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ORIGINAL ARTICLE

Impact of physical activity levels and diet on central obesity among civil servants in Tamale metropolis

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This study sought to assess the prevalence of central obesity using Waist to Hip ratio as well as establishing the relationship between physical activity, diet and central obesity among civil servants in the Tamale metropolis. This cross-sectional study was conducted between January and July, 2011. One hundred and eighty six (186) subjects were involved with 121 being males and 65 being females. The study participants were recruited from an adult population between the ages of 20-59 years. Dietary pattern was assessed using food frequency questionnaires whilst physical activity was determined using the WHO Global physical activity levels questionnaire. The prevalence of central obesity was found to be 31.2%. The prevalence of central obesity was significantly higher ($p=0.031$) among females (41.5%) compared to men (25.6%) and also increased with age ($p<0.0001$). The prevalence of obesity significantly decreased ($p = 0.018$) from 70.7% via 25.6% to 3.4% as the level of physical activity increased from low through moderate to high. There were no significant associations between dietary pattern and central obesity from this study. Central obesity from this study is high and more common in females. The level of physical activity appears to be a key determinant of the prevalence of central obesity in this study. Preventive actions such as exercise and active lifestyles have to be implemented to reduce the tendency for central obesity in this population in particular and the general Ghanaian populace at large.

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INTRODUCTION

There is a worldwide obesity epidemic with the prevalence of overweight and obesity exceeding 50% in almost all the regions of the world (Balkau *et al.*, 2007). Over the past three decades, this epidemic has affected the industrialized countries, with some areas of North America, Europe and Asia having more than threefold increase in the prevalence of obesity (Fezeu *et al.*, 2008; Parikh *et al.*, 2007; Wang *et al.*, 2007). In recent times, low- and medium-income countries have joined the obesity epidemic,

and the increase has been faster in these countries (Fezeu *et al.*, 2008; Wang *et al.*, 2007).

Genetics and lifestyle changes such as diet and physical activity level can greatly modify the prevalence and severity of obesity in adults (Helge, 2002). In a healthy adult population, there is an inverse relationship between the level of physical activity and obesity (Ford *et al.*, 2002; Katzmarzyk *et al.*, 2004; Owiredo *et al.*, 2011). The causes and prevalence of obesity vary from population to population based on individual life style, diet, cultural background, genetic make-up as well as the type of instrument used in the assessment of obesity.

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The prevalence of obesity was found to be 5.5% in Ghana and higher among women (7.4%) compared to men (2.8%) using body mass index (Biritwum *et al.*, 2005). In a study conducted by Owiredu *et al.*, (2011) in Kumasi, the prevalence of obesity was found to be higher among sedentary workers (17.4%) compared to active subjects (5.9%) using body mass index. On the other hand, using waist to hip ratio, the prevalence of central obesity was found to be 27.9% among sedentary workers as compared to 2.9% in active subjects (Owiredu *et al.*, 2011). In Ghana there is paucity of data on the prevalence of central obesity among civil servants. However, with the increasing westernization of Ghanaian lifestyle and eating habits the prevalence of obesity has been reported to be on the increase across the country. This study therefore sought to find the prevalence of central obesity among civil servants in the Tamale Metropolis as well as the impact of diet and physical activity level.

MATERIALS AND METHODS

Subjects

This cross-sectional study was conducted between January and July, 2011. The subjects comprised of 186 adult civil servants in the Tamale Metropolis in the Northern Region of Ghana. All personnel from the 13 civil service departments in the Tamale Metropolis were eligible to participate in the study. The subjects were selected using a proportionate random sample that included more subjects from larger departments with the use of a random numbers statistical table. The participation of the subjects was voluntary and informed consent was obtained from each subject. Subjects who were on medication known to modify serum lipid or carbohydrate metabolism were excluded from the study. The study was approved by the Ethics Committee of the University for Development Studies, School of Medicine and Health Sciences, Ghana.

Anthropometric variables

Anthropometric measurements included waist circumference, measured midway between the inferior angle of the ribs and the suprailiac crest. Hip circumference, measured as the maximal circumference over the buttocks in centimetres. Both meas-

urements were measured to the nearest 1 cm using a non-stretchable fibre-glass measuring tape (Butterfly, China). During both measurements, subjects were made to stand upright, with arms relaxed at the side, feet evenly spread apart and body weight evenly distributed in accordance with the WHO expert consultation report on waist circumference and waist-hip ratio (WHO, 2004). Waist to Hip Ratio (WHR) was calculated by dividing the waist circumference (cm) by the hip circumference (cm). Men with WHR < 0.90, 0.90–0.99 and ≥ 1.0 were classified as normal, overweight or obese respectively, while women were classified in the same categories on the basis of WHR of < 0.80, 0.80 – 0.84 and ≥ 0.85 (WHO, 2004).

Physical activity level

The level of physical activity was assessed using the Global Physical Activity Questionnaire (GPAQ) (WHO, 2009). The GPAQ comprised of 19 questions about physical activity level in a typical week. The frequency and duration of time spent doing physical activity is measured in 3 domains: activity at work, travel to and from places and recreational activities.

The GPAQ was selected as a result of its standardization, easiness to administer, relative unobtrusiveness and inexpensiveness. Its reliability has been found to be 0.67–0.81 and the validity was 0.54 (Armstrong *et al.*, 2006). The questionnaire was fully adapted for the study, without any modifications. However, to suit the Ghanaian context, local examples of types and intensity of activities were used. The collection of all data and processing followed the GPAQ analysis protocol (WHO, 2009). All durations were converted into minutes. Energy expenditure, measured in MET (Metabolic equivalents), was estimated using duration, intensity and frequency of physical activities performed within 7-days. MET, is the ratio of specific physical activity metabolic rates to the resting metabolic rate (1 MET = the energy cost of sitting quietly, and was equivalent to a caloric consumption of 1 kcal/kg/hour). A MET-minute showed the total activity volume on weekly basis, and calculated by multiplying time spent on each activity during a week by the

MET-values of each level of activity. Using the compendium of physical activities (Ainsworth *et al.*, 2000), MET-values for various levels of activities was established. MET values of 4 and 8 were set for moderate-intensity (transport-related walking or cycling) and vigorous-intensity physical activity, respectively. Total MET/minutes/week was computed by the sum of all moderate- to vigorous-intensity physical activities performed at work, transport and recreation. Based on the total met/minutes/week, subjects were classified into low, moderate, and high physical activity levels as defined by the GPAQ analysis framework (WHO, 2009).

High: A subject found within any of the following categories: Vigorous-intensity activity on at least three days achieving at least 1,500 MET-minutes/week OR seven or more days of any combination of walking, moderate or vigorous intensity activities achieving at least 3,000 MET-minutes per week.

Moderate: A subject not achieving the criteria for the high category but either of the following three criteria: (a) 3 or more days of vigorous-intensity of at least 20 minutes per day OR (b) 5 or more days of moderate-intensity and/ or walking of at least 30 minutes per day OR (c) 5 or more days of any combination of walking, moderate-or vigorous- intensity activities accumulating at least 600 MET minutes/week.

Low: Subject's reported activity is lower than the categories outlined above or no activity is reported at all.

Dietary intake

A validated FFQ was used for assessing usual dietary intakes (Ovaskainen *et al.*, 2006; Rolls *et al.*, 2006). The FFQ was adapted and modified from the Willett food frequency questionnaire in which 38 food items were listed (Willett *et al.*, 1987). FFQs have been shown to be valuable tools for evaluating long-term dietary intake, especially in the context of epidemiological studies such as this (Baer *et al.*, 2005). The questionnaire considered the number of times subjects ate some listed food items. The listed food items were put into six food groups: carbohydrates (e.g. rice, tuo zaafi (T.Z), etc), proteins (animal source: meat, beans, fish, and plant source: beans,

nuts, etc), fats and oils (vegetable oil, palm oil, etc), fruits and vegetables (pineapple, mango, etc), non-alcoholic beverages (soft drinks, etc) and alcoholic drinks (beer, whisky, wine, etc).

Statistical Analysis

The results are expressed as proportion and compared using Fischer's exact test or χ^2 for trend analysis as appropriate. A level of $p < 0.05$ was considered as statistically significant. GraphPad Prism version 5.00 (GraphPad software, San DiegoCalifornia USA, www.graphpad.com) for windows was used for statistical analysis.

RESULTS

The general characteristics of the studied population are as shown in Table 1. Majority of the studied population were within 20 to 39 years i.e. 150/186 (80.6%) and have attained a high level of education (89.8%). Even though, male participants represent 65.1% of the studied population, there is no significant difference in the age distribution and educational level between the male and female subjects (Table 1). From this study, the prevalence of obesity and overweight as determined by WHR was 31.2% and 12.4% respectively. When the study population was stratified based on gender, 64.5% of the male subjects significantly ($p = 0.0004$) had normal body weight compared to female subjects (41.5%). However, the prevalence of obesity was significantly ($p = 0.0312$) higher among the female participants (41.5%) compared to males (25.6%) (Table 1).

Whereas about 10% of the studied population was engaged in high level of physical activity, about 30% were engaged in moderate level of physical activity and about 60% were engaged in low level of physical activity as shown in Table 1. There was no significant difference in the levels of physical activity when the studied population was classified by gender. About 27%, 44%, 10% and 5% of the studied population consumed at least one form of carbohydrate rich foods, protein rich foods of animal source, protein rich foods of plant source as well as fats and oils rich foods respectively for more than once a day. Gender had no significant impact on the type of food groups consumed by the studied population (Table 1).

Table 1: General characteristic of the study population stratified by gender

Variable	Total (n=186)	Male (n=121)	Female (n=65)	P value
Age				
20-29	73(39.2%)	43(35.5%)	30(46.2%)	0.161
30-39	77(41.4%)	51(42.1%)	26(40.0%)	0.876
40-49	17(9.1%)	13(10.7%)	4(6.2%)	0.425
50-59	19(10.2%)	14(11.6%)	5(7.7%)	0.458
Educational level				
High	167(89.8%)	108(89.1%)	59(90.8%)	0.805
Central adiposity				
Normal	105(56.4%)	78(64.5%)	27(41.5%)	0.001
Overweight	23(12.4%)	11(9.1%)	12(8.5%)	0.099
Obese	58(31.2%)	31(25.6%)	27(41.5%)	0.031
Physical activity level				
Low	106(57.0%)	68(56.2%)	38(58.5%)	0.876
Moderate	60(32.3%)	39(32.2%)	21(32.3%)	1.000
High	20(10.8%)	14(7.5%)	6(9.5%)	0.805
Diet frequency				
Carbohydrate-rich foods	50(26.9%)	34(28.1%)	16(24.6%)	0.729
Animal protein	82(44.1%)	54(44.6%)	28(43.1%)	0.839
Plant protein	18(9.7%)	11(9.1%)	7(10.8%)	0.712
Fats & Oils	9(4.8%)	5(4.1%)	4(6.2%)	0.540
Fruits & vegetables	29(15.6%)	17(14.0%)	12(18.5%)	0.429
Alcoholic beverages	4(2.2%)	4(3.3%)	0(0.0%)	0.138
Non-Alcoholic beverages	26(14.0%)	15(12.4%)	11(16.9%)	0.396

Data are presented as proportion and analyzed using Fischer's exact test.

From Table 2, using chi-square for trend analysis, there is no significant association between central obesity as determined by WHR and food groups consumed by the studied population using chi square for trend analysis. In Table 3, the type of food groups consumed was stratified based on age.

The proportion of the studied population that consumed at least one form of carbohydrate rich food, protein rich food of animal source, fats and oils rich food as well as those that consumed fruits and vegetables for more than once a day generally decreased with age (Table 3).

Table 2: Diet frequency and central adiposity

Variable	Normal (n=105)	Overweight (n=23)	Obese (n=58)	P value
Carbohydrate-rich foods	32(30.5%)	4(17.4%)	14(24.1%)	0.324
Animal protein	46(43.8%)	7(30.4%)	29(50.0%)	0.542
Plant protein	12(11.4%)	0(0.0%)	6(10.3%)	0.689
Fats & Oils	6(5.7%)	0(0.0%)	3(5.2%)	0.783
Fruits & vegetables	16(15.2%)	3(13.0%)	10(17.2%)	0.765
Alcoholic beverages	2(1.9%)	0(0.0%)	2(3.4%)	0.570
Non-Alcoholic beverages	15(14.3%)	4(17.4%)	7(12.1%)	0.737

Table 3: The rate of consumption of the various diet group stratified by age

Variable	20-29 (n=73)	30-39 (n=77)	40-49 (n=17)	50-59 (n=19)	P value
Carbohydrate-rich	26(35.6%)	21(27.3%)	2(11.8%)	1(5.3%)	0.002
Animal protein	44(60.3%)	31(40.3%)	3(17.6%)	4(21.1%)	0.001
Plant protein	7(9.6%)	8(10.4%)	1(5.9%)	2(10.5%)	0.945
Fats & Oils	7(9.6%)	2(2.6%)	0(0.0%)	0(0.0%)	0.025
Fruits & vegetables	17(23.3%)	10(13.0%)	1(5.9%)	1(5.3%)	0.016
Alcoholic beverages	2(2.7%)	2(2.6%)	0(0.0%)	0(0.0%)	0.385
Non-alcoholic beverages	13(17.8%)	9(11.7%)	2(11.8%)	2(10.5%)	0.313

Data was presented as proportion and analyzed using chi-square for trend

The association between age and physical activity level with body weight is presented in figure 1. From the chi-square for trend analysis, the prevalence of

obesity significantly increased with age ($p < 0.001$) but decreased with level of physical activity ($p = 0.018$).

