterine growth restriction (IUGR), placental abruption and foetal death, as well as maternal mortality and morbidity. Pregnancy-Induced Hypertension is one of the commonest pregnancy complications in Ghanaian hospitals today with a 7.03% incidence rate for preeclampsia being reported by Obed and Patience (2006).

To date, the aetiology of PIH remains unknown, however, a number of risk factors have been identified (Roberts and Lain, 2002; Zhang *et al.*, 1997). Primiparas are known to be at markedly greater risk of preeclampsia than multiparas. Preeclampsia is a complication in 25-30% of nulliparous pregnancies. It is more common in nulliparous than in multiparous women and as such the first pregnancy is a risk factor for preeclampsia (Serhal *et al.*, 2003). The use of barrier contraceptives, young maternal age, change of partner (Duckitt and Harrington, 2005; Skjaerven *et al.*, 2002; Trupin *et al.*, 1996), have all been reported to amplify the risk of PIH or preeclampsia, but these observations await corroboration or refutation most especially in the black African community.

Previous studies reported hypertension in pregnancy to be associated with an increased risk of preterm delivery (Hauth *et al.*, 2000; Sibai *et al.*, 1998). It is also known that the condition reverses after delivery of the placenta. As such, placental hormonal imbalance in hypertensive pregnant women has been studied (Bhansali and Eugere, 1992; Friedman *et al.*, 1991). Human placental lactogen and cortisol levels have been reported to be either unchanged or decreased (Garoff and Seppala, 1976; Salem *et al.*, 1983). It is therefore essential to identify patients with Pregnancy-Induced Hypertension in whom the foetus is at greatest risk. One approach to this is the assessment of the size and growth rate of the foetus by clinical or ultrasonic means. A second approach based on the fact that foetal morbidity and mortality can be largely attributed to placental dysfunction, is the measurement, in the maternal circulation of substances such as Human Placental Lactogen (hPL) produced by the feto-placental unit (Letchworth and Chard, 1972a; Letchworth and Chard, 1972b).

Human placental lactogen is a member of a structurally and biologically overlapping family of polypeptide hormones (Corbacho *et al.*, 2002; Goffin *et al.*, 1996). They are known to play an important role in lactation, reproduction, osmoregulation, immunomodulation and growth of tissues and blood vessels (Corbacho *et al.*, 2002; Karabulut *et al.*, 2001). Human placental lactogen, due to its effect on blood vessels, has been implicated in the aetiology of PIH due to the fact that endothelial and vascular dysfunctions are associated with the disorder.

There is need for follow-up studies in the black populations in Africa and as such, studying the patterns of risk factors for PIH among Ghanaian women is essential since publications on this subject in Ghana are very scanty. This study, therefore, seeks to identify the relationship between some putative risk factors of PIH among Ghanaian pregnant women and to determine if placental hormonal imbalance is associated with PIH among Ghanaians with the aim of making inputs to available literature and also as a step to possibly finding in-

terventions for reducing the prevalence of this clinical condition, which is associated with a high maternal as well as perinatal morbidity and mortality worldwide.

MATERIALS AND METHODS

Subjects

This cross-sectional study was conducted at the Komfo Anokye Teaching Hospital in Kumasi, Ashanti Region of Ghana between November, 2006 and December, 2007. Women within the age group of 17- 45 years visiting the Obstetrics and Gynaecology Department of the Hospital were recruited for the study. One hundred pregnant women with Pregnancy–Induced Hypertension (seventy with gestational hypertension and thirty with preeclampsia) served as cases; and fifty normotensive pregnant women with uncomplicated pregnancy served as study control. Only one woman with PE declined to participate in the study. Most importantly, women with known renal disease, diabetes and hypertension prior to pregnancy or cardiovascular diseases were excluded from this study among both case and control participants. Women with a history of twin birth or who were carrying twin pregnancy as observed from their ultrasound scan were also excluded from the study.

Pregnancy–Induced Hypertension was diagnosed according to the criteria of the National High Blood Pressure Education Program Working Group for PIH as assessed by a single qualified Obstetrician/ Gynaecologist. Briefly, the presence of high blood pressure on two occasions six hours apart was considered GH while pregnant women who had proteinuria level of 2+ positive result on a dipstick (using early morning midstream urine), were considered as presenting with PE (Forest *et al.*, 2005). Finally, GH and PE were collectively considered as PIH. All the subjects were Ghanaians and their participation was voluntary and informed consent was obtained from each subject. The study was approved by the School of Medical Sciences and Komfo Anokye Teaching Hospital Committee on Human Research Publications and Ethics (CHRPE/ KNUST/KATH/15_03_08).

