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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.

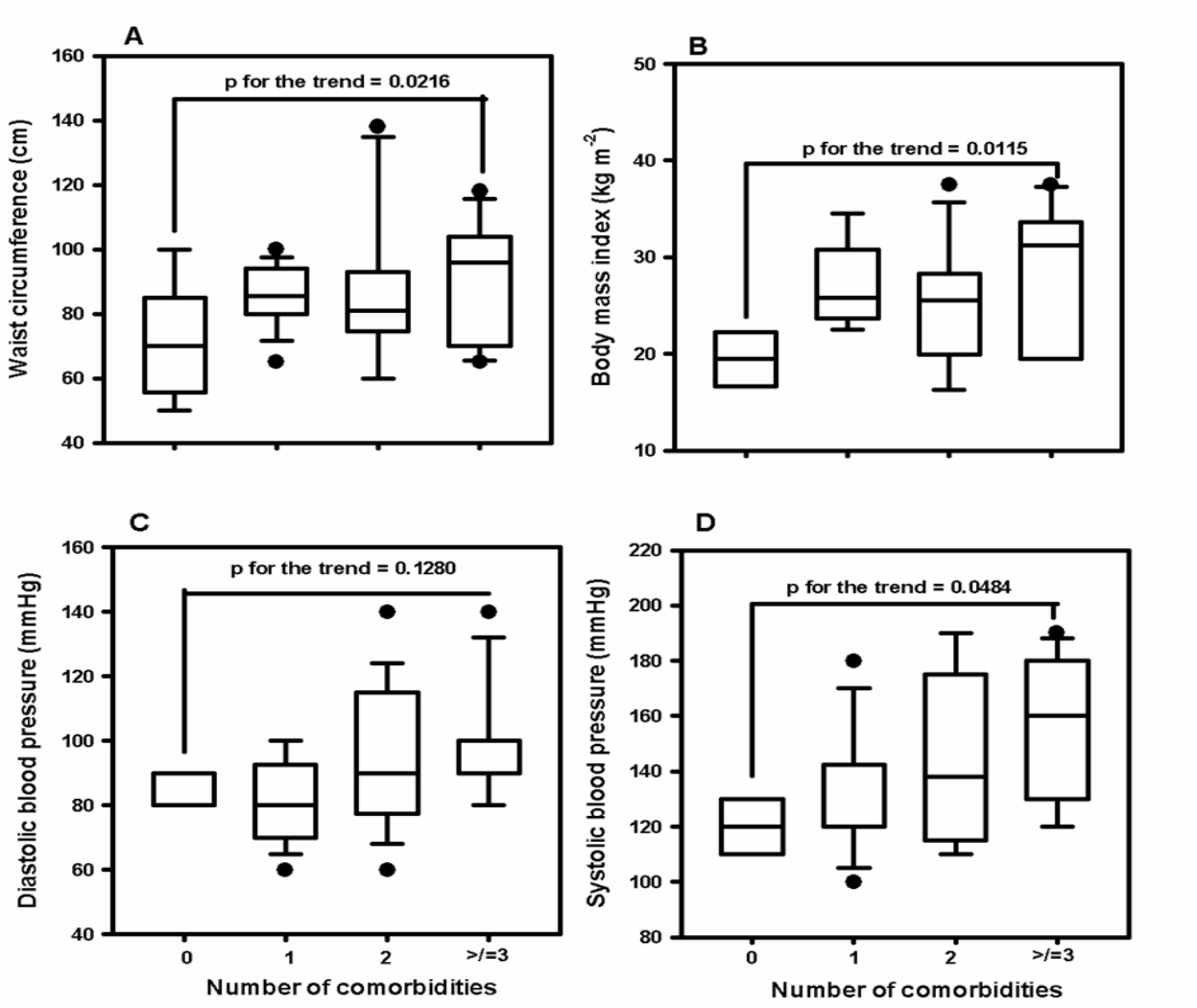

Figure 1: Comparisons of BMI, DBP, SBP and WC between participants with a different number of comorbidities of the MetS in CKD. The lower and upper margins of the box represent the 25th and 75th percentiles, with the extended arms representing the 10th and 90th percentiles, respectively. The median is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

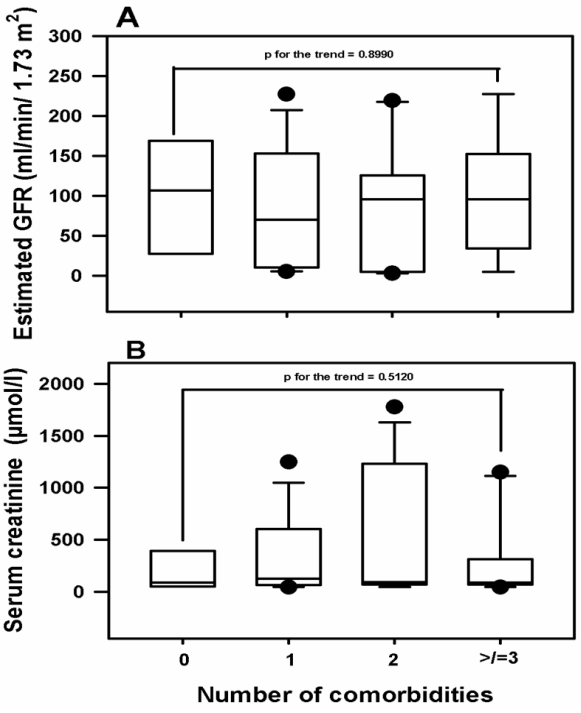

Figure 2: Comparisons of eGFR and serum Creatinine between participants with different number of comorbidities of MetS in CKD. The lower and upper margins of the box represent the 25th and 75th percentiles, with the extended arms representing the 10th and 90th percentiles, respectively. The median is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