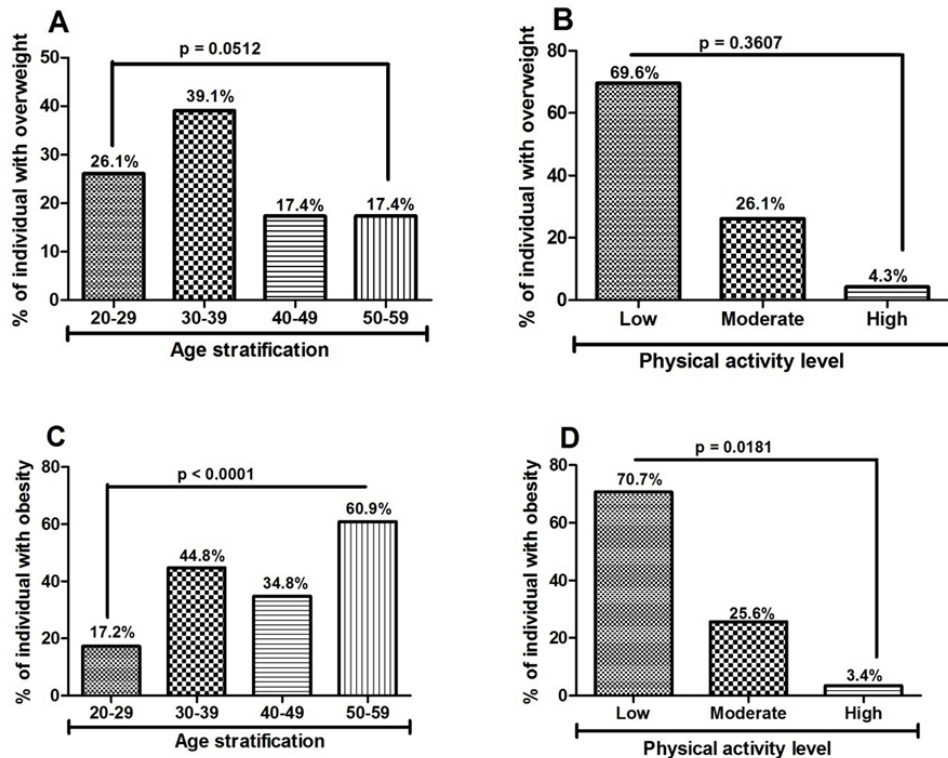


Figure 1: Association between age and overweight (A), physical activity level and overweight (B), age and obesity (C) as well as physical activity level and obesity (D). Data was analyzed using chi-square for trend.

DISCUSSION

From the study, the overall prevalence of central obesity was found to be 31.2%. This is consistent with a central obesity prevalence of 31.9% reported in a study among an industrial population in India (Reddy *et al.*, 2006). The prevalence of central obesity in this study was higher than the 20% reported by Addo *et al.*, (2009) among civil servants in Accra and the 14.4% (defined by WHR) reported by Owiredu *et al.*, (2011) in Kumasi. Addo *et al.*, (2009) used Body Mass Index (BMI) to define obesity and this could have accounted for the difference in prevalence. Ferland *et al.*, (1989) reported that WHR was a good predictor of intra-abdominal adipose tissue than BMI. Furthermore, BMI does not account for the wide variation in body fat distribution and has considerable limitations in predicting intra-abdominal fat accumulation (Chen *et al.*, 2000). BMI is therefore not able to differentiate between subcutaneous from visceral fat accumulation (Fujimoto *et al.*, 1999). On the other hand, WHR provides an index of both subcutaneous and intra-abdominal adipose tissue (Bjorntorp, 1987), making the central obesity rates defined by it being higher than rates measured by BMI. Owiredu *et al.*, (2011) conducted their study among active sportsmen/sportswomen and sedentary workers and this could have contributed to the lower prevalence of central obesity. The subjects in that study included active sportsmen and women, who were generally physically active. Subjects in this research however had lower levels of physical activity demonstrated by the inverse relationship between the level of physical activity and central obesity observed in this study.

Central obesity was significantly higher in women than men and this significant finding is in agreement with several studies done elsewhere in Ghana (Hill *et al.*, 2007) and in other African countries (Maher *et al.*, 2011; Shayo *et al.*, 2011; Wahab *et al.*, 2011). Participation in regular physical activity was generally low in this study and was even lower among females. This fact, coupled with the general perception among African women that being obese is a sign of affluence perhaps accounts for such a high obesity prevalence rate among women involved in this study. In a study by Ojofeitimi and colleagues in

south western Nigeria, subjects in a University community believed that being obese is a sign of well-being and gives respect (Ojofeitimi *et al.*, 2007).

Generally, obesity increased significantly with increase in age category in this study. These findings are in consonance with prevalence of central obesity found among senior civil servants in Nigeria (Odenigbo *et al.*, 2008), South-African adults in South Africa (Puoane *et al.*, 2002) and Cuban adults in Cuba (Diaz *et al.*, 2009). The proportion of fat deposited in the abdomen increases as body shape becomes more android with age, due to decreasing height and increasing slackness of abdominal wall muscles. During adulthood, weight gain occurs in the abdominal region, emphasizing the importance of hypertrophic obesity, which is generally android (Kaye *et al.*, 1990). This change in the adult figure may influence the positive association between age and excess abdominal adiposity, measured by waist-to-hip ratio (Lanska *et al.*, 1985). This fact possibly explains the increase in obesity prevalence with increasing age category in this study.

In a study on the association of body fat distribution with lifestyle and reproductive factors in postmenopausal women, Kaye *et al.*, (1990) showed that waist-to-hip ratio was negatively associated with physical activity. In addition, a study of subjects from two urban and one rural community in Accra, Ghana, found high levels of overweight and obesity among subjects who were sedentary and engaged in light activity (Amoah, 2003). These findings are consistent with the findings of this current study which showed that subjects with low physical activity levels had significantly high prevalence of overweight (69.6%) and central obesity (70.7%) and the prevalence of both overweight and obesity decreased with increase in the level of physical activity. Similarly, a population based cross-sectional study conducted by Al-Nozha and colleagues among Saudis aged 30-70 years, reported that inactive males and females had a significantly higher waist circumference than active participants of both sexes (Al-Nozha *et al.*, 2007). In a study, in which CT scan was used to assess abdominal obesity, physical activity was shown to be strongly associated with lower

visceral adipose tissue in men from 30-70 years (Hunter *et al.*, 1997). One can therefore not overemphasize the well-established protective role of physical activity for the development of obesity and associated co-morbidities. The role of physical activity in the prevention and management of overweight and obesity is linked, in part, to the impact of physical activity on energy expenditure, body composition, and substrate oxidation and metabolism (Donnelly *et al.*, 2004). Policies that would help individuals build a positive attitude towards exercise should be instituted so as to cultivate the habit of engaging in regular and beneficial physical exercises.

There was no significant association between dietary intake and obesity. As indicated in the results of this study, subjects consumed less of both high energy dense foods and fruits and vegetables. This suggests that the high prevalence rate of central obesity found in this research cannot be attributed to dietary intake, but could be due to low levels of physical activity and age. In contrast to the works of other researchers, no relationship was found between alcohol and obesity in this study (Breslow *et al.*, 2005; Colditz *et al.*, 1991; Schroder *et al.*, 2007; Wannamethee *et al.*, 2003). The lack of association could be explained by the small proportion of study participants who indulged in alcohol drinking. The small proportion of alcohol drinkers could be due to the fact that a greater majority of the inhabitants in the Tamale metropolis are moslems (Ghana Government Official Portal, 2012) and as required by the Islamic faith, do not consume alcoholic beverages of any kind.

CONCLUSION

Prevalence of central obesity is high in this study. In consonance with most studies, female subjects were more at risk compared to their male counterparts. Level of physical activity and age category were major determinants of central obesity. Individuals should be encouraged to engage in regular physical exercise.

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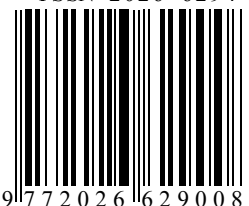
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ORIGINAL ARTICLE

Haematopoietic effect of an ethanolic leaf extract of *Ipomoea involucrata* P. Beauv in phlebotomized New Zealand White Rabbits

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Ipomoea involucrata is used by some religious bodies (that do not believe in the use of allopathic medicine) in Ghana to treat haematological conditions particularly anaemia. The aim of this study therefore is to determine the haematopoietic effect of the ethanolic leaf extract of *Ipomoea involucrata* to establish the scientific bases for its use. The haematological profile of healthy New Zealand rabbits, phlebotomized rabbits, and phlebotomized rabbits treated with 0.23 ml/kg Ferglobin®, 100, 300, and 1000 mg/kg *I. involucrata* ethanolic leaf extract, or 0.23 ml normal saline were determined at 20-day intervals for 40 days using the Cell Dyn 1800 Automatic Analyzer®. Data obtained was analyzed using GraphPad Prism version 5. The 300 and 1000 mg/kg dose of the extract and the reference hematinic caused significant increments ($P \leq 0.01$ -0.001) in white blood cells, red blood cells, haemoglobin concentration, hematocrit, and platelets counts within 20 days of treatment. The mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration, and the red cell distribution width recorded in all categories were not significantly different. This indicates that the ethanolic leaf extract of *Ipomoea involucrata* has some haematopoietic activity and thus could be effective in managing anaemia and other blood cell deficiency disorders.

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Keywords: Phlebotomy, Haemoglobin, RBC, Haematological Profile

INTRODUCTION

Anaemia, the most predominant blood cell deficiency disorder, is a global public health problem affecting both developing and developed countries with major consequences for human health as well as social and economic development (de Benoist *et al.*, 2008). It occurs at all stages of life, but is more prevalent in pregnant women and young children. More frequently it coexists with a number of other causes, such as malaria, parasitic infection, nutritional deficiencies, and haemoglobinopathies (de Benoist *et al.*, 2008). Other blood cell deficiency disorders are neutropenia and thrombocytopenia.

Severe anaemia (prevalence exceeds 2%) is a problem in most countries in Africa and South Asia and some countries in East Asia and the Pacific (Galloway, 2003; Stoltzfus, 2003). In most parts of Africa the main causes of anaemia are usually due to poor nutrition and malaria. An earlier study has shown prevalence of anaemia in southern Ghana to be fairly common, particularly in children and women (Commye and Dekyem, 1995).

Low income levels in Ghana (Obeng, 2008) pose a challenge in the affordability of orthodox haematinics. Access to health centers in rural areas is also a problem; meanwhile as much as 80 % of people in developing countries are said to depend on traditional medicines for primary healthcare (Bodekar, 1994; WHO, 2008). In fact, the Alma Ata declara-

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tion of 1978 encouraged the use of all available resources for primary healthcare and recommended that government should give high priority to using traditional health practices and incorporate proven traditional remedies into National Drug Policy and Regulations (Zhang, 1994). One widely used traditional remedy for anaemia is *Ipomoea involucrata*.

Ipomoea involucrata P. Beauv. var. *involucrata* (Family: Convolvulaceae) commonly known as Dutchman's pipe is a slender but vigorous, sprawling or twining annual or perennial herb, of grassland, secondary scrub and forest origin very common throughout tropical Africa. Its several medicinal uses are as follows: an infusion of the whole plant is drunk as a stimulant, or preventative of fever; a decoction of the fresh sap is taken as a remedy for gonorrhoea; the leaves are used for asthma (Oliver, 1960); a plant preparation is added to baths or made into a lotion for treating jaundice (Bouquet & Debray, 1974); the leaf-sap is applied and rubbed into areas of localized edema and is instilled into the eyes for filarial infection; an aqueous decoction is taken by women for dysmenorrhoea and at child-birth to hasten expulsion of the after-birth; and a compress of pounded up stems is used for headache (Bouquet, 1969).

It is in this light that this study has been conducted to determine the haematopoietic effect of *Ipomoea involucrata* to establish the scientific bases for its use and to add to the armamentarium of herbs that can be used to manage anaemia in Ghana.

MATERIALS AND METHODS

Plant Collection

Ipomoea involucrata was collected from KNUST campus in August 2008 and authenticated by Mr. G. H. Sam of the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi, Ghana where a voucher specimen with number KNUST/HM1/2011/R004 has been deposited. The leaves were harvested, air dried, and powdered using a hammer mill (Schutte Buffalo, New York, USA).

Preparation of Extract

A 220 g quantity of the leaf powder of *Ipomoea involu-*

crata was soxlet extracted with 70 % ethanol. The liquid extract obtained was condensed under low temperature and pressure using a Buchi Rotor Evaporator (Rotavapor R-210, Switzerland), and further dried over a hot water bath (B-TEX Laboratory Engineering, Gujarat India) at 40 °C. The dried extract weighing 18.5 g (percentage yield: 8.4) was labeled and stored in a desiccator. Required quantities taken and dissolved in distilled water for use in this study will be referred to as the ethanolic extract of *Ipomoea involucrata* or EIE.

Phytochemical Screening

The ethanolic leaf extract of *Ipomoea involucrata* was subjected to phytochemical screening in accordance with the standard procedure (Harborne 1998).

Animals and Husbandry

Male New Zealand White Rabbits (4.8 – 5.2 kg) obtained from Kwadaso Agricultural College, Kumasi, Ghana were kept in the Department of Pharmacology, KNUST, animal house for this study. They were fed with normal rabbit chow (GHAFCO, Tema, Ghana), fresh herbs like *Aspilia Africana* and rough meadow-grass (*Poa trivialis*) from around the KNUST campus, and clean water. During the experimental period, the rabbits were kept in clean, well-ventilated, individual, front-opening stainless steel cages (that had grid plastic floor) in the laboratory under ambient dark-light cycle and relative humidity. The rabbits were allowed to acclimatize to the laboratory environment for one week prior to experimentation. During this period, the animals were periodically assessed for gross morphological and behavioral changes.

All procedures and techniques used in these studies were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH, Department of Health Services publication No. 83-23, revised 1985). The protocols for the study were approved by the Departmental Ethics Committee.

Reference Haematinic

Feroglobin® (Vitabiotics Ltd, Great Britain), a liquid tonic containing Iron 0.2 %, Zinc 0.06 %, Cop-

per 0.02 %, Manganese 0.025 %, and vitamin B-Complex 0.39 % in a blend of honey and malt, was used as the reference hematinic. The dose of Feroglobin® administered to experimental animals in this study is equivalent to the adult human dose (0.23 ml/kg/day) stated by the manufacturers.

Phlebotomization of Rabbits

Phlebotomization of the New Zealand White rabbits was achieved by following a rabbit bleeding protocol (McGuill and Rowan, 1989) after their basal haematological profile had been determined using the Abbot Cell Dyn 1800 Automatic Analyzer® (Abbott Diagnostics, USA). In this protocol, the rabbits were sedated using sodium thiopentone (60 mg/kg, i.p). The left ears were shaved to expose the auricular artery (which runs medially in the ear). This was disinfected with 70% ethanol and rubbed gently with 30% limonene in 95% ethanol to dilate it. A hypodermic needle (22, or 20 gauge, 1 inch long) attached to a calibrated tube was inserted into the central artery about two-thirds the way up the central artery, with the tip of the needle pointing towards the base of the ear (McGuill and Rowan, 1989). About 15-20 % of the total volume of blood, calculated based on 60 ml/kg per rabbit (Suckow *et al.*, 2002), in the rabbit were collected into the calibrated tube. When blood collection was completed, the needle was removed and the artery held off firmly with cotton wool for 3 minutes until the bleeding stopped. Drops and smears of blood were cleaned with 70% ethanol to prevent infection of punctured site. The rabbits were returned to the cage when there was no further bleeding which is indicative of restored circulation. Phlebotomization was considered satisfactory and normocytic anaemia was considered induced when blood cell counts reduced by at least 30% after the haematological profile were determined after 24 hours.