Each subject had a questionnaire-based interview, which was conducted privately and in person and lasted approximately 45 minutes. Information on maternal lifestyle factors such as smoking and alcohol consumption during pregnancy, demographic data, recent medical history, a complete obstetric history, contraceptive use, occupational factors, exercise and social data was obtained. Each participant reported the outcomes of all previous pregnancies as live births, stillbirths, spontaneous abortions or induced abortions. Information extracted from questionnaire included socio-demographic characteristics, medical and previous reproductive history as well as social information and lifestyle habits. The veracity of the information extracted from the questionnaire on reproductive history and socio-demography obtained during the interview was verified through a review of hospital records.

Exercise was defined as at least a conscious effort to stroll around participant's home for not less than 20-30 minutes daily. Change of partner was defined as a change in spouse at least four months before the participant's current pregnancy. Alcohol consumption related to alcohol intake in the months preceding pregnancy as well as during the current pregnancy.

Sample Collection and Preparation

Biochemical analysis

Fasting Blood Glucose (FBG) was determined using the glucose oxidase/peroxidase method (Trinder, 1969) and hPL was analyzed using Enzyme–Linked Immunoassay specific for hPL using a DRG® hPL Enzyme Immunoassay kit (DRG® Instruments, GmbH Germany) and results calculated from the standard curve. Human placental lactogen and fasting glucose were estimated between 29-31 weeks of gestation in all the participants.

Urinalysis

Early morning midstream urine was collected in plastic containers from the respondents and urine protein was analyzed using the dip-stick qualitative method (CYBOW™ DFI Co Ltd, Gimhae-City,

Republic of Korea). Proteinuria values of 0, 0.1, 0.3, 1 and 5 g L^{-1} corresponded to qualitative dipstick testing attributes of negative, trace, +, ++ and +++ respectively.

Anthropometric variables
Subjects were weighed on a bathroom scale (Zhongshan Camry Electronic Co. Ltd, Guangdong, China) and their height measured with a wall-mounted ruler. BMI was calculated by dividing weight (kg) by height squared (m^2) and obesity defined as BMI greater or equal to 30 kg m^{-2}.

Blood Pressure
Blood pressure was taken by trained personnel using a mercury sphygmomanometer and a stethoscope. Measurements were taken from the left upper arm after subjects had been sitting for >5 minutes in accordance with the recommendation of the American Heart Association (Kirkendall *et al.*, 1967). Duplicate measurements were taken with a 5 minute rest interval between measurements and the mean value was recorded to the nearest 2.0 mm Hg.

Statistical Analysis
Statistical analyses were performed using GraphPad Prism version 5.00 for windows (GraphPad software, San Diego California USA, www.graphpad.com). Continuous variables are expressed as their mean ± SEM while categorical variable were expressed as proportion. Comparisons of the women with PIH (gestational hypertension and preeclampsia separately and combined) against the control group were carried out using, unpaired *t*-tests, $\chi 2$ tests or fisher exact tests where appropriate. A level of $p<0.05$ was considered as statistically significant.

SAS System for windows, version 6.12 was used to examine other putative risk factors for possible confounding effects on PIH. Abortion variables were similarly examined by analysis to estimate the risk on PIH (preeclampsia and gestational hypertension). We examined abortion by type; spontaneous or induced. Subjects were categorized by the type of abortion history, as follows: (a) women with no prior history of abortion (reference group), (b) women with prior history of spontaneous abortion, (c) women with prior history of induced abortion. Crude odds ratios (*c*OR) and 95% confidence interval for PIH were calculated for all studied risk factors. Crude ORs were then adjusted by taking into account possible influence of other covariates, with the use of multiple logistic regression analysis to obtain adjusted (*a*OR). The *c*OR were adjusted for age, family history of hypertension, condom use, contraceptive use and change of partner. Three models were constructed one for each of the outcomes (GH, PE, and PIH). Variables with zero cells in some of the categories were not included in the models. Variables were entered into the model if $p < 0.05$ and a stepwise procedure was applied using the Cornfield exact method.

RESULTS

Clinical Characteristics
The demographic and clinical characteristics of the study population are as shown in Table 1. The mean value and the percentage prevalence of components of the metabolic syndrome were significantly increased in the entire studied group compared to the control group. The frequency distributions of the various putative risk factors are as shown in Table 2. As shown in Figure 1, the concentration of Human Placental Lactogen was significantly decreased when the PIH group was compared to the control group. The mean concentration of hPL was 4.9±0.3 g L^{-1} for the PIH subjects, 4.8±0.4 g L^{-1} for GH subjects, 4.4±0.6 g L^{-1} for PE subjects and that of the control group was 8.6±1.1 g L^{-1}.