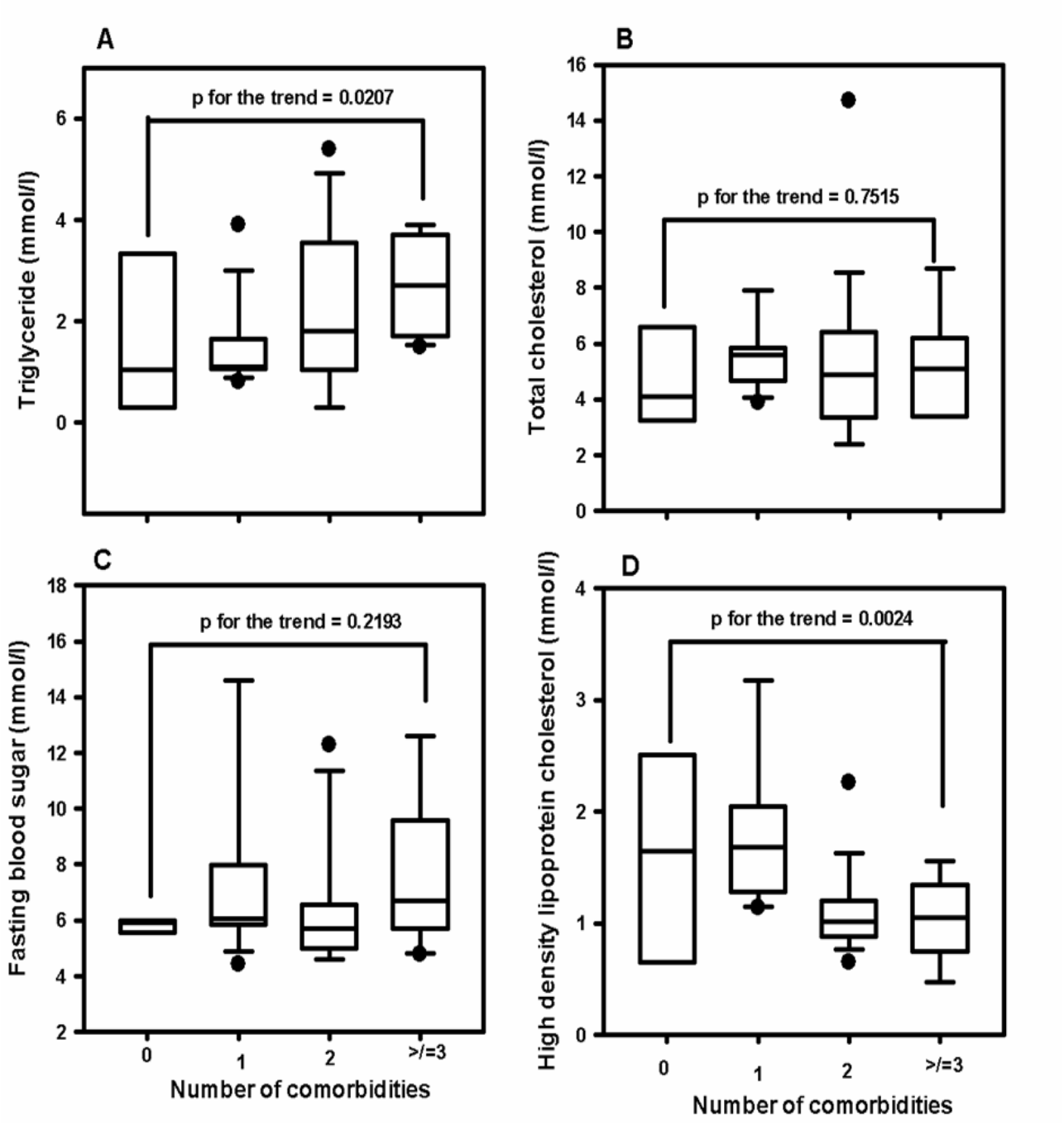

Figure 3: Comparisons of FBG, TG, TC and HDL-C between participants with different number of comorbidities of MetS in CKD. The lower and upper margins of the box represent the 25th and 75th percentiles, with the extended arms representing the 10th and 90th percentiles, respectively. The median is shown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