Experimental Procedure

The phlebotomized rabbits were weighed and put into five groups (A-E) with five animals per group for the study. The groups were treated with 0.23 ml/kg Feroglobin®, 100, 300, or 1000 mg/kg EIE, or 0.23 ml/kg normal saline for 40 days. A sixth group, F, with 5 healthy rabbits was also kept under

the laboratory conditions but these were not given any form of treatment. At day 20 and day 40, blood samples from animals in all groups were collected into MediPlus K3 EDTA tubes (Sunphoria Co. Ltd., Taiwan) for haematological analysis using the Abbot Cell Dyn 1800 Automatic Analyzer®. Data obtained on white blood cell count (WBC), red blood cell count (RBC), haemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red blood cell distribution width (RDW), and platelet count (PLT) were recorded for analysis.

Statistical Analysis

Significant changes in measured parameters between normal, phlebotomized, and phlebotomized but treated rabbits were determined using One-Way Analysis of Variance followed by Dunnett's Multiple Comparison Test. Statistical estimates were made at a confidence limit of 95% and probability values ($P \leq 0.05$) were considered significant. The analysis was done using GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA).

RESULTS

Phytochemical Screening

Results from the phytochemical screening of EIE is as shown in Table 1.

Table 1: Results obtained from phytochemical screening of ethanolic extract of *Ipomoea involucrata* (EIE)

Components	EIE
Tannins	+
Flavonoids	-
Alkaloids	+
Sterols	-
Glycosides	+
Saponins	+
Triterpenoids	-

(+) indicates the presence of; (-) indicates the absence of the phytochemicals tested

Phlebotomization

There was a very significant reduction ($P \leq 0.001$) in RBCs, HGB, HCT and PLT after phlebotomy. The WBC was also significantly ($P \leq 0.01$) reduced; however, there were insignificant changes ($P > 0.05$) in MCV, MCH, MCHC, and RDW (Table 2).

Haematopoietic effect

Treatment of phlebotomized rabbits with 300 mg/kg EIE for 20 days resulted in significant increments ($P \leq 0.05$) in WBC, RBCs, and very significant increments ($P \leq 0.001$) in HGB, HCT and PLT. The 1000 mg/kg EIE also caused significant increments ($P \leq 0.05$) in WBC and very significant increments ($P \leq 0.01$) in RBC and PLT. HGB, and HCT were also very significantly ($P \leq 0.001$) elevated. The reference hematonic showed very significant elevation ($P \leq 0.01$) in WBC, RBC, and PLT as well as very significant elevation ($P \leq 0.001$) in HGB and HCT. Changes in MCV, MCH, MCHC, and RDW with treatments were however insignificant (Table 3). After 40 days of treatment, the reference drug-treated and the 1000 mg/kg-treated groups showed very significant increments ($P \leq 0.01$) in WBC, and even more significant increments ($P \leq 0.001$) in RBCs, HGB, HCT and PLT. The 100 mg/kg EIE treatment also showed some significant increments ($P \leq 0.05$) in WBC and PLT and also significant increments in RBC, HGB, and HCT (not seen with the normal saline-treated group). With the exception of WBC which increased significantly ($P \leq 0.05$), RBC, HGB, HCT, and PLT increased very significantly ($P \leq 0.001$) in the 300 mg/kg-treated group. However there were still no significant changes in MCV, MCH, MCHC, and RDW with the treatments (Table 4).

DISCUSSION

The significant reduction in the blood cells in circulation after phlebotomy is an indication that this protocol causes blood cell deficiency which will require haematopoiesis for correction. The very significant elevation of WBC, RBC, and PLT caused by the 300 and 1000 mg/kg doses of EIE and the reference hematonic indicates enhanced haematopoiesis. This indicates that EIE can be used to correct anaemia resulting from active bleeding, malnutrition, pregnancy, and chronic disease; leucopenia caused by viral

Table 2: The haematological profiles of New Zealand White rabbits prior to phlebotomization (Normal I), phlebotomized rabbits (PBTM), and normal rabbits kept under experimental conditions over the 40-day study period (Normal II).

Variables	Normal I	PBTM	Normal II
WBC (K/ μ L)	8.7 \pm 1.5	5.3 \pm 1.7 ^{††}	8.0 \pm 1.8 ^{ns}
RBC (M/ μ L)	6.6 \pm 0.8	4.0 \pm 0.8 ^{†††}	6.3 \pm 0.9 ^{ns}
HGB (g/dL)	13.2 \pm 0.7	8.3 \pm 0.8 ^{†††}	12.9 \pm 1.0 ^{ns}
HCT (%)	42.3 \pm 3.5	27.3 \pm 3.9 ^{†††}	40.8 \pm 3.6 ^{ns}
MCV (fL)	64.2 \pm 2.3	63.4 \pm 3.5 ^{ns}	64.9 \pm 3.2 ^{ns}
MCH (pg)	21.5 \pm 2.6	21.9 \pm 3.0 ^{ns}	22.5 \pm 3.6 ^{ns}
MCHC (g/dL)	33.9 \pm 2.3	35.2 \pm 2.9 ^{ns}	34.5 \pm 3.5 ^{ns}
RDW (%)	16.2 \pm 1.9	16.6 \pm 2.1 ^{ns}	16.4 \pm 1.8 ^{ns}
PLT (K/ μ L)	379 \pm 55.8	232 \pm 43.6 ^{†††}	342 \pm 48.8 ^{ns}

Values quoted are means \pm SD. Levels of significance between phlebotomized/normal II and normal I was determined using One-Way Analysis of Variance (ANOVA) followed by Dunnett's Multiple Comparisons Test. For significant decrements: ^{††} implies $P \leq 0.01$; ^{†††} implies $P \leq 0.001$.

infection, rheumatoid arthritis and autoimmune disorders; and thrombocytopenia caused by diminished platelet survival. Haematopoiesis requires iron and the B-complex vitamins (Koury and Ponka, 2004), folic acid, some mineral such as copper, zinc and manganese, and the action of haematopoietic growth factors or haematopoietic cytokines. Similarity in effect between the higher doses of the extract and the reference hematonic suggests that the extract could have some of the components stated or could possibly stimulate the synthesis and release of the haematopoietic growth factors (which are synthesized by activated cells under required conditions rather than being produced constitutively all the time) to cause the proliferation and differentiation of haematopoietic stem cells of the bone marrow into the various blood cells.

Although the body's homeostatic mechanisms could induce haematopoiesis (seen as the slight elevation of blood cells in the normal saline treated rabbits), the extracts and the reference hematonic induced haematopoiesis very significantly in a

Table 3: A comparison of the haematological profiles of phlebotomized (PBTM) rabbits and phlebotomized rabbits treated with 0.23 ml/kg normal saline (NS), 0.23 ml/kg Ferroglobin®, and 100, 300, and 1000 mg/kg EIE for 20 days.

Variables	PBTM	NS	Ferroglobin®	EIE (100)	EIE (300)	EIE (1000)
WBC(K/ μ l)	5.3 \pm 1.7	6.3 \pm 1.4 ^{ns}	9.0 \pm 1.3 ^{††}	7.2 \pm 1.2 ^{ns}	8.0 \pm 1.3 [†]	8.0 \pm 1.5 [†]
RBC(M/ μ l)	4.0 \pm 0.8	4.4 \pm 0.6 ^{ns}	5.8 \pm 0.6 ^{††}	4.8 \pm 0.7 ^{ns}	5.4 \pm 0.7 [†]	5.6 \pm 0.9 ^{††}
HGB (g/dl)	8.3 \pm 0.8	9.0 \pm 0.8 ^{ns}	11.6 \pm 0.8 ^{†††}	9.8 \pm 0.7 [†]	10.9 \pm 0.7 ^{†††}	11.3 \pm 0.9 ^{†††}
HCT (%)	27.3 \pm 3.9	30.7 \pm 2.9 ^{ns}	39.6 \pm 1.9 ^{†††}	32.1 \pm 2.4 [†]	37.0 \pm 2.4 ^{†††}	37.1 \pm 1.8 ^{†††}
MCV (fl)	65.4 \pm 3.5	66.8 \pm 2.9 ^{ns}	65.5 \pm 3.2 ^{ns}	65.2 \pm 3.5 ^{ns}	66.8 \pm 2.6 ^{ns}	65.4 \pm 3.8 ^{ns}
MCH (pg)	20.0 \pm 1.0	19.5 \pm 1.4 ^{ns}	19.9 \pm 1.9 ^{ns}	19.3 \pm 1.1 ^{ns}	19.8 \pm 1.2 ^{ns}	19.9 \pm 1.0 ^{ns}
MCHC (g/dl)	31.2 \pm 2.9	31.6 \pm 2.1 ^{ns}	31.2 \pm 2.6 ^{ns}	31.3 \pm 2.4 ^{ns}	31.7 \pm 2.9 ^{ns}	31.4 \pm 2.7 ^{ns}
RDW (%)	16.6 \pm 2.1	16.4 \pm 1.7 ^{ns}	16.4 \pm 2.2 ^{ns}	16.8 \pm 1.5 ^{ns}	16.0 \pm 1.9 ^{ns}	16.6 \pm 2.1 ^{ns}
PLT (K/ μ l)	232 \pm 43.6	278 \pm 51.8 ^{ns}	365 \pm 62.7 ^{††}	291 \pm 47.8 ^{ns}	380 \pm 56.2 ^{††}	388 \pm 72.5 ^{††}

Values quoted are means \pm SD. Levels of significant between the phlebotomized and the phlebotomized but treated rabbits were determined using One-Way Analysis of Variance (ANOVA) followed by Dunnet's Multiple Comparisons Test. For significant increments: † implies $P \leq 0.05$; †† implies $P \leq 0.01$; ††† implies $P \leq 0.001$; ns implies $P > 0.05$.

Table 4: A comparison of the haematological profiles of phlebotomized (PBTM) rabbits treated with 0.23 ml/kg normal saline (NS), 0.23 ml/kg Ferroglobin®, 100, 300, and 1000 mg/kg EIE for 40 days.

Variables	PBTM	NS	Ferroglobin®	EIE (100)	EIE (300)	EIE (1000)
WBC(K/ μ l)	5.3 \pm 1.7	6.9 \pm 1.3 ^{ns}	8.8 \pm 1.3 ^{††}	7.8 \pm 1.5 [†]	8.1 \pm 1.4 [†]	8.5 \pm 1.2 ^{††}
RBC(M/ μ l)	4.0 \pm 0.8	5.5 \pm 0.9 [†]	7.3 \pm 0.9 ^{†††}	5.9 \pm 0.9 ^{††}	6.5 \pm 0.7 ^{†††}	6.8 \pm 0.9 ^{†††}
HGB (g/dl)	8.3 \pm 0.8	10.8 \pm 1.3 [†]	14.5 \pm 1.2 ^{†††}	11.9 \pm 1.7 ^{††}	13.3 \pm 1.1 ^{†††}	14.1 \pm 1.9 ^{†††}
HCT (%)	27.3 \pm 3.9	36.1 \pm 3.4 ^{††}	48.7 \pm 3.9 ^{†††}	40.4 \pm 2.8 ^{†††}	43.7 \pm 2.7 ^{†††}	45.9 \pm 3.7 ^{†††}
MCV (fl)	65.4 \pm 2.0	66.2 \pm 1.6 ^{ns}	66.4 \pm 1.5 ^{ns}	66.1 \pm 1.7 ^{ns}	66.7 \pm 1.4 ^{ns}	65.8 \pm 1.7 ^{ns}
MCH (pg)	20.0 \pm 1.0	19.3 \pm 1.6 ^{ns}	19.5 \pm 1.5 ^{ns}	19.9 \pm 1.2 ^{ns}	20.1 \pm 1.8 ^{ns}	19.2 \pm 1.1 ^{ns}
MCHC(g/dl)	31.2 \pm 2.9	29.8 \pm 1.1 ^{ns}	30.4 \pm 1.3 ^{ns}	30.7 \pm 1.4 ^{ns}	29.9 \pm 1.1 ^{ns}	30.1 \pm 1.2 ^{ns}
RDW (%)	16.6 \pm 2.1	16.9 \pm 1.9 ^{ns}	16.1 \pm 1.4 ^{ns}	16.6 \pm 2.1 ^{ns}	16.5 \pm 1.7 ^{ns}	16.2 \pm 1.8 ^{ns}
PLT (K/ μ l)	232 \pm 43.6	303 \pm 49.7 ^{ns}	419 \pm 52.2 ^{†††}	324 \pm 48.4 [†]	362 \pm 58.6 ^{††}	388 \pm 64.5 ^{†††}

Values quoted are means \pm SD. Levels of significant between the phlebotomized and the phlebotomized but treated rabbits were determined using One-Way Analysis of Variance (ANOVA) followed by Dunnet's Multiple Comparisons Test. For significant increments: † implies $P \leq 0.05$; †† implies $P \leq 0.01$; ††† implies $P \leq 0.001$.

shorter duration of time. The insignificant changes in haematological profile between the normal animals prior to phlebotomy and normal rabbits kept under experimental conditions indicates that the experimental conditions had no adverse effect on the haematological profile of experimental animals.

Preliminary phytochemical screening carried out in this study indicated that *Ipomoea involucrata* leaves contain tannins, alkaloids, glycosides, and saponins in its ethanolic extract. The haematopoietic potential of the leaf extract may be related to its phytochemicals present. Glycosides and saponins have been documented to significantly increase the proliferation abilities of bone marrow cells (Kirby and Bentley, 1991; Gao et al., 1992; Li et al., 2011). Alkaloids enhance the restoration of haematopoiesis (Boyko and Belskiy, 1998). Tannins have been reported to inhibit the formation of superoxide ions and hydroxy radicals, which are two strong peroxidation agents (Facino et al., 1990; Uboh et al., 2010). This antioxidant activity may protect both the haematopoietic committed stem and the formed blood cells from the attack of the reactive free radicals in the body.

CONCLUSION

The ethanolic leaf extract of *Ipomoea involucrata* has some haematopoietic effects and thus can be used to treat anaemia and other blood cell deficiency disorders. Its safety for use however needs to be ascertained.