Putative Risk Factors

Pregnancy-Induced Hypertension
Women aged 24 years or less did not have a significantly increased risk of developing PIH compared to women between 25–29 years of age (*a*OR 2.2; 95% CI 0.6-7.6). However, women between 35-39 years of age had about 9 times increased risk of developing PIH compared to women between 25-29 years (95% CI 2.5–34.7). Nulliparity was not a significant risk factor for PIH from this study (*a*OR

Table 1: Demographics and clinical characteristics of Ghanaian pregnant women, 2006-2007

Parameters	Control	PIH	P value	GH	P value	PE	P value
n	*50*	*100*		*70*		*30*	
Maternal age (yrs)	30.9±0.7	31.8±0.6	0.004	32.4±0.7	0.000	30.4±1.3	0.280
GP (weeks)	31.0±0.9	30.4±0.8	0.110	29.4±0.7	0.220	30.7±1.0	0.100
Proteinuria (g L^{-1})	0.0±0.0	0.4±0.1	0.001	0.0±0.0	0.320	1.4±0.3	0.000
SBP (mmHg)	105.8±1.5	149.0±1.7	0.000	147.1±1.6	0.000	153.3±3.9	0.000
DBP (mmHg)	67.2±1.1	95.6±1.2	0.000	94.1±1.1	0.000	99.0±2.9	0.000
BMI (kg m^{-2})	27.0±0.5	29.5±0.6	0.009	29.2±0.8	0.032	30.1± 0.9	0.001
Obesity	9(18.0%)	51(51.0%)	0.000	32(45.7%)	0.002	19(63.3%)	0.000
FBS (mmol L^{-1})	3.5±0.1	4.0±0.2	0.060	3.9±0.2	0.200	4.2±0.2	0.000
Diabetes	0(0.0%)	7(7.0%)	0.100	6(8.57%)	0.039	1(3.3%)	0.370

Continuous data are presented as mean ± SEM and categorical variable as number with percentage in parenthesis. GP = gestational age, PIH = pregnancy-induced hypertension subjects, PE = preeclampsia group, GH = gestational hypertension group, SBP = systolic blood pressure, DBP = diastolic blood pressure, BMI = body mass index and FBS = fasting blood sugar

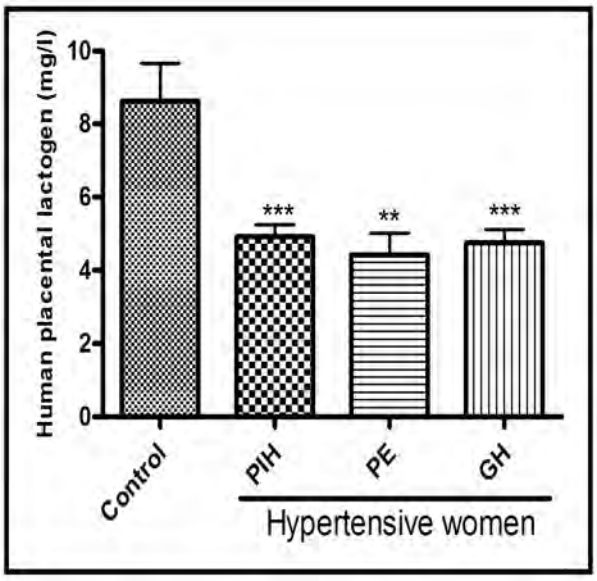

Figure 1: Distribution of human placental lactogen (hPL) in control and pregnancy-induced hypertension subjects (PIH). PE = preeclampsia group, GH = gestational hypertension group. Values are mean ± SEM and significantly different from control using unpaired *t*-test:P < 0.01 and ***P < 0.001**

1.8; 95% CI 0.8–4.0). Women who were obese were about 5 times at risk of developing PIH compared to the reference BMI group (95% CI 1.7-12.5). Marital status, the consumption of alcoholic beverages and educational attainment did not significantly influence the risk of developing PIH from this study (Table 3).

As shown in Table 4, lack of exercise and prior abortion did not pose any significant risk for the development of PIH from this study. However, women with a family history of hypertension were about 7 times at risk of developing PIH as compared to women without family history of hypertension (95% CI 2.3-19.6). Also, the risk of developing PIH was about 6 times among women whose partners used condom during coitus (95% CI 1.2-23.0), about 2 times among women who used contraceptives (95% CI 1.2-3.9) and about 2 times among women who changed sexual partners (95% CI 1.1-5.8) compared to women whose partners did not use condom, women who did not use contraceptives and women who did not change sexual partners respectively. Women with prior preterm delivery were not at risk of developing

Table 2 - Distribution of the Risk Factors for Ghanaian pregnant women, 2006-2007