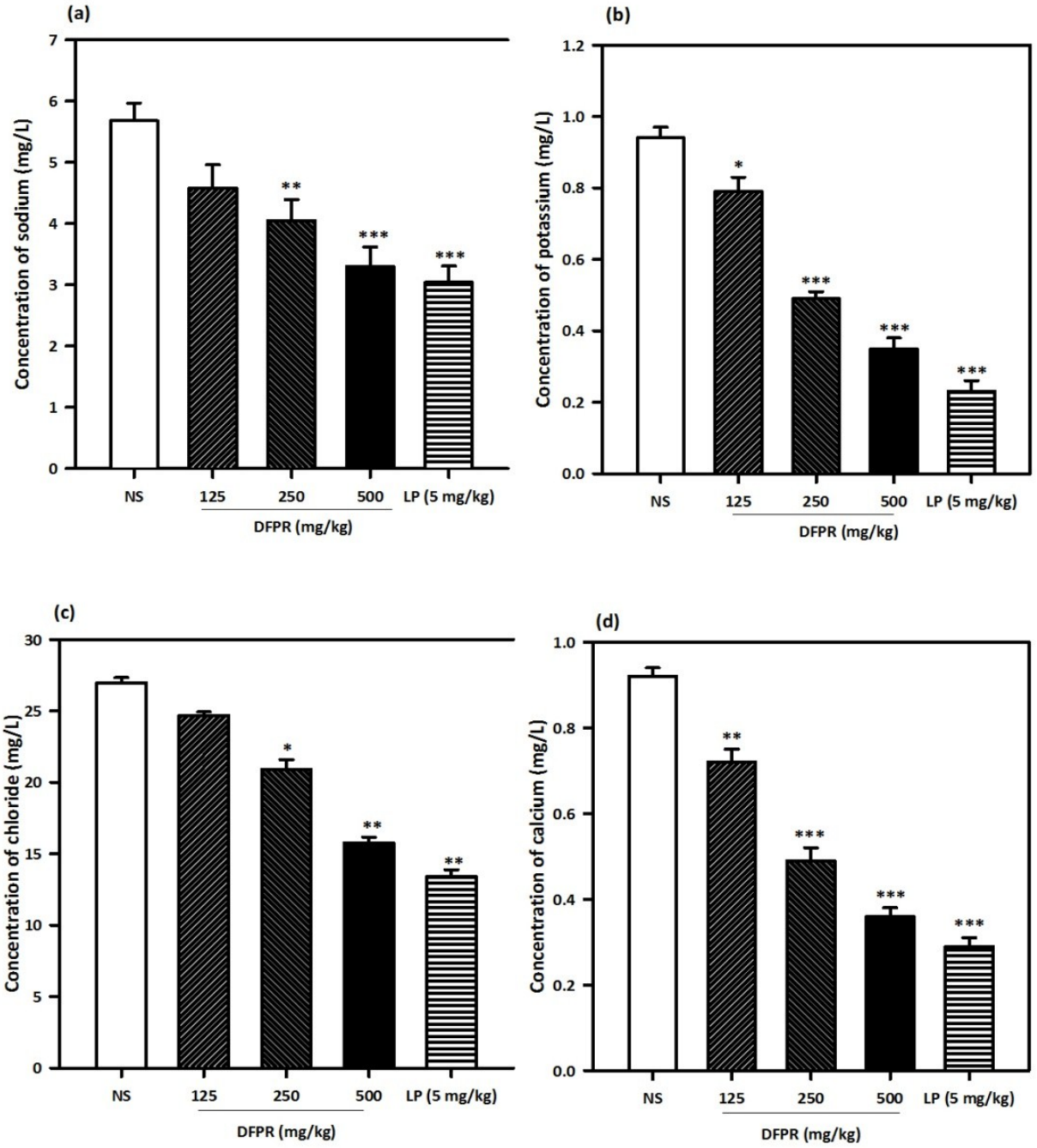

Figure 1: Effects of the dichloromethane fraction of *Piliostigma reticulatum* (DFPR) and loperamide (LP) on faecal concentration of (a) sodium; (b) potassium; (c) chloride and (d) calcium (mg/L). Data are mean ± SEM (n=6). *p < 0.05, **p < 0.01, ***p < 0.001 compared to vehicle treated group (one-way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

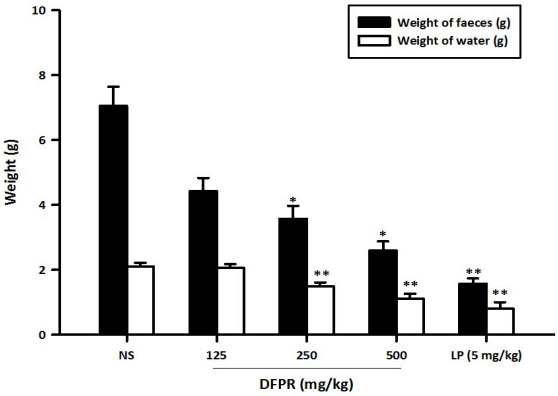

Figure 2: Effects of the dichloromethane fraction of *Piliostigma reticulatum* (DFPR) and loperamide (LP) on the weight of faeces and water contained in the faeces of rats. Data are mean ± SEM (n=6). *p < 0.05, **p < 0.01, ***p < 0.001 compared to vehicle treated group (one-way ANOVA followed by a Dunnett's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