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ORIGINAL ARTICLE

Residual Risk of Transmission of Hepatitis B Virus through Blood Transfusion in Ghana: Evaluation of the performance of Rapid Immunochromatographic Assay with Enzyme Linked Immunosorbent Assay

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Blood transfusion necessitates screening of transmissible infectious pathogens such as hepatitis B virus (HBV) to curtail post transfusion risk of infection. The study re-examined this approach by evaluating the efficiency of solely testing for hepatitis B surface antigen (HBsAg) marker for blood transfusion, the efficacy of the various immunochromatographic assays in the screening process and the residual risk of hepatitis B viral transmission through transfusion in Ghana. A convenient purposive sampling technique was used in selecting ten hospitals, from each of the 10 regions. A total of 480 aliquots of blood were collected anonymously, from blood already tested for HBsAg with immunochromatographic assay in the blood banks of the chosen facilities and declared negative. Plasma from the blood was obtained through centrifugation, separated into well labeled microtubes and transported in cold boxes to the Molecular Medicine Department-KNUST. The samples were then re-examined for all six hepatitis B virus (HBV) (HBsAg, anti-HBs, HBeAg, anti-HBe, anti-HBc and IgM anti-HBc) serological markers using ELISA assay. When a total of 480 plasma samples from the blood banks of the ten chosen facilities were re-examined with the ELISA assay, 39(8.13%) samples reacted positive for HBsAg, 60(12.5%) reacted for Anti-HBs, 13(2.71%) reacted for HBeAg, 51(10.63%) for Anti-HBe and 329(68.54%) reacted positively for Anti-HBc. None of the samples reacted positive for IgM anti-HBc. The estimated sero-prevalence for all HBV serological markers is 76.67% whereas the estimated residual risk of HBV infection through blood transfusion caused by the use of immunochromatographic methods in the screening of blood for transfusion was 8.47%(5.98% - 10.94% at 95% CI). An additional risk of 3.10%(1.54% - 4.62% at 95% CI) of HBV infection through transfusion was also estimated for the non-testing of other HBV infectious serological markers. The total residual risk for transfusion transmitted HBV was 11.16%(8.34% - 13.95% at 95% CI). The study revealed that neither the kits in use nor the testing strategy in place now is adequate to prevent transmission of hepatitis B virus through transfusion in Ghana due to the high residual risk of transmission of HBV. There is therefore an urgent need for a sustainable quality control system on the screening of HBsAg in blood for donation in Ghana.

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INTRODUCTION

Transfusion transmitted infections are still a major global public health concern confronting the worldwide delivery of transfusion services and this is

even more apparent in the under resourced transfusion centres operating in the third world countries (Aach *et al.*, 1981). Blood transmitted infections involving pathogenic viruses are the most renowned in transfusion medicine (Niederhauser *et al.*, 2005). In spite of all the scientific advancement geared towards blood donation safety, Hepatitis B virus residual risk remains the single most transmitted infectious pathogenic disease passed on from blood donors to their recipients through transfusion (El-Sherif *et al.*, 2007).

Hepatitis B virus infection leads to a sequence of serological markers or viral protein in the bloodstream of infected individuals. Hepatitis B viral deoxyribonucleic acid (DNA) is the first to appear in the blood, then hepatitis B viral surface antigen (HBsAg), followed by viral polymerase DNA and then hepatitis B viral e antigen (HBeAg). Host's response results in production of antibodies to various hepatitis B viral antigens. The first detectable antibody in the serum of an infected person is antibody to the core antigen (Anti-HBc); this antigen is not expressed in the bloodstream and is only found in HBV-infected hepatocytes in the liver. Antibodies to hepatitis B viral e antigen (Anti-HBe) are then secreted into the serum and followed finally by antibody to hepatitis B viral surface antigen (Anti-HBs) (Kumar *et al.*, 2007; Kukka, 2008).

The issues with blood safety are essentially in two parts. First and most important is the identification of tainted or infectious blood units and the onward prevention of its transfusion. In the presence of safeguarding the recipient, steps must also be taken to prevent false positives which could lead to wastage of blood. The donor selection policy adopted by the facility should therefore be able to exclude donors from high risk groups. Also the specificity and sensitivity of the assay technology in use should be high enough to identify all true positive and true negative blood donors or blood units (Allain and Lee, 2005).

Despite the institution of mandatory screening for HBsAg, the issue of transfusion-associated HBV is still a major health problem plaguing most third

world and resource poor countries (Dhawan *et al.*, 2008). Blood donors in Ghana are selected on the basis of a health check questionnaire coupled with a visual physical examination and a mandatory microbiological screening which includes Syphilis, HIV, Hepatitis C and HBsAg test (Ampofo *et al.*, 2002).

Among the pathogenic markers which are screened before transfusion in Ghana, human immunodeficiency virus screening is the only marker that has a nationally developed standardised guideline procedure for testing, supervision and centralised provision of test kits (Allain *et al.*, 2003). Unlike HIV, Hepatitis B virus surface antigen (HBsAg) screening in Ghana does not have any nationally developed supervised procedures and thus the choice of assay technology and brand of test kit used is the sole prerogative of health facilities (Ampofo *et al.*, 2002).

Retrospective studies carried out in other countries on blood donors using first generation tests such as immunodiffusion and/or immunochromatography in the detection of HBsAg, found that 52%-69% of recipients of blood developed hepatitis B infection (Alter and Chalmers, 1981). Allain and co-workers in 2003 observed that neither the latex agglutination nor the dipstick assays currently in use for HBsAg screening had sufficient sensitivity in excluding HBV infected blood from transfusion. Latex agglutination method presented a false negative rate of 46%, whilst dipstick recorded a rate of 29% false negative (Allain *et al.*, 2003). HBsAg sero-negative test however does not rule out the risk of transmission of hepatitis B infection (Al-Mekhaizeem *et al.*, 2001; Brechot *et al.*, 2001; Kumar *et al.*, 2007), and this can be as a result of undetected infected donors with early acute infection in the "window period", resolving infection, silent or occult infection or infection with atypical variants or mutation (Reesink *et al.*, 2008).

The accurate estimation of residual risk of transfusion-transmissible infectious diseases are essential for monitoring the safety of blood supply and the evaluation of the potential effect of screening tests and the risk reduction procedures under implementation (Schreiber *et al.*, 1996). The most direct way of estimating the residual risk of viral agents transmissible

through transfusion would be a prospective study that evaluates the rate of viral infection in blood recipients (Aach *et al.*, 1981; Donegan *et al.*, 1990). However such a prospective study would require testing a large patient population for HBV before transfusion and then follow up testing after transfusion for the risk to be measured accurately and this would be both resource and time demanding. There is also an obvious implication of responsibility for health facilities where recipient after transfusion later test positive for HBV and these make such research impracticable in most settings. The alternative therefore is further retesting for the rate of infection in samples of donated blood that test negative on routine screening by the use of more sensitive assays for the viral agents (Schreiber *et al.*, 1996). The primary objective of this study therefore was to evaluate the residual risk of transmission of hepatitis B virus through blood transfusion and thus assess the efficacy of the test methods being used for HBsAg screening and the efficiency of using HBsAg as sole hepatitis B viral marker for blood transfusion in Ghana.

MATERIALS AND METHODS

Sampling

A convenient, purposive cross-sectional study was carried out from October 2007 to June 2008 in ten laboratories, one in each of the ten regions in Ghana. The study was anonymous and non-linked. Aliquots of blood were collected from donated blood units that tested sero-negative for HBsAg upon screening with immunochromatography assays in the facilities. The plasma was separated into well labelled micro-tubes and transported in cold boxes to the Molecular Medicine Laboratory of the Kwame Nkrumah University of Science and Technology in Kumasi and stored at -20 degrees. The types and brand names of the immunochromatographic assays used for the initial screening at the various facilities were recorded. Samples were re-examined for HBsAg and the five other serological markers of HBV (Anti-HBs, HBeAg, Anti-HBe, Anti-HBc and IgM Anti-HBc) using an Intec advanced 3rd generation ELISA test kit according to the manufacturers instruction. All blood donors in Ghana are of consenting age and consented to the

retesting of HBV. Approval for this study was granted by the research and coordinating units of all ten laboratory facilities involved in the conduct of the study.

Dipstick assay methods

All samples from the Central, Ashanti and Brong Ahafo regions were pre-screened with DiaSpotTM one step test strip (DiaSpot World of Health Biotech Co.; USA). Samples from the Volta and Upper East regions were screened with VirucheckTM dipstick, (Orchid Biomedical Systems, India). Samples from the Greater Accra and the Eastern regions were screened with ClinotechTM HBsAg Dipstick, (Clinotech Diagnostics, Canada). Samples from the Northern regions were screened with one step HBsAg dipstick (WondfoTM Guangzhou Wondfo Biotech Co., Ltd. China). Samples from the Upper West were screened with NovatecTM dipstick (Novatec Immundiagnostica GmbH, Technologie & Waldpark. Germany) and samples from the Western region were screened with Accu-TellTM test kits. (AccuBiotech Co. Ltd. China). The strips were dipped in the serum or plasma for about 10 seconds and read for the visibility of one test and one procedural control line after about 10 minutes.

Enzyme Linked Immunosorbent Assay (ELISA)

HBsAg, Anti-HBs and HBeAg were determined by a double sandwich enzyme-linked immunosorbent assay (ELISA) using AdvancedTM 3rd generation assay kits (InTec Products Inc. China). Anti-HBe and Anti-HBc were determined by competitive immunoassay using AdvancedTM 3rd generation assay kits (InTec Products Inc. China). Anti-HBc IgM was tested with an immunoassay using AdvancedTM 3rd generation assay kits (InTec Products Inc. China). Reactive samples were retested in duplicate and considered to be reactive if at least 1 of the 2 repetitions also gave a positive result.

Statistical analysis

Statistical analysis was performed using MedCalc Version 10.2.0.0 for Windows 98/NT/Me/2000/XP/Vista (Vienna, Ausra. <http://www.medcalc.be>). The Bland-Altman analysis was used to compare

the immunochromatographic method for the screening of HBsAg and ELISA screening for HBsAg for each region. The calculation of the residual risk of HBV transmission through blood transfusion was done using the Modified Wald analysis for categorical outcomes as recommended by Agresti and Coull, (1998).

RESULTS

Reactivity of Hepatitis B viral marker in the various regions upon re-examination

As shown in Table 1, out of the total number of 480 samples that had tested sero-negative upon testing with rapid immunochromatographic test kit in the various facilities and thus the blood declared safe for transfusion, 39(8.13%) of the samples reacted sero-

positive for HBsAg upon retesting with ELISA. Sixty (12.5%) of the sample showed sero-positivity for Anti-HBs, 13(2.71%) reacted for HBeAg, 51 (10.63%) for Anti-HBe, 329(68.54%) reacting sero-positively for Anti-HBc with 226(47.08%) reacting sero-positive for isolated or only Anti-HBc. None of the samples however reacted sero-positive to IgM anti-HBc. The Volta region 10(20.00%), the Northern region 6(13.95%), the Upper East region 7(13.46%) and the Eastern region 5(10.00%) all showed a higher HBsAg seroreactivity than the total percentage seroreactivity of 39(8.13%) from all centres.

Comparison of dipstick methods with ELISA method using Bland-Altman analysis

Table 1: Reactivity of Hepatitis B viral marker in the various regions upon re-examination

Region	Sample	HBsAg	Anti-HBs	HBeAg	Anti-HBe	Anti-HBc	Isolated Anti-HBc
Eastern	50	5(10.00)	10(20.00)	1(2.00)	0(0.00)	40(80.00)	25(50.00)
Volta	50	10(20.00)	1(2.00)	3(6.00)	1(2.00)	19(38.00)	13(26.00)
Central	50	0(0.00)	3(6.00)	0(0.00)	1(2.00)	22(44.00)	19(38.00)
Northern	43	6(13.95)	0(0.00)	3(6.98)	6(13.95)	37(86.05)	29(67.44)
Greater Accra	50	0(0.00)	5(10.00)	1(2.00)	11(22.00)	35(70.00)	22(44.00)
Ashanti	50	3(6.00)	12(24.00)	2(4.00)	8(16.00)	28(56.00)	15(30.00)
Western	42	3(7.14)	6(14.29)	0(0.00)	6(14.29)	31(73.81)	22(52.38)
Upper West	43	2(4.65)	9(20.93)	1(2.33)	5(11.63)	32(74.42)	25(58.14)
Brong Ahafo	50	3(6.00)	8(16.00)	2(4.00)	8(16.00)	40(80.00)	26(52.00)
Upper East	52	7(13.46)	6(11.53)	0(0.00)	5(9.62)	45(86.54)	30(57.69)
Total	480	39(8.13)	60(12.50)	13(2.71)	51(10.63)	329(68.54)	226(47.08)

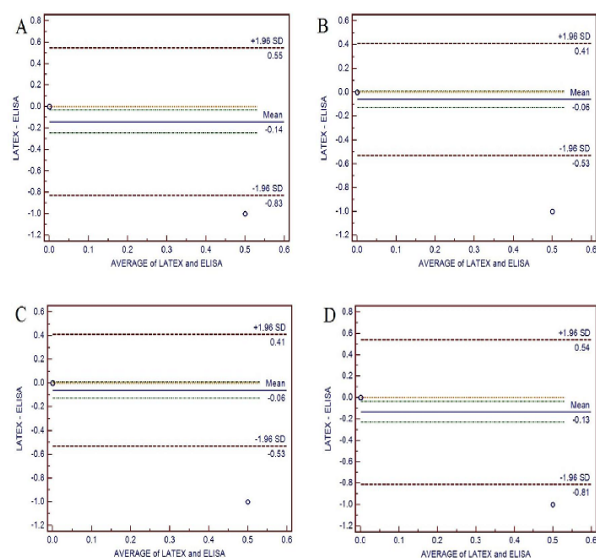
Data are presented as number or number (percentage). HBsAg = Hepatitis B virus Surface antigen, Anti-HBs = Antibody to Hepatitis B virus surface antigen, HBeAg = Hepatitis B virus e antigen, Anti-HBe = Antibody to Hepatitis B virus e antigen, Anti-HBc = Antibody to hepatitis B virus core antigen

When the results for HBsAg screening using the rapid latex immunochromatographic method was compared with the ELISA assay using the Bland-Altman analysis and as shown in figures 1 and 2. The Volta region (VirusCheck) showed the highest underestimation with a bias of -0.20 with a 95% limit of agreement of -0.99 to 0.59 (figure 2D). Northern Region (Wondfo), also underestimated presence of HBsAg in the samples (-0.14 and the 95% limit of agreement was -0.83 to 0.55, Figure 1A). Upper East region (Viruscheck) generated underestimated

HBsAg sero-reactivity with a bias of -0.13 and 95% limit of agreement of -0.81 to 0.54 (Figure 1D). This was followed by Eastern region (Clinotech) generating a bias of -0.10 and the 95% limit of agreement was -0.69 to 0.49 (Figure 2B). Western region (Acull-Tell) showed a bias of -0.07 and the 95% limit of agreement was -0.58 to 0.44 (Figure 2C). The bias was -0.06 and the 95% limit of agreement were -0.53 to 0.41 for both Ashanti and Brong Ahafo regions (DiaSpot) (Figure 1B and 1C respectively). A bias of -0.05 and a 95% limit of

agreement of -0.46 to 0.37 was generated in the Upper West region (Novatec) (Figure 2A). There was perfect agreement between the immunochromato-

graphic methods used in both Central and Greater Accra region facilities and the ELISA method.