PARAMETERS	CG (50)	PIH (100)	GH (70)	PE(30)
Maternal Age (yrs)				
<25	8(16.0%)	11(11.0%)	7(10.0%)	4(13.3%)
25-29	19(38.0%)	17(17.0%)	9(12.9%)	8(26.7%)
30-34	17(34.0%)	35(35.0%)	26(37.1%)	9(30.0%)
35-39	4(8.0%)	28(28.0%)	22(31.4%)	6(20.0%)
40-44	2(4.0%)	9(9.0%)	6(8.6%)	3(10.0%)
Parity				
0	20(40.0%)	43(43.0%)	43(61.4%)	0(0.0%)
1+	30(60.0%)	57(57.0%)	27(38.6%)	30(100.0%)
Body Mass Index (BMI)				
< 19	0(0.0%)	4(4.0%)	3(4.3%)	1(3.3%)
19-24.9	14(28.0%)	17(17.0%)	13(18.6%)	4(13.3%)
25-29.9	27(54.0%)	28(28.0%)	22(31.4%)	6(20.0%)
≥ 30	9(18.0%)	51(51.0%)	32(45.7%)	19(63.3%)
Marital Status				
Single	2(4.0%)	8(8.0%)	4(5.7%)	4(13.3%)
Married	48(96.0%)	92(92.0%)	66(94.3%)	26(86.7%)
Alcohol Consumption				
Yes	15(30.0%)	42(42.0%)	28(40.0%)	14(46.7%)
No	35(70.0%)	58(58.0%)	42(60.0%)	16(53.3%)
Educational Background				
None at all	3(6.0%)	8(8.0%)	5(7.14%)	3(10.0%)
Basic	33(66.0%)	74(74.0%)	50(71.4%)	24(80.0%)
Secondary	7(14.0%)	12(12.0%)	9(12.9%)	3(10.0%)
Tertiary	7(14.0%)	6(6.0%)	6(8.6%)	0(0.0%)
Exercise				
Yes	28(56.0%)	45(45.0%)	31(44.3%)	14(46.7%)
No	22(44.0%)	55(55.0%)	39(55.7%)	16(53.3%)
Family history of hypertension				
Yes	5(10.0%)	38(38.0%)	26(37.1%)	12(40.0%)
No	45(90.0%)	62(62.0%)	44(62.9%)	18(60.0%)
Prior adverse birth outcome				
No prior adverse birth	47(94%)	93(93%)	70(100%)	24(80%)
Prior caesarian section	3(6%)	3(3%)	0(0%)	3(10%)
Prior preterm	0(0%)	5(5%)	0(0%)	5(16.7%)
Prior still birth	2(4%)	6(6%)	0(0%)	6(20%)
Prior abortion				
No prior abortion	32(64%)	62(62%)	52(74.3%)	8(26.7%)
Prior spontaneous abortion	17(34%)	31(31%)	14(20%)	17(56.7%)
Prior induced abortion	16(32%)	22(22%)	18(25.7%)	4(13.3%)
Condom Use				
Yes	2(4%)	19(19%)	12(17.1%)	7(23.3%)
No	48(96%)	81(81%)	58(82.9%)	23(76.7%)
Contraceptive Use				
Yes	13(26.0%)	37(37.0%)	25(35.7%)	12(40.0%)
No	37(74.0%)	63(63.0%)	45(64.3%)	18(60.0%)
Change of Partner				
Yes	10(20.0%)	37(37.0%)	21(30.0%)	16(53.3%)
No	40(80.0%)	63(63.0%)	49(70.0%)	14(46.7%)

PIH (cOR 0.0; 95% CI 0.0-0.4) (Table 4).

Gestational Hypertension

Women <25 years were not significantly at risk of GH when compared to women between 25–29 years of age (*a*OR 1.9; 95% CI 0.4-8.3). The risk of developing GH was about 4, 15, and 8 times higher among women in the age range 30-34 (95% CI 1.2-11.4), 35-39 (95% CI 3.6-63.2) and 40-44 (95% CI 1.0-55.0) respectively when compared to women between 25-29 years (Table 3). Nulliparity was a significant risk factor for GH (95% CI 1.2–7.4). However, pregnant women who were obese (i.e. BMI > 30 kg m^{-2}) were about 4 times at risk of developing GH compared to women with normal BMI (19-24.9 kg m^{-2}) (95% CI 1.3-10.9) (Table 3).

Pregnant women who did not engage in exercise and those with a prior history of adverse birth outcome as well as those with a history of abortion were not significantly at risk of developing GH when compared to their respective reference groups (Table 4). On the other hand, women with a family history of hypertension were significantly at risk of developing GH (95% CI 2.2-22.7). The risk of developing GH was about 4 times among women whose partners used condom during coitus (95% CI 1.1-2.1), about 2 times among women who used contraceptive (95% CI 1.1-2.6) and about 3 times among women who changed sexual partners (95% CI 1.1-8.0) when compared to women whose partners did not use condom, women who did not use contraceptives and women who did not change sexual partners respectively (Table 4).

Preeclampsia

The risk of developing PE significantly increased with maternal age, from 2 times among women 35-39 years old (95% CI 1.2-8.1) to about 3 times among women who were between 40-44 years old (95% CI 1.2-47.1) when compared to women who were between 25-29 years old. Women < 25 years were not significantly at risk of PE as compared to women between 25–29 years of age (95% CI 0.2-7.5) (Table 3). Nulliparity was not a significant risk factor for PE (cOR 0.0; 95% CI 0.0–0.2) from this study. Obese women were about 7 times at risk of developing PE compared to the reference BMI group (95% CI 1.9-27.7). Marital status, the consumption of alcoholic beverages and educational status did not significantly influence the risk of developing PE from this study (Table 3).

Pregnant women with a family history of hypertension were about 10 times at risk of developing PE compared to those without a family history of hypertension (95% CI 2.2-42.6). Women with a prior history of spontaneous abortion were at about 4 times at risk of developing PE (95% CI 1.2-12.2) when compared to the reference group **(**Table 4). The risk of developing PE was about 5 times among women whose partners used condom during coitus (95% CI 1.3-33.0), about 3 times among women who used contraceptives (95% CI 1.2-16.1) and about 9 times among women who changed sexual partners (95% CI 2.4-30.3) when compared to women whose partners did not use condom, women who did not use contraceptives and women who did not change sexual partners respectively (Table 4).

DISCUSSION

Hypertension is regarded as a major public health problem (Cappuccio *et al.*, 2004). It is the most common medical complication of pregnancy, which occurs in 3% to 10% of pregnancies (Saudan *et al.*, 1998). Several epidemiological studies have indicated that a family history of chronic hypertension is an independent risk factor for preeclampsia (Eskenazi *et al.*, 1991; Kobashi *et al.*, 2001; Qiu *et al.*, 2003). This study has established a high risk for preeclampsia, gestational hypertension as well as PIH among women with a family history of hypertension indicating a familial inheritance.

Components of the metabolic syndrome increased significantly among the study population. The results of this study after adjusting for all other confounding risk factors indicates that advanced age posed a substantial risk factor for the development of PIH (GH+ PE). Although, nulliparity posed as risk for the development of GH, this risk was not observed for PIH and PE subjects. Obesity as as-

Table 3: Risk model of the putative socio-demographic risk factor for Ghanaian pregnant women, 2006-2007

PARAMETERS	PIH				GH				PE			
	cOR(95% CI)	P value	aOR(95% CI)	P val-ue	cOR(95% CI)	P value	aOR(95% CI)	P value	cOR(95% CI)	P value	aOR(95% CI)	P value
Age (yrs)												
<25	1.5(0.5-4.7)	0.45	2.2(0.6-7.6)	0.20	1.8(0.5-6.5)	0.35	1.9(0.4-8.3)	0.38	1.2(0.3-4.8)	0.82	1.1(0.2-7.5)	0.89
25-29*	1	*	1	*	1	*	1	*	1	*	1	*
30-34	2.3(0.9-5.5)	0.06	2.7(1.0-7.0)	0.05	3.2(1.2-8.7)	0.02	3.7(1.2-11.4)	0.02	1.3(0.4-3.9)	0.70	0.8(0.2-3.4)	0.81
35-39	7.8(2.4-25.6)	0.001	9.2(2.5-34.7)	0.001	11.6(3.1-42.0)	0.0001	15.1(3.6-63.2)	0.0000	3.6(0.8-15.3)	0.04	2.4(1.2-8.1)	0.03
40-44	5.0(1.0-26.6)	0.06	5.6(0.9-34.0)	0.06	6.3(1.1-32.9)	0.03	7.5(1.0-55.0)	0.04	3.6(0.6-21.6)	0.04	2.8(1.2-47.1)	0.04
Parity												
0	1.1(0.6-2.2)	0.86	1.8(0.8-4.0)	0.19	2.4(1.1-5.0)	0.02	3.0(1.2-7.4)	0.02	0.0(0.0-0.2)	0.0001	§	§
1+*	1	*	1	*	1	*	1	*	1	*	§	§
Body Mass Index (BMI)												
< 19	undefined		§	§	undefined		§	§	undefined		§	§
19-24.9*	1	*	§	§	1	*	§	§	1	*	§	§
25-29.9	0.9(0.4-2.1)	0.82	§	§	0.9(0.3-2.2)	0.79	§	§	0.8(0.2-3.0)	0.73	§	§
≥ 30	4.7(1.7-12.5)	0.004	§	§	3.8(1.3-10.9)	0.01	§	§	7.4(1.9-27.7)	0.003	§	§
Marital Status												
Single	2.1(0.4-20.8)	0.36	3.5(0.6-21.3)	0.18	undefined		2.4(0.3-17.2)	0.39	3.7(0.6-21.5)	0.19	4.0(0.4-44.8)	0.27
Married*	1	*	1	*	1	*	1	*	1	*		
Alc Consumption												
Yes	1.7(0.8-3.5)	0.15	1.1(0.5-2.6)	0.80	1.6(0.7-3.3)	0.26	1.3(0.5-3.4)	0.54	2.0(0.8-5.2)	0.13	1.3(0.4-4.3)	0.66
No*	1	*	1	*	1	*	1	*	1	*	1	*
Educational Background												
None at all	1.6(0.3-7.2)	0.59	2.8(0.5-17.5)	0.27	1.3(0.2-6.7)	0.77	1.2(0.1-10.4)	0.85	2.3(0.3-17.3)	0.42	§	§
Basic	1.3(0.5-3.5)	0.60	1.8(0.5-6.7)	0.36	1.2(0.4-3.3)	0.76	1.3(0.3-5.0)	0.75	1.7(0.4-6.6)	0.47	§	§
Secondary*	1	*	1	*	1	*			1	*	§	§
Tertiary	0.5(0.1-2.0)	0.34	0.5(0.2-4.9)	0.91	0.7(0.2-2.8)	0.59	1.7(0.3-9.2)	0.51	0.0(0.0-1.6)	0.23	§	§