A = Northern region, B = Ashanti region, C = Brong Ahafo region and D = Upper east region

Figure 1: Bland-Altman graphs of difference scores for the screening of the Hepatitis B viral antigen by the various Rapid immunochromatographic assays evaluated with the ELISA Assay

Residual risk of transfusion

The modified Wald analysis was used to estimate the residual risk of transmission of HBV through blood transfusion for the use of rapid immunochromatographic test kits in screening for the HBsAg before transfusion. As shown in Table 2, the national mean residual risk of transmission of HBV was 8.47% (5.98% to 10.94% at 95% CI). The facility at the Volta region (VR) generated the highest residual risk of transmission, 22.22% (11.05% to 33.23% at 95% CI) and followed in a descending order of magnitude, the residual risk of HBV transmission through blood transfusion in the facility at the Northern region (NR), was 17.02% (6.18% to 27.64% at 95% CI), the Upper East region (UE), 16.07% (6.37% to 25.58% at 95% CI), the Eastern region (ER), 12.96% (3.91% to 21.79% at 95% CI), the Western region

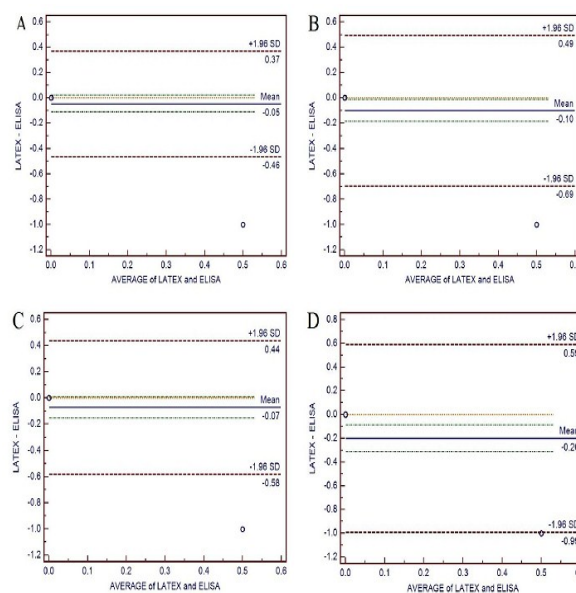


Figure 3: Bland-Altman graphs of difference scores for the screening of the Hepatitis B viral antigen by the various Rapid immunochromatographic assays evaluated with the ELISA Assay. A = Upper West region, B = Eastern region, C = Western region, D = Volta region

(WR), 10.87% (1.77% to 19.70% at 95% CI). The facility in the Ashanti region (AR) as well as that in the Brong Ahafo region generated a residual risk of 9.26% (1.44% to 16.84% at 95% CI). The facility in the Upper West region (UW) generated a residual risk of 8.51% (0.44% to 16.30% at 95% CI). With the exceptions of the two facilities in the Central and the Greater Accra regions which generated a residual risk of 3.70% (0.00% to 8.52% at 95% CI) each, all the other facilities had a residual risk higher than the national mean residual risk of HBV transmission through transfusion.

To assess the risk of transmission of HBV for solely testing for hepatitis B surface antigen (HBsAg) marker for blood transfusion without testing the other HBV serological markers (anti-HBs, HBeAg,

anti-HBe, anti-HBc and IgM anti-HBc), two serological markers which are indicators of the presence of the virus and thus a risk of transfusion hepatitis infections were considered in the Modified Wald analysis. These were reactivity of HBeAg which is serologically indicative of high HBV replication and IgM anti-HBc which indicates an acute infection of HBV. Thus samples that showed sero-reactivity for HBeAg and IgM anti-HBc without HBsAg were considered.

The total percentage residual risk of HBV transmission for using the HBsAg as the sole HBV marker to transfused blood was 3.10 % (1.54% to 4.62% at 95% CI). The facility at the Northern region (NR) generated the highest residual risk of transmission, 10.64% (1.72% to 19.29% at 95% CI) and followed in a decreasing order of magnitude, the residual risk of HBV transmission through blood transfusion in the facility at the Volta region (VR), was 9.26% (1.44% to 16.84% at 95% CI). The facility in the Ashanti region (AR) as well as that in the Brong Ahafo region (BA) generated a residual risk of 7.41% (0.34% to 14.22% at 95% CI) for non inclusion of the two markers in the testing of HBV blood transfusion. Again the facilities in the Greater Accra region (GA) and the one in the Eastern region (ER) each generated a residual risk of 5.56% (0.01% to 11.47% at 95% CI). The residual risk for the facility in the Western region (WR) was 4.35% (0.00% to 9.99% at 95% CI) and that for the Central region (CR) was 3.70% (0.00% to 8.52% at 95% CI). A residual risk of 3.57% (0.01% to 13.16% at 95% CI) and 3.57% (0.00% to 8.22% at 95% CI) were obtained for the two facilities in the Upper West region (UW) and the Upper East region (UE) respectively. As shown in table 2. All the individual facilities generated a residual risk higher than the total average.

In the estimation of the total residual risk of HBV transmission through transfusion, the samples that reacted sero-positive for any of the infectious HBV serological markers (HBsAg, HBeAg and IgM anti-HBc) were considered and counted only once. Though the risk of transmission is not nil for isolated anti-HBe sero-positive samples in the absence of HBsAg positivity, however the inability to establish the presence or otherwise of viral DNA in these

samples by the test method (ELISA) led to the exclusion of such samples. Also sero-reactivity for anti-HBc was excluded due to the inability of the test method (ELISA) to distinguish between samples that tested sero-positive for anti-HBc and sero-negative for all other HBV markers indicative of infectivity (HBsAg, HBeAg, and IgM anti-HBc) but contained viral DNA and thus infectious, from those that exhibited the same serological profile but lack viral DNA.

As shown in Table 2, the total percentage residual risk was estimated as 11.16% (8.34% to 13.95% at 95% CI). The total residual risk of transmission of the individual facilities were 27.78% (15.76% to 39.67% at 95% CI) for VR, 23.40% (11.21% to 35.42% at 95% CI) for NR, 16.07% (6.37 to 25.58% at 95% CI) for UE. A total residual risk of 16.07% (1.72% to 19.29% at 95% CI) and 14.82% (5.25% to 24.17% at 95% CI) for the Upper West (UW) and the Eastern regions (ER) respectively. The facility in the Ashanti region (AR) as well as that in the Brong Ahafo region (BA), each generated a total residual risk of 12.96% (3.91% to 21.79% at 95% CI). The facilities Western, Greater Accra and Central regions all had their total residual risk below the national average, 10.87% (1.77% to 19.70% at 95% CI) for WR, 5.56% (0.01% to 11.47% at 95% CI) for GA and 3.70% (0.00% to 8.52% at 95% CI) for CR.

Reactivity Profile of ELISA upon Retesting

The reactivity profile of the samples upon retesting with enzyme linked immunosorbent assay showed disparity between the number of samples that reacted for a particular antigen and its corresponding antibody. Figure 3A and Figure 3B represents regional reactivity profile of HBV surface antigen and its corresponding regional reactivity profile of surface antibody respectively, whilst Figure 3C and Figure 3D represents regional reactivity for HBV e antigen and e antibody respectively. There were a higher number of samples that reacted for anti-HBc only (Figure 3F) compared to the sample that reacted for anti-HBc and other serological markers (Figure 3E). There was high discordance between HBsAg and HBeAg with only three of the samples

Table 2: Estimated Residual Risk of Hepatitis B virus transmission through blood transfusion

LOCATION	RRT for testing HBsAg with Immunochromatographic Assays	RRT for the non testing of other HBV serological markers	Total RRT of HBV through Transfusion
Eastern	12.96(3.91 - 21.79)	5.56(0.01 - 11.47)	14.82(5.25 - 24.17)
Volta	22.22(11.05 - 33.23)	9.26(1.44 - 16.84)	27.78(15.76 - 39.67)
Central	3.70(0.00 - 8.52)	3.70(0.00 - 8.52)	3.70(0.00 - 8.52)
Northern	17.02(6.18 - 27.64)	10.64(1.72 -19.29)	23.40(11.21 - 35.42)
Greater Accra	3.70(0.00 - 8.52)	5.56(0.01 - 11.47)	5.56(0.01 - 11.47)
Ashanti	9.26(1.44 - 16.84)	7.41(0.34 - 14.22)	12.96(3.91 - 21.79)
Western	10.87(1.77 - 19.70)	4.35(0.00 - 9.99)	10.87(1.77 - 19.70)
Upper West	8.51(0.44 - 16.30)	3.57(0.01 - 13.16)	16.07(1.72 - 19.29)
Brong Ahafo	9.26(1.44 - 16.84)	7.41(0.34 - 14.22)	12.96(3.91 - 21.79)
Upper East	16.07(6.37 -25.58)	3.57(0.00 - 8.22)	16.07(6.37 - 25.58)
Total	8.47(5.98 - 10.94)	3.10(1.54 - 4.62)	11.16(8.34 - 13.95)

Data are presented in percentage, RRT Residual risk of transmission

from the northern region showing concordance for HBsAg (Figure 3G). There was high reactivity correlation between Anti-HBs to the Anti-HBc (Figure 4D). Anti-HBe also showed higher reactivity correlation to Anti-HBc (Figure 4G) than Anti-HBs.

DISCUSSION

The year 2012 has been targeted as the year for safe blood and the achievement of 100% testing for infectious markers by the World Health Organization (WHO) (Tagny *et al.*, 2008). Though these may look achievable in the developed world, sub-

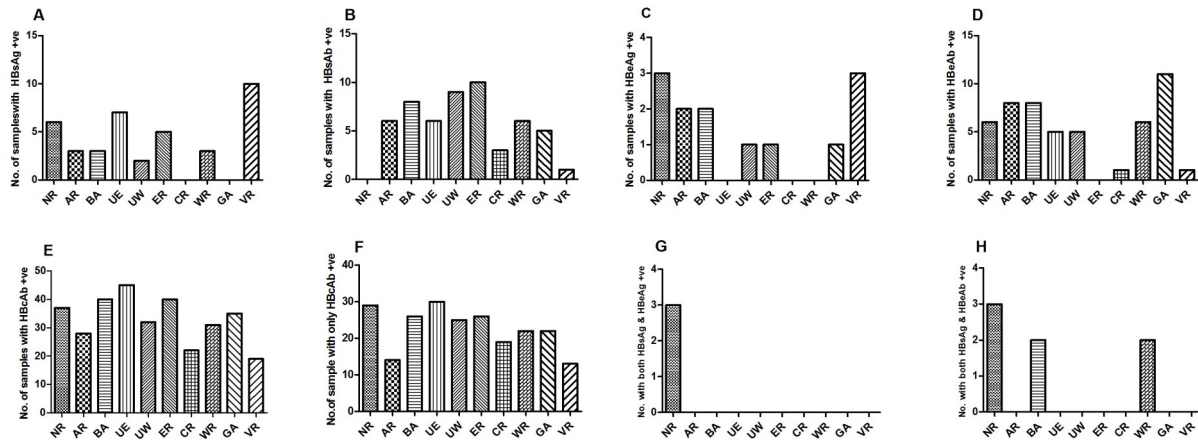


Figure 3: Hepatitis B Viral Serological Marker Reactivity Profile of Enzyme Linked Immunosorbent Assay upon Retesting

East and Volta regions.

Although an attempt to do an inter-laboratory comparison of biases would be limited because different samples were used in each facility, and also the fact that performance of an assay is greatly affected by factors such as the technique and the storage of the test kits. However, the impact of lack of centralised control on standardised guideline on acquisition of test kits, coupled with a non-existence of a national policy geared toward ensuring the quality of pre-screening of HBsAg before transfusion cannot be underestimated (Allain and Lee, 2005). Again even in a facility it was revealed that different brands of the rapid latex immunochromatographic assay were used depending on what was available in the open market. It was estimated that each facility uses an average of four (4) different hepatitis B virus rapid latex immunochromatographic assays within a year, thus the inaccuracy of a low performing assay would be wide spread in different laboratories and this may contribute to the high risk of being infected with HBV through blood transfusion in sub-Saharan Africa estimated by Jayaraman et al., (2010) to be over four times the risk of HIV infection through blood transfusion.

Using the Modified Wald method (Agresti and Coull, 1998), the estimated residual risk of HBV transmission for the use of the rapid immunochromatographic assays for screening HBsAg in Ghana was 8.47%. This estimated risk percentage is similar to what Allain *et al.*, reported in 2003 (9.09%) as the risk of transmission of transfusion borne HBV to recipients under 10 years by the use of immunochromatographic assay in Kumasi. Thus all these years not much improvement in the quality and reliability of pre-transfusion screening for HBV has been achieved since the screening regimen has not improved to date. The presence of HBeAg in the bloodstream is an indication of active HBV replication associated with large quantity of HBV-DNA in the bloodstream and therefore an enhanced infectivity (Lee, 1997; Yuen *et al.*, 2004; El-Sherif *et al.*, 2007; Kukka, 2008; Zahn *et al.*, 2008). Whilst the positivity for serum Anti-HBe is an indication of seroconversion with a low viral load and unlikely to produce a

viable infectivity through transmission portals such as needle stick, sexual contact and household contact, it has a high potential of producing a viable infectivity through blood transfusion because of the large volume of viral DNA that is passed on to the recipient (Kukka, 2008).

HBV infection exhibits a broad spectrum of serologic patterns associated with HBV-DNA persistence. It is rare to find sero-positivity for HBeAg, anti-HBe nor both in the serum of subjects who are sero-negative for HBsAg (Brecht *et al.*, 2001; Allain *et al.*, 2003; Kidd-Ljunggren *et al.*, 2004). But as was evident in this study, out of the 13 samples that reacted sero-positive for HBeAg, only 3 were also sero-positive for HBsAg and out of the 51 samples that reacted sero-positive for Anti-HBe, only 7 also reacted sero-positive for HBsAg. Though rare undetectable HBsAg in the presence of detectable HBeAg has been reported and most of these cases had been attributed to escape mutants with point mutations in the preS1, preS2 and preS regions (Osioy, 2002; Yang *et al.*, 2003). Chronic hepatitis with ongoing HBV replication in presence of anti-HBe has frequently been observed (Lai *et al.*, 1991), Kidd-Ljunggren *et al.*, (2004) reported that 65% of anti-HBe sero-positive patients who might be considered most likely to be non viremic had HBV viral DNA. Thus the non-testing of HBeAg/anti-HBe during HBV pre-donation screening may increase the residual risk of HBV transmission through blood transfusion in Ghana.