****Reference group, CG = control group, PIH = pregnancy-induced hypertension subjects, PE = preeclampsia group, GH = gestational hypertension group, cOR = crude odds ratio, aOR = adjusted odds ratio and CI = confidence interval, §= variables with 0 cells not included in the multivariable model, Alc.= Alcohol***

Table 4 - Risk model of the other putative risk factor for Ghanaian pregnant women, 2006-2007

PARAMETERS	PIH				GH				PE			
	cOR(95% CI)	P value	aOR(95% CI)	P value	cOR(95% CI)	P value	aOR(95% CI)	P value	cOR(95% CI)	P value	aOR(95% CI)	P value
Exercise												
Yes*	1	*	1	*	1	*	1	*	1	*	1	*
No	1.6(0.8-3.1)	0.20	1.3(0.6-2.9)	0.48	1.6(0.8-3.3)	0.21	1.4(0.6-3.4)	0.47	1.5(0.6-3.6)	0.42	1.0(0.3-3.3)	0.94
Family history of hypertension												
Yes	5.5(2.0-14.6)	0.0004	6.8(2.3-19.6)	0.001	5.3(1.9-14.6)	0.0008	7.0(2.2-22.7)	0.001	6.0(1.9-18.8)	0.002	9.7(2.2-42.6)	0.003
No*	1	*	1	*	1	*	1	*	1	*	1	*
Prior adverse birth outcome												
NPAB*	1	*	§	§	1	*	§	§	1	*	§	§
PCS	0.5(0.1-2.3)	0.41	§	§	0.0(0.0-0.8)	0.07	§	§	1.7(0.3-9.2)	0.66	§	§
PP	0.0(0.0-0.4)	0.005	§	§	undefined		§	§	undefined		§	§
PSB	1.5(0.3-7.5)	1.00	§	§	0.0(0.0-1.3)	0.17	§	§	6.0(1.1-32.0)	0.05	§	§
Prior abortion												
NPA*	1	*	1	*	1	*	1	*	1	*	1	*
PSA	0.9(0.5-1.9)	0.87	0.8(0.5-1.9)	1.00	0.5(0.2-1.2)	0.10	0.5(0.2-1.3)	0.16	4.0(1.5-11.0)	0.006	3.7(1.2-12.2)	0.02
PIA	0.7(0.3-1.5)	0.38	0.5(0.3-1.8)	0.49	0.7(0.3-1.5)	0.41	0.6(0.2-1.5)	0.51	1.0(0.3-3.7)	1	1.0(0.3-3.6)	1.00
Condom Use												
Yes	5.6(1.2-25.2)	0.01	5.8(1.2-23.0)	0.01	5.0(1.1-3.3)	0.04	3.8(1.1-2.1)	0.04	7.3(1.4-37.9)	0.01	4.5(1.3-33.0)	0.02
No*	1	*	1	*	1	*	1	*	1	*		
Contraceptive Use												
Yes	1.7(0.7-3.5)	0.03	1.7(1.2-3.9)	0.03	1.6(0.8-3.5)	0.04	1.7(1.1-2.6)	0.04	1.9(0.7-5.0)	0.0411	3.4(1.2-16.1)	0.0391
No*	1	*	1	*	1	*	1	*	1	*	1	*
Change of Partner												
Yes	2.3(1.1-5.2)	0.03	2.3(1.1-5.8)	0.04	1.7(0.7-4.0)	0.22	2.6(1.1-8.0)	0.04	4.6(1.7-12.2)	0.002	8.5(2.4-30.3)	0.001
No*	1	*	1	*	1	*	1	*	1	*	1	*

Reference group, CG = control group, PIH = pregnancy-induced hypertension subjects, PE = preeclampsia group, GH = gestational hypertension group, cOR = crude odds ratio, aOR = adjusted odds ratio and CI = confidence interval. NPAB= No prior adverse birth, PCS= Prior caesarian section, PP= Prior Preterm, PSB= Prior still birth, NPA=No prior abortion, PSA=Prior spontaneous Abortion, PIA= Prior Induced abortion, §= variables with 0 cells not included in the multivariable model.

sessed by BMI posed a significant risk factor for all three clinical conditions. Socio-economic status (SES) (evaluated by marital status and education) was not associated with PIH (including GH and PE). Alcohol consumption and lack of exercise during pregnancy did not significantly influence the risk of PIH, GH and PE. Although, history of prior adverse birth outcome and a history of prior abortion did not influence the risk of PIH and GH; an increased risk for PE was observed among women with prior preterm delivery and prior spontaneous abortion. Family history of hypertension significantly increased the risk for PIH, GH and PE; the use of contraceptives in either the male or the female partner as well as the change of sexual partner similarly, posed as risk factors for the development of the clinical conditions.

Some studies have reported increased risk of preeclampsia in younger women who are ≤ 21 years (Anorlu *et al.*, 2005; Sibai, 1990) and others have reported an association of increased risk of preeclampsia with women who are 35 years or older (Conde-Agudelo and Belizan, 2000; Sibai, 1990). This study found older mothers were at greater risk for the pathology. Indeed the relationship was not only observed for preeclampsia but also for GH. Stone *et al.,* (1995) and later Hartikainen *et al.,* (1998) have also demonstrated that older mothers were at increased risk of GH. The observed risk could be attributed to biological changes associated with maturity.

In this study, the risk of developing PIH, GH or PE positively associated with maternal obesity as measured by maternal Body Mass Index (BMI). This corroborates the findings of several studies where a strong association between increased maternal body mass and risk of preeclampsia has been reported (Anorlu *et al.*, 2005; Bodnar *et al.*, 2005; Eskenazi *et al.*, 1991; Villamor and Cnattingius, 2006). Obesity is associated with insulin resistance, dyslipidaemia, chronic inflammation and oxidative stress (Reilly and Rader, 2003), all of which have been demonstrated in women presenting with PIH (Ahenkorah *et al.*, 2008; Roberts and Lain, 2002; Turpin *et al.*, 2008). As a result of the strong relationship observed, the association between increasing changes in BMI and risk of PIH may support the theory that obesity-mediated inflammatory changes may play a role in the pathogenesis of PIH (Getahun *et al.*, 2007).

From this study, nulliparity was a risk factor for GH, as also reported by Hernandez-Diaz *et al.,* (2002). Preeclampsia is considered to be a disease largely associated with nulliparous women (Roberts and Redman, 1993). Serhal and Craft, (1987) also reported that first pregnancy is a risk factor for preeclampsia and its occurrence is more common in nulliparous than multiparous women (Eskenazi *et al.*, 1991; Roberts and Redman, 1993). Contrary to these and other reports, this study did not find nulliparity as a risk factor for women presenting with PE and PIH. The lack of association between nulliparity and risk of preeclampsia observed in this study is in agreement with the findings of Funai *et al.,* (2005). Although, the mechanism for this lack of association cannot be fully explained, Funai *et al.,* (2005) have proposed that, the degree to which preeclampsia would chiefly be a disease of nulliparity would depend on the fraction of patients seen who were nulliparous. Further research with emphasis on nulliparous women may be required to corroborate or refute this assertion.

Previous spontaneous abortion increased the risk for preeclampsia in this study. Contrary to this, Eras *et al.,* (2000) have reported that a history of abortion reduced the risk against both gestational hypertension and preeclampsia. Although, abortion reduced the risk for PIH and GH in this study, it however did not reach significant levels. In one of the few reported studies that evaluated the timing of abortion, Campbell *et al.,* (1985) showed and reported that late spontaneous abortion, defined as 13-27 weeks, conferred protection against preeclampsia in the subsequent pregnancy, whereas early spontaneous abortions did not. This might in part explain why spontaneous abortion (i.e. miscarriage) which usually occurs in the early weeks of pregnancy could not reduce the risk of preeclamp-

sia in this study.