The study also revealed a high reactivity for Anti-HBc 68.54%, and this was consistent with the findings of Allain, (2006) who estimated that 75% of the Ghanaian population by the age of 16 tested sero-positive for Anti-HBc. Among the Anti-HBc sero-positive samples 8.82% also reacted sero-positive for Anti-HBs and these represent the proportion of donors that had cleared the virus and hence had developed immunity (Al-Mekhaizeem *et al.*, 2001), contrary evidence however suggests this accession may not be the case at all times (Yotsuyanagi *et al.*, 1998; Owiredu *et al.*, 2001; Dhanwan *et al.*, 2008; Allain *et al.*, 2009). Among the isolated Anti-HBc sero-positive, two possible donor

groups exist, those chronically infected with persistence of HBV-DNA but had undetectable level of HBsAg and therefore produce viable viraemic infection, and those who had eventually recovered from the infection and no longer carry HBV-DNA but show no detectable Anti-HBs (Reesink *et al.*, 2008).

The ELISA test method could not be used in distinguishing between the two groups of Anti-HBc only positive donors mentioned in the preceding paragraph, however, Zahn *et al.*,(2008) estimates that the Ghanaian donor population includes 1.5% of the first group. It should be acknowledged however, that despite the indicative potential of this serological marker in identifying donors who have been naturally exposed to HBV, its practical use as a marker for pre-screening of blood in Ghana may not be adequate, essentially because of the high prevalence rate in the donor population (Reesink *et al.*, 2008). Though in estimating the residual risk of transfusion for the non testing of other HBV markers, this group (Anti-HBc sero -positivity) was not considered and was also not included in the total residual risk estimation, the high sero -positivity of Anti-HBc in this study even heightens the risk of getting transfused with HBV infectious blood unit in Ghana.

CONCLUSIONS

This study evaluated the performance of test kits used in pre-donation screening for HBV, and the efficiency of the screening strategy among donors in ten facilities in Ghana. The indication was that neither the kits in used nor the testing strategy in place now, is adequate to prevent transmission of hepatitis B virus through transfusion in Ghana due to high residual risk of transmission of HBV through blood transfusion. There is therefore an urgent need for an effective quality control system for the pre-donation screening of blood for HBV in Ghana.

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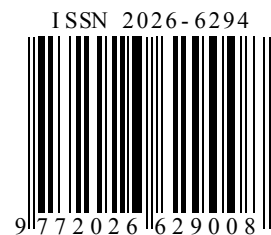
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ORIGINAL ARTICLE

The efficacy of aqueous and ethanolic leaf extracts of *Pistia stratiotes* linn in the management of arthritis and fever

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Arthritic pain and disability are at or near the top of the list of reasons adult patients seek medical attention. This study therefore attempts to establish the efficacy of an aqueous and ethanolic leaf extract of *Pistia stratiotes* Linn (Araceae) in a rodent experimental model of arthritis and fever to ascertain its importance in the traditional management of this inflammatory disorder. The aqueous and ethanolic extracts of *P. stratiotes* at doses of 30, 100, and 300 mg/kg as well as 0.3 mg/kg methotrexate, 0.46 mg/kg diclofenac and 1 mg/kg dexamethasone were administered to formalin-induced arthritic rats. The same doses of the extracts in comparison to 150 mg/kg acetaminophen were also administered to rats in which fever had been induced with lipopolysaccharides. Data obtained was analyzed using GraphPad Prism 5.0. The results obtained indicated significant reduction ($P \leq 0.05-0.01$) in paw thickness of formalin-induced arthritic animals treated with both aqueous and ethanolic leaf extracts with effects comparable to that of methotrexate, diclofenac, and dexamethasone. Lipopolysaccharide-induced fever in rats was also significantly reduced ($P \leq 0.05-0.01$) at all dose levels of aqueous and ethanolic treated animals in a manner similar to that of acetaminophen. The aqueous and ethanolic leaf extracts of *P. stratiotes* have anti-arthritic and antipyretic effect in formalin-induced arthritis and LPS-induced fever in Sprague-Dawley rats.

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INTRODUCTION

Arthritis is a form of joint disorder that involves inflammation of one or more joints characterized by varied levels of pain, swelling, joint stiffness, and sometimes a constant ache around the joint(s) (CDC, 2011). The world prevalence of arthritis is estimated to be around 0.3–1.2 % (Silman and Horchberg, 1993). At least 47.8 million US residents have arthritis. In Europe, the magnitude of the problem is similar, affecting 8 million in the United Kingdom and 108 million across the continent (VanItallie, 2010). The story is no different in Africa. It is the number one disabling disease in South Africa; affecting an estimated one in every seven people. About

132 Million East Africans have minimal rheumatological care (Arthritis Africa, 2012). Although there is no study on the prevalence of arthritis in Ghana the situation is projected to be no better (WHO, 2000). Arthritis makes it very difficult for individuals to be physically active and many become home bound. Arthritic pain and disability are at or near the top of the list of reasons adult patients seek medical attention. Arthritis makes it very difficult for individuals to be physically active and many become home bound.

Although non-steroidal anti-inflammatory agents remain the mainstay treatment for this degenerative inflammatory disorder, its prolonged clinical use elicits numerous side effects, notable amongst them are gastric erosion, ulceration, hemorrhage, bronchospasm, kidney and liver dysfunction (Lin *et al.*, 2004). Studies have shown that asymptomatic mu-

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cosal damage is initially evident in 80 % of subjects after non-steroidal anti-inflammatory drug (NSAID) therapy (Ehsanullah *et al.*, 1988) however, upon continuous use of NSAIDs 15-20% of treated patients develop ulcer (Singh *et al.*, 1996), and 1-3% received hospital treatment for gastrointestinal, (GI) bleeding or perforation. Fever is one of the most prominent systemic manifestations of acute inflammation, especially when an inflammation is associated with infection (Romanovsky *et al.*, 2005).

A population based study projects a phenomenal increase in the consumption of non-steroidal anti-rheumatic drug from 380 million to 600 million consumers in the next two decades among the geriatric population alone (Steineyer, 2000). This consolidates the need for an urgent search for new safer and efficacious anti-inflammatory agents. One medicinal plant commonly used traditionally for inflammatory disorders is *Pistia stratiotes*. Commonly known as water lettuce or water cabbage, it is an aquatic plant, stoloniferous, floating on lakes, streams, and stagnant water ponds. It is distributed in the tropical and subtropical region of Asia, Africa, and America. (Arber, 2002). Several medicinal prowesses have been ascribed to this plant. These include anti-helminthic, anti-microbial and anti-fungal properties (Prem kumar and Shyamsundar, 2005; Achola and Indalo, 1997; Sundeep Kumar *et al.*, 2000). The anti-inflammatory and anti-pyretic activity of ethanolic extract of *P. stratiotes* has been demonstrated using carrageenan, cotton-pellet-induced granuloma model and brewer's yeast fever model (Sundeep Kumar *et al.*, 20011).

This study therefore attempts to establish the efficacy of an aqueous and ethanolic leaf extracts of *Pistia stratiotes* in a rodent formalin-induced model of arthritis as it closely depict corresponding human disease state (Greenwald, 1991) and lipopolysaccharides-fever model to enhance understanding into the possible mechanism of action. This is to ascertain its importance in the traditional management of inflammatory disorders and to predict its possible mechanism in curbing fever commonly associated with inflammation (Abbiw, 1990).

MATERIALS AND METHODS

Plant Collection

Pistia stratiotes was collected from the Fosu lagoon, Cape Coast in the Central Region of Ghana (5°7' N & 1°16' W) in December 2010. It was identified and authenticated by Mr. G H Sam of the Department of Herbal Medicine, CHS, KNUST, where a voucher specimen bearing the number KNUST/HM1/11/W002 has been deposited at the herbarium for future reference.

Preparation of Extracts

The leaves of *P. stratiotes* were washed thoroughly with tap water and sun-dried. The dry leaves were milled into coarse powder by a hammer mill (Schutte Buffalo, New York, USA). In preparing the aqueous leaf extract of *P. stratiotes*, 700 g of the leaf powder was mixed with 1litre of water. The mixture was maintained at 80 °C (in a round bottom flask fitted with a reflux condenser) in a thermostatically controlled water bath for 24 h and then filtered. The filtrate was freeze dried with a Hull freeze dryer /lyophilizer 140 SQ FT (model 140FS275C, USA) into powder (percentage yield 4.7%) and stored at a temperature of 4 °C in a refrigerator. This powder was reconstituted in normal saline to a desired concentration and labeled as AQ PSE for dosing in this study. Similarly, 700 g of the leaf powder was soaked with one liter of 70 % ethanol at room temperature (27-29 °C) for 72 h and filtered. The filtrate obtained was freeze-dried into powder (percentage yield 5.2 %). Quantities of this powder was reconstituted in normal saline at desired concentrations to be referred to and used in this study as the ethanolic leaf extract of *P. stratiotes* or ET PSE.

Drugs and Chemicals

Formaldehyde (Yash Chemicals, India) was used to induce arthritis while LPS (Sigma-Aldrich, USA) was used to induce pyrexia. Diclofenac sodium (KRKA, d.d., Novo mesto, Solvenia), dexamethasone sodium (Anhui Medihel Co. Ltd), and methotrexate sodium (Dabur Pharma, New Delhi, India) were the reference anti-inflammatory agent in this study. Acetaminophen (Simpex Pharamchem Inc. USA) was the reference antipyretic.

Preparation of Reference Drugs

The reference anti-inflammatory drugs were dissolved in normal saline for the study. The drugs were freshly prepared as follows 0.3 mg/kg methotrexate, 0.46 mg/kg, 1 mg/kg dexamethasone which was administered in volumes not exceeding 10 ml/kg.

Animals

Six to eight-week old Sprague Dawley rats of either sex (180-200 g) purchased from the Centre for Scientific Research into Plant Medicine (CSIRPM), Mampong-Akwapim, Ghana, were maintained in the Animal House of Department of Pharmacology, KNUST, Ghana. The animals were housed in polyacrylic cages (34cm × 47cm × 18cm) with soft wood shaving as bedding, under ambient laboratory conditions (temperature $28 \pm 2^\circ\text{C}$, relative humidity 60-70 %, and normal light-dark cycle). Females were non-pregnant. They were fed with normal commercial pellet diet (GAFCO, Tema) water *ad libitum*. All procedures and techniques used in these studies were in accordance with the National Institute of Health for the Care and Use of Laboratory Animals (NIH, Department of Health and Human Services publication no. 85-23, revised 1985). The protocols for the study were approved by the Departmental Ethics Committee.

Preliminary Phytochemical Screening

Screening was performed on AQ PSE and ET PSE to ascertain the presence of phytochemicals using standard procedures described by Wagner and Bladt (1996), Glasl (1983), Harborne (1998), and Kujur *et al.*, (2010).

Formaldehyde-Induced Arthritis and Treatment

The test was performed according to the technique developed by Brownlee in 1950. Pedal inflammation was induced by injecting 0.1 ml of 4 % formalin solution below the plantar aponeurosis of the right hind paw of the rats after measuring their paw thickness. The arthritic animals were divided into ten groups of five and treated with either 30, 100, or 300 mg/kg AQ PSE or ET PSE, orally 30 minutes after intra-plantar injection with formalin on day 1, and then daily), 0.3 mg/kg methotrexate intraperitoneally

(i.p every four days), 0.46 mg/kg diclofenac (i.p, daily), 1 mg/kg dexamethasone (i.p, every other day), 1 ml/kg normal saline (p.o, daily), the control, over the experimental period.

Lipopolysaccharide-Induced Fever and Treatment

The method of Santos and Rao (1998) was modified and used for the assessment of the anti-pyretic activity of the aqueous and ethanolic extracts of *P. stratiotes*. Animals were fasted overnight prior to induction of fever, but given water *ad libitum*. Rectal temperature was measured using a lubricated ECT-1 digital thermometer (Estar Electronic And Instrument Co., Ltd., Zhejiang, China) inserted 3cm deep into the rectum of the rats. Fever was induced by injecting intramuscularly, 1 mg/kg of LPS into the right thigh of each rat. Rectal temperature was measured again and animals that showed an increase in temperature of 0.5°C and more were selected for the study. The animals with fever were put into eight groups of five and were treated with either 30, 100, or 300 mg/kg AQ PSE or ET PSE, 150 mg/kg acetaminophen, or 1 ml/kg normal saline solution (the control), orally, two hours after LPS-induced fever. Rectal temperature was measured at 1 h intervals for 6h. All experiments were carried out between 08.00 h and 18.00 h in a quiet laboratory with an ambient temperature of $25 \pm 2^\circ\text{C}$.

Statistical Analysis

Results were analyzed using one way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test by using GraphPad Prism; version 5.03. Values were expressed as mean \pm SEM and P values ≤ 0.05 were considered statistically significant.

RESULTS

Preliminary Phytochemical Screening

Results for the initial phytochemical screening are as shown in Table 1.

Formalin-induced Arthritis

There were significant reductions ($P \leq 0.01-0.001$) in paw thickness of formalin-induced arthritic ani-

Table 1: Results of phytochemical screening of the aqueous and ethanolic extracts of *P. stratiotes* Linn

Components	AQ PSE	ET PSE
Tannins	+	+
Flavonoids	+	+
Alkaloids	+	-
Sterols	+	+
Glycosides	+	+
Saponins	-	-
Triterpenoids	-	-

“+” implies present, “-“implies absent

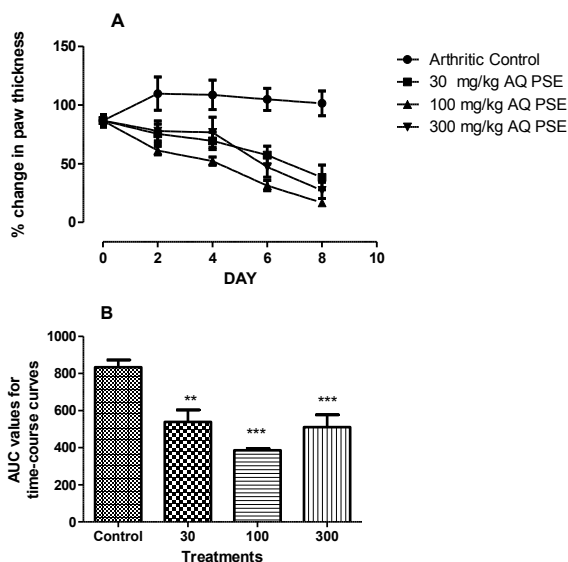


Figure 1: Plots of (A) the time-course curves and (B) the area under the time-course curves (AUC) of the effects of 30, 100, and 300 mg/kg of AQ PSE on formalin-induced arthritis in Sprague-Dawley rats. Data are presented as mean \pm SEM (n=5). ** implies $P \leq 0.01$, *implies $P \leq 0.001$: the level of significance of paw thickness reduction (compared to the control) analyzed by One-way ANOVA followed by Dunnet’s test *post hoc*. Percentage change in paw thickness was computed using the formula $V=(V_t-V_o) / V_o \times 100$ where V is percentage in paw thickness, V_t is paw thickness after formalin challenge, V_o is the initial paw thickness before formalin challenge**

imals treated with both aqueous and ethanolic leaf extracts of *P. stratiotes* compared to the normal saline-treated arthritic animals. Similar significant reductions ($P \leq 0.001$) in paw thicknesses were observed among the methotrexate, diclofenac, and dexamethasone treated arthritic animals (Figure 1, 2 and 3).