Contraceptive use in either the male or the female partner as well as change of partner by the female partner positively associated with the risk of PIH, (PE and GH inclusive). Pregnancy-Induced Hypertension has long been considered to have an immunological basis. The results of several studies suggest that repeated exposure to the male partner's spermatozoa prior to conception reduces the risk of Pregnancy-Induced Hypertension in the first pregnancy (Marti and Herrmann, 1977). This implies that if extensive periods of cohabitation with the partner protects against Pregnancy-Induced Hypertension, it could be deduced that the mechanism may be related to the contact of spermatozoa with the female genital tract. This might explain the high risk observed in women whose spouses used condom as a means of contraception because the use of condom reduces or limits exposure of the female genital tract to the male partner's spermatozoa.

Similarly, women who used oral contraceptives prior to this study were not protected from Pregnancy-Induced Hypertension. From the face-to-face interview conducted in this study, most of the women used oral contraceptives. Oral contraceptives are known to act at different levels of the female reproductive tract, i.e. cervical mucus thickening, tubal motility, changes in endometrial lining (i.e. by decreasing the possibility of implantation, should conception occur) and ovulation suppression. However, in terms of exposure to spermatozoa, the main difference in a woman taking contraceptives is that the characteristics of cervical mucus may confine the semen to the vagina (Gratacos *et al.*, 1996). The non-protective effect of oral contraceptives might therefore be as a result of the reduced exposure to spermatozoa which is brought about by the effect of oral contraceptives on the female genital tract.

Various studies have reported paternity change as a risk factor for preeclampsia (Feeney and Scott, 1980; Li and Wi, 2000; Trupin *et al.*, 1996). This current study did not only find change of partner to be a strong risk factor for preeclampsia but also for gestational hypertension. Indeed, it is presumed that a previous normal pregnancy is associated with a reduced risk of Pregnancy-Induced Hypertension, but other studies have reported that this protective effect is lost with the change of partner (Dekker, 2002; Robillard *et al.*, 1999; Robillard *et al.*, 1993). Lie *et al.,* (1998) have also reported on the existence of what they termed 'dangerous' father, where they established that men who fathered one preeclamptic pregnancy were nearly twice as likely to father a preeclamptic pregnancy in a different woman regardless of whether she had already had a preeclamptic pregnancy or not (Dekker, 2002; Lie *et al.*, 1998). Furthermore, it has been reported in some studies that multiparous women who have had a change of partner before the index pregnancy have an increased risk of preeclampsia (Eras *et al.*, 2000; Li and Wi, 2000). Therefore, preeclampsia might be a problem of primipaternity (Robillard *et al.*, 1999; Robillard *et al.*, 1993) rather than primigravidity as most studies suggest.

In assessing the levels of placental hormone hPL among Ghanaian women presenting with pregnancy-induced hypertension, decreased levels of hPL was observed in all the subject cases (PIH, PE, GH) with the least concentration noted among the PE subjects. The observed decrease in hPL level in this study is consistent with the findings of other studies (Bersinger *et al.*, 2002; Letchworth and Chard, 1972a; Westergaard *et al.*, 1984). Human placental lactogen levels are a valuable indicator of foetal well-being (Letchworth and Chard, 1972b), clinical signs of foetal growth retardation and very low hPL values seem to indicate high foetal risk (Lindberg and Nilsson, 1973) and appears to satisfy all the criteria of a good test of placental function (Genazzani *et al.*, 1971; Spellacy *et al.*, 1971).

The disclosure of information on abortion type, previous obstetric history and contraceptive use was obtained from personal interviews. Although verified with medical records, a drawback of the data is that, these matters bother on sexuality and thus considered personal and sensitive in this part of the world and as such, most women may not be forthcoming with accurate answers. Reporting errors would have resulted in non-differential mis-

classification that may have introduced a bias and thus potentially underestimate observed associations and thereby serving as a limiting factor to the outcomes of the study.

Another limitation of this study relates to the limited power to detect associations between previous adverse birth outcome and PIH, due to low numbers for some of the subtypes of previous adverse birth outcome. Further scientific enquiry may be required involving a larger sample size in order to corroborate or refute the findings in this report.

CONCLUSION

This study has shown that obesity, older women, family history of hypertension, contraceptive use are some significant risk factors for PIH among women in Ghana and possibly in other black African countries. Paternal and placental roles have also been observed in this cohort of Ghanaian subjects. These risk factors are not very different from what has been reported in studies conducted outside Ghana.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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