Lipopolysacharride –Induced Fever

Lipopolysacharride- induced fever in rats was significantly reduced ($P \leq 0.01-0.001$) at all dose levels of AQ PSE and ET PSE treatment; the effect was similar to that observed for acetaminophen treatment (Figures 4 and 5).

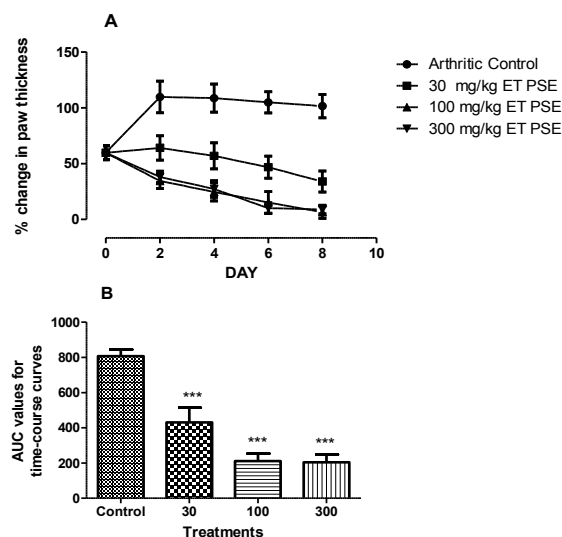


Figure 2: Plots of (A) the time-course curves and (B) the area under the time-course curves (AUC) of the effects of 30, 100, and 300 mg/kg of ET PSE on formalin-induced arthritis in Sprague-Dawley rats. Data are presented as mean \pm SEM (n=5). * implies $P \leq 0.001$; the level of significance of paw thickness reduction (compared to the control) analyzed by One-way ANOVA followed by Dunnet’s test *post hoc*. Percentage change in paw thickness was computed using the formula $V=(V_t-V_o) / V_o \times 100$ where V is percentage in paw thickness, V_t is paw thickness after formalin challenge, V_o is the initial paw thickness before formalin challenge**

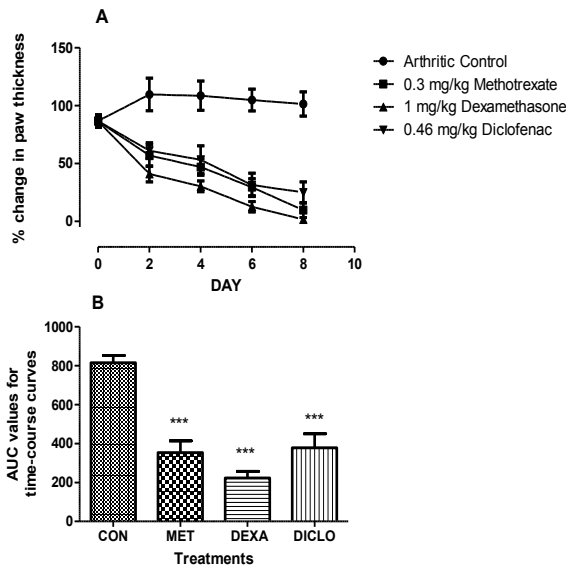


Figure 3: Plots of (A) the time-course curves and (B) the area under the time-course curves (AUC) of the effects of 0.3 mg/kg methotrexate, 1 mg/kg dexamethasone, and 0.46 mg/kg diclofenac on formalin-induced arthritis in Sprague-Dawley rats. Data are presented as mean \pm SEM (n=5). *** implies $P \leq 0.001$; the level of significance of paw thickness reduction (compared to the control) analyzed by One-way ANOVA followed by Dunnet's test *post hoc*. Percentage change in paw thickness was computed using the formula $V = (V_t - V_o) / V_o \times 100$ where V is percentage in paw thickness, V_t is paw thickness after formalin challenge, V_o is the initial paw thickness before formalin challenge

DISCUSSION

It is established that inhibition of formalin-induced paw oedema in rats is one of the most appropriate modus operandi to screen for anti-arthritis and anti-inflammatory agents as it closely resembles human arthritis (Greenwald, 1991). Injection of formalin subcutaneously into hind paw of rats produces localized inflammation and pain. The nociceptive effect of formalin is biphasic, an early neurogenic component followed by a later tissue mediated response (Wheeler-Aceto and Cowan, 1991). Thus formalin-

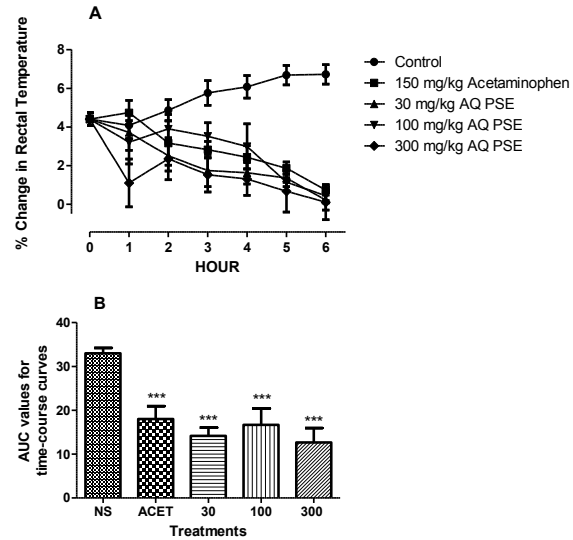


Figure 4: Plots of (A) the time-course curves and (B) the area under the time-course curves (AUC) of the effects of 30, 100, and 300 mg/kg of AQ PSE and 150 mg/kg acetaminophen on LPS-induced fever in Sprague-Dawley rats. Data plotted are means \pm SEM (n=5). ***implies $P \leq 0.001$; the level of significance of rectal temperature reduction (compared to the normal saline-treated) analyzed by One-way ANOVA followed by Dunnet's test *post hoc*. Percentage change in rectal temperature was computed using the formula $T = (T_t - T_o) / T_o \times 100$ where T is percentage in rectal temperature, T_t is rectal temperature after LPS challenge, T_o is the initial rectal temperature before LPS challenge

induced arthritis is a model used for the evaluation of an agent with probable anti-proliferative activity. This experiment is associated with the proliferative phase of inflammation (Banerjee *et al.*, 2000).

The reference drugs and both extracts of *P. stratiotes* significantly suppressed formalin-induced arthritis. Dexamethasone is acknowledged to inhibit the release of pro-inflammatory cytokines (TNF- α , Tumor Necrosis Factor- α and IL-1 β , interleukin-1 β), which are known to play a central role in the prop-

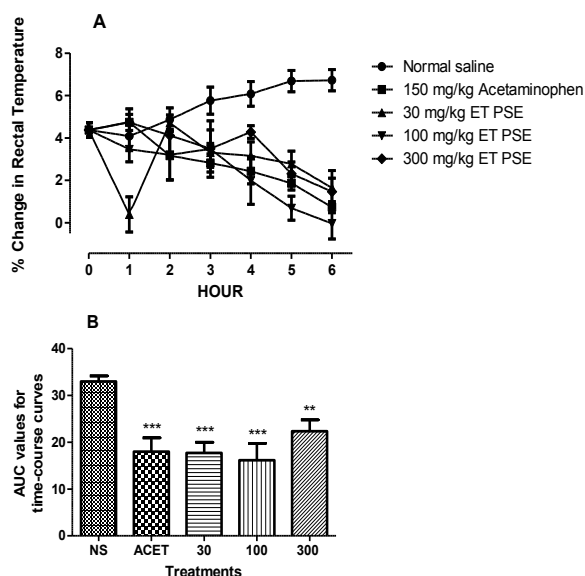


Figure 4: Plots of (A) the time-course curves and (B) the area under the time-course curves (AUC) of the effects of 30, 100, and 300 mg/kg of ET PSE and 150 mg/kg acetaminophen on LPS-induced fever in Sprague-Dawley rats. Data plotted are means \pm SEM (n=5). ** implies $P \leq 0.01$; ***implies $P \leq 0.001$: the level of significance of rectal temperature reduction (compared to the normal saline-treated) analyzed by One-way ANOVA followed by Dunnet's test *post hoc*. Percentage change in rectal temperature was computed using the formula $T = (T_t - T_o) / T_o \times 100$ where T is percentage in rectal temperature, T_t is rectal temperature after LPS challenge, T_o is the initial rectal temperature before LPS challenge

agation of the disease process in arthritis thus its ability to arrest paw swelling (Issekutz and Issekutz, 1991). Methotrexate inhibits proliferation of the lymphocytes and other cells responsible for inflammation in the joint (Gubner, 1951). The anti-inflammatory effect of diclofenac is mediated mainly through inhibition of COX and prostaglandin production (Furst and Manning, 2001).

Fever is one of the most prominent systemic manifestations of acute inflammation, especially when an

inflammation is associated with infection (Romanovsky *et al.*, 2005). These reactions represent the primary host defense response to infection; collectively called the “acute-phase reaction” (Blatteis, 1992). The usual view of the mechanism by which infectious fevers are produced stipulates that infectious noxa e.g. bacterial endotoxic lipopolysaccharides (LPS) that invade the body activate mononuclear phagocytes that then produce and release pyrogenic cytokines including IL-1 β and TNF- α . These are transported via the bloodstream to the ventromedial preoptic area of the anterior hypothalamus, the “fever producing center”, where they operate (Saper, 1998; Roth and De Souza, 2001; Dunn, 2002; Dinarello, 2004). It is, however, doubtful how cytokines, as hydrophilic peptides, could penetrate the brain. That is to say, it is generally believed that, rather than acting directly, the cytokines induce the local generation and release of prostaglandin E₂, a lipid mediator that is obviously thermogenic when injected centrally (Blatteis, 1997; Ivanov and Romanovsky, 2004). Its production is dependent on the activation of two enzymes, cyclooxygenase (COX)-2 and microsomal PGE synthase-1, which catalyze its conversion from arachidonic acid present in the membranes of cells (Ivanov *et al.*, 2002).

Acetaminophen is a reputable antipyretic analgesic agent, often administered therapeutically to ease pain and fever (Ayoub *et al.*, 2004). The main mechanism proposed is the inhibition of COX, and recent findings suggest that it is highly selective for COX-2 (Hinz *et al.*, 2008). Paracetamol reduces the oxidized form of the COX enzyme, preventing it from forming pro-inflammatory chemicals (Roberts *et al.*, 2001; Högestätt *et al.*, 2005). This leads to a reduced amount of Prostaglandin E₂ in the CNS, thus lowering the hypothalamic set-point in the thermoregulatory centre.

Oral administration of both aqueous and ethanolic leaf extracts of *P. stratiotes* as earlier indicated could possibly be inhibiting COX-2 and subsequent production of prostaglandins thereby exhibiting potent hypothermic effect in LPS-induced fever in Sprague-Dawley rats at much lower doses. (Sundeeep Kumar

et al., 2011).

The presence of biologically active phytochemicals present in both the aqueous and ethanolic extracts of *P. stratiotes* could have contributed to the anti-inflammatory activity. Tannins (Mota *et al.*, 1985; Owoyele *et al.*, 2010), flavonoids (Borissova *et al.*, 1994; Hämäläinen *et al.*, 2007), sterols (Bouic *et al.*, 1996; Bouic, 1998; Akihisa *et al.*, 2007), alkaloids (Barbosa-Filho *et al.*, 2006) and glycosides (Odontuya *et al.*, 2005; Liu and Wang, 2011) have been documented to have anti-inflammatory effect via several mechanisms.

CONCLUSION

P. stratiotes has anti-arthritic and antipyretic effect in formalin-induced arthritis and LPS-induced fever in Sprague-Dawley rats.

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ORIGINAL ARTICLE

Prevalence of metabolic syndrome among Psychiatric Patients in the Kumasi Metropolis, Ghana

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This cross-sectional study seeks to find the prevalence of the metabolic syndrome (MetS), its individual components and oxidative stress in psychiatric patients on antipsychotic medication compared to newly diagnosed patients attending the Psychiatric Department of the Komfo Anokye Teaching Hospital (KATH), Kumasi, Ghana, between February 2009 and July 2010. A total of 200 psychiatric patients comprising 100 newly diagnosed antipsychotic-naïve patients and 100 patients on antipsychotic medication were sampled for the study. MetS was diagnosed using the World Health Organization (WHO), International Diabetes Federation (IDF) and the National Cholesterol Education Programme, Adult Treatment Panel III (NCEP ATP III) criteria. The overall prevalence of MetS was 11.5%, 13.5% and 15.5% using NCEP ATP III, WHO and IDF criteria respectively. The prevalence was significantly higher among psychiatric patients on treatment as compared to treatment-naïve group using NCEP ATP III (21.0% vs. 2.0%; $p < 0.0001$) and IDF (29.0% vs. 2.0%; $p < 0.0001$) criteria but not WHO (13.0% vs. 14.0%; $p = 0.8372$). These overall prevalence rates were higher compared to the general Ghanaian population prevalence rates of 3.9%, 2.2% and 7.8% determined with the NCEP ATP III, WHO and IDF criteria respectively. Regular monitoring of metabolic parameters should be considered as a standard part of their medical care.

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INTRODUCTION

Within individuals in the general population as well as those with psychiatric illnesses such as schizophrenia, MetS is known to be a leading cause of cardiovascular disease (CVD)-related deaths and morbidity (Eckel *et al.*, 2005; Meyer *et al.*, 2005). Current studies based on the general population in Ghana have established that MetS, determined with the three definition criteria was 3.9% (NCEP ATP III), 2.2% (WHO), and 7.8% (IDF) predominant (Owiredu *et al.*, under review).

The contributions that led to studies on MetS within

psychiatric individuals include the current invigoration of concern in medical comorbidity in mentally deranged individuals (Holt *et al.*, 2004; Lambert and Chapman, 2004) as well as the release of viable MetS definitions that are physician-friendly (NCEP, 2001; Alberti *et al.*, 2006). MetS prevalence rates of 28% to 37% in psychiatric subjects has been reported in diverse research works in Europe (De Hert *et al.*, 2006; Bobes *et al.*, 2007) but in the United States and Canada, more elevated rates of 43% and 46% respectively have been reported (Cohn *et al.*, 2004; McEvoy *et al.*, 2005).

In Ghana, psychiatric disorders occur more frequently due to the apparent adverse use of psychoactive substances like cannabis, heroin etc. (Turkson, 1998). In an earlier research by Owiredu

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et al., (2009), an interrelation between diabetes, dyslipidaemia and mental disorders was established. Within a research which analyzed ensuing dyslipidaemia after antipsychotic treatment, there was not only the correlation between hypertriglyceridaemia, reduced HDL cholesterol and the newly diagnosed psychiatric subjects but these factors became more pronounced among those on antipsychotic treatment (Owiredu *et al.*, 2009). Abnormalities in other factors, including blood pressure, body mass index and waist-to-hip ratio have also been associated with mental illness.

Globally, the MetS predominance has been poorly investigated. Several factors of lifestyle related to CVD like poor diet, inactive lifestyle, substance abuse and smoking, are common within individuals with psychotic illnesses (Taylor and MacQueen, 2006; Suvisaari *et al.*, 2007). Furthermore, contemplations are rising about the effect of some atypical antipsychotic drugs at the onset of MetS (Holt *et al.*, 2004; Lambert and Chapman, 2004).

Due to paucity of studies on MetS as a whole in psychiatric patients in Ghana, this study seeks to find the prevalence of MetS and associated co-morbid conditions in psychiatric patients on antipsychotics (conventional and atypical) compared to newly diagnosed psychiatric patients using the World Health Organization (WHO), International Diabetes Federation (IDF) and the National Cholesterol Education Programme, Adult Treatment Panel III (NCEP ATP III) criteria for defining MetS.

MATERIALS AND METHODS

Study population and setting

This case related study was carried out at the Psychiatric department of the Komfo Anokye Teaching Hospital, (KATH), Kumasi, Ghana. Patients attending the psychiatric department between February 2009 and July 2010 were eligible for recruitment. A total of 200 psychiatric patients comprising 100 newly diagnosed antipsychotic-naïve patients and 100 patients on antipsychotic medication were invited through a written informed consent to participate in the study.

Sampling

About 5 ml of venous blood sample was collected from the antecubital fossa of the study participants after an overnight fast (12 – 16 hours). One milliliter (1 ml) of the blood sample was dispensed into fluoride oxalate tube and the other 4 ml into vacutainer plain tubes. The sera and plasma were obtained as supernatants after centrifugation at 500 g for 15 minutes. With the aid of Pasteur pipettes, the sera and plasma were transferred into well-labelled cryotubes and stored at -80°C until assay was performed. Assay parameters include: fasting blood glucose (FGB), total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL) cholesterol and uric acid. Serum low density lipoprotein (LDL) cholesterol was estimated with the Friedewald equation. Reagent manufacturer's protocol was adopted for the estimation of analytes. Malondialdehyde (MDA) concentration was determined by the method described by Kamal *et al.*, (1989).

Anthropometric variables

Height to the nearest centimetre without shoes was measured against a wall-mounted ruler and weight to the nearest 0.1 kg in light clothing on a bathroom scale (Zhongshan Camry Electronics Co. Ltd. Guangdong, China). The body mass index (BMI) was calculated by dividing weight (kg) over the height squared (m²). Waist circumference (to the nearest centimetre) was measured with a Gulick II spring-loaded measuring tape (Gay Mill, WI) midway between the inferior angle of the ribs and the suprailiac crest. Hip circumference was measured as the maximal circumference over the buttocks in metres and the waist to hip ratio (WHR) calculated by dividing the waist circumference (m) by the hip circumference (m).

Definitions for MetS

MetS was diagnosed using three sets of criteria as stated below:

National Cholesterol Education Program, Adult Treatment Panel III (NCEP ATP III) Criteria: The NCEP ATP III criteria mandates that individuals with MetS should have three or more of

the following five components of MetS: (1) Abdominal obesity (waist circumference >102 cm for men or >88 cm for women); (2) Raised triglyceride (≥ 1.7 mmol L⁻¹); (3) Low HDL-cholesterol (<0.9 mmol L⁻¹ in men or <1.0 mmol L⁻¹ in women); (4) High Blood Pressure (systolic BP ≥ 130 mmHg or diastolic BP ≥ 85 mmHg or treatment of hypertension) and (5) Raised fasting glucose (≥ 6.1 mmol L⁻¹) (NCEP, 2002).

International Diabetes Federation (IDF) Criteria: The IDF criteria mandates that MetS be diagnosed if Central obesity (waist circumference >90 cm for men or >80 cm for women) is accompanied by any two (2) of the following four (4) factors: (1) Triglyceride level ≥ 1.7 mmol L⁻¹; (2) HDL cholesterol <1.03 mmol L⁻¹ for men or <1.29 mmol L⁻¹ for women; (3) Blood pressure $\geq 130/85$ mmHg or treatment of previously diagnosed hypertension and (4) Fasting blood glucose (FBG) ≥ 5.6 mmol L⁻¹ or previously diagnosed type 2 diabetes (Alberti *et al.*, 2006).

World Health Organization (WHO) Criteria: The WHO criteria mandates the presence of diabetes mellitus, impaired glucose tolerance or insulin resistance and any two (2) of the following: (1) Body mass index (BMI) ≥ 30 kg m⁻² and/or waist to hip ratio >0.90 for males or >0.85 for females; (2) Blood pressure $\geq 140/90$ mmHg or on medication; (3) Triglyceride ≥ 1.7 mmol L⁻¹ and (4) HDL cholesterol <0.91 mmol L⁻¹ in males or <1.01 mmol L⁻¹ in females (World Health Organization, 1999).

All three study definition criteria were used to assess the relationship between MetS and patient variables.

Statistical Analysis

Results are presented as Means \pm SD. Unpaired *t*-test was used to compare the means of all continuous variables. The Chi-square test statistic was used to assess the statistical significance of categorical variables. Logistic regression test statistic was used to estimate the crude (c) and adjusted (adj) odds ratio (OR) for risk factors of MetS. A *p*-value < 0.05 was considered to be statistically significant. All sta-

tistical analyses were performed using MedCalc® version 10.2.0.0 (www.medcalc.be) for windows.

RESULTS

General Characteristics

Table 1 presents the general characteristics of the study population stratified by treatment. Patients on antipsychotic (AP) medication were significantly older (37.9 ± 1.4 years) and heavier (66.1 ± 1.4 kg) than the newly diagnosed antipsychotic-naïve patients (26.2 ± 1.0 years and 61.9 ± 1.3 kg respectively) (*p* < 0.000). The mean waist circumference in patients on AP medication (85.2 ± 1.3 cm) as a marker of central obesity was significantly higher compared to the newly diagnosed AP-naïve patients (76.7 ± 1.0 cm) (*p* < 0.000) likewise the body mass index of 24.7 ± 0.5 kg m⁻² and 22.7 ± 0.6 kg m⁻² respectively (*p* = 0.009). The mean systolic (125.2 ± 2.0 mmHg) and diastolic (79.5 ± 1.0 mmHg) blood pressure in patients on treatment was also significantly higher in comparison to the newly diagnosed AP-naïve patients (117.8 ± 1.8 mmHg, 75.3 ± 0.9 mmHg respectively).

The mean concentrations of the lipid profile including total cholesterol (4.7 ± 0.1 mmol L⁻¹, *p* = 0.003), low density lipoprotein cholesterol (2.9 ± 0.1 mmol L⁻¹, *p* < 0.000) and very low density lipoprotein (0.3 ± 0.0 mmol L⁻¹, *p* = 0.012) in patients on treatment was significantly higher than in the newly diagnosed AP-naïve patients with mean concentrations of 4.3 ± 0.1 , 2.4 ± 0.1 and 0.2 ± 0.0 mmol L⁻¹ respectively. Conversely, the mean concentrations of high density lipoprotein (1.2 ± 0.0 mmol L⁻¹, *p* = 0.001) and MDA (0.8 ± 0.0 mmol L⁻¹, *p* < 0.000) in patients on treatment were significantly lower compared to that in newly diagnosed AP-naïve patients (1.4 ± 0.0 mmol L⁻¹ and 0.9 ± 0.0 mmol L⁻¹ respectively) while the mean triglyceride, fasting blood sugar concentrations and waist to hip ratio showed no statistically significant differences (Table 1).

Co-morbid conditions

Odds analysis to evaluate the risk of developing certain physical co-morbidities and lifestyle associated with MetS in psychiatric patients based on

Table 1: General characteristics of the study population stratified by treatment

Variables	Total (n = 200)	On Treatment (n = 100)	Newly Diagnosed (n = 100)	P value
Age (yrs)	32.0 ± 1.0	37.9 ± 1.4	26.2 ± 1.0	< 0.000
Weight (kg)	64.0 ± 1.0	66.1 ± 1.4	61.9 ± 1.3	0.028
Height (m)	1.6 ± 0.0	1.6 ± 0.0	1.6 ± 0.0	0.054
WC (cm)	81.2 ± 0.9	85.7 ± 1.3	76.7 ± 1.0	<0.000
HC (cm)	98.7 ± 1.0	100.3 ± 1.4	97.1 ± 1.1	0.073
WHR	1.1 ± 0.2	1.3 ± 0.5	0.8 ± 0.0	0.268
BMI (kg m ⁻²)	23.7 ± 0.4	24.7 ± 0.5	22.7 ± 0.6	0.009
SBP (mmHg)	121.5 ± 1.4	125.2 ± 2.0	117.8 ± 1.8	0.006
DBP (mmHg)	77.4 ± 0.7	79.5 ± 1.0	75.3 ± 1.0	0.002
FBS (mmol L ⁻¹)	5.4 ± 0.2	5.5 ± 0.3	5.3 ± 0.2	0.426
Uric acid (µmol L ⁻¹)	236.3 ± 5.1	255.3 ± 7.9	217.4 ± 5.8	0.000
TC (mmol L ⁻¹)	4.5 ± 0.1	4.7 ± 0.1	4.3 ± 0.1	0.003
TG (mmol L ⁻¹)	1.4 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	0.138
HDL-C (mmol L ⁻¹)	1.3 ± 0.0	1.2 ± 0.0	1.4 ± 0.0	0.000
LDL-C (mmol L ⁻¹)	2.6 ± 0.1	2.9 ± 0.1	2.4 ± 0.1	<0.000
VLDL (mmol L ⁻¹)	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.012
MDA (µmol L ⁻¹)	1.0 ± 0.0	0.8 ± 0.0	0.9 ± 0.0	<0.000

Results are presented as mean ± SD. P value defines the level of significance when study population on treatment was compared to the newly diagnosed. WC = waist circumference, HC = hip circumference, WHR = waist to hip ratio, BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, FBS = fasting blood sugar, TC = total cholesterol, HDL-C = high density lipoprotein cholesterol, LDL-C = low density lipoprotein cholesterol, VLDL = very low density lipoprotein, MDA = malondialdehyde.

treatment classification (AP naïve and those on AP) are shown in Table 2. Patients on treatment are approximately 12 times at risk of indulging in alcoholism ($p=0.024$) and 26 times at risk of developing hyperuricaemia ($p = 0.0001$) when compared to the newly diagnosed patients. The odds of being obese (2.5 times), developing hypercholesterolaemia (3 times), having low high density lipoprotein (HDL) cholesterol (3 times), developing high low density lipoprotein (LDL) cholesterol (3 times) in patients on treatment were all statistically significant compared to the newly diagnosed AP-naïve patients. Being on treatment however conferred some level of protection against oxidative stress ($p = 1.000$) while sex, smoking, hypertension, diabetes and hypertriglyceridaemia showed no statistical significance when patients on treatment were compared to the newly diagnosed AP-naïve patients.

Prevalence of MetS

Table 3 presents a general overview of the prevalence of MetS and its score in the study population defined by the three different classification criteria. When defined by the National Cholesterol Education Program, Adult Treatment Panel III (NCEP ATP III) criteria, 21.0% of the patients on treatment compared to 2.0% of the newly diagnosed AP-naïve patients are at risk of developing MetS (score ≥ 3) with an odds ratio of 13.0 ($p < 0.000$). On using the International Diabetic Federation (IDF) criteria, 29.0% of the patients on treatment compared to 2.0% of the newly diagnosed AP-naïve patients were at risk of developing MetS (OR = 20.0, $p < 0.000$). Contrary to the results from the two criteria stated above, the World Health Organization (WHO) criteria gave percentage prevalence of 13.0% and 14.0% for patients on AP

Table 2: Co-morbid conditions stratified by treatment among the studied population

Variables	Total (n = 200)	On Treatment (n = 100)	Newly Diagnosed (n = 100)	OR(95% CI)	P value
Females	121(60.5%)	63(63.0%)	58(58.0%)	1.2(0.7-2.2)	0.563
Smokers	1(0.5%)	1(1.0%)	0(0.0%)	3.0(0.1-75.3)	1.000
Alcohol	5(2.5%)	5(5.0%)	0(0.0%)	11.6(0.6-212.3)	0.024
Hypertension	28(14.0%)	17(17.0%)	11(11.0%)	1.7(0.7-3.7)	0.308
Diabetes	53(26.5%)	27(27.0%)	26(26.0%)	1.1(0.6-2.0)	1.000
Obesity	33(16.5%)	16(16.0%)	7(7.0%)	2.5(1.0-6.5)	0.046
Hypercholesterolaemia	43(21.5%)	30(30.0%)	13(13.0%)	2.9(1.4-5.9)	0.006
Hypertriglyceridaemia	58(29.0%)	28(28.0%)	30(30.0%)	0.9(0.5-1.7)	0.876
Low HDL-cholesterol	51(25.5%)	36(36.0%)	15(15.0%)	3.2(1.6-6.3)	0.001
High LDL-cholesterol	168(84.0%)	91(91.0%)	77(77.0%)	3.0(1.3-6.9)	0.011
Hyperuricaemia	11(5.5%)	11(11.0%)	0(0.0%)	25.8(1.5-444.9)	0.000
Oxidative stress	189(94.5%)	90(90.0%)	99(99.0%)	0.1(0.0-0.7)	1.000

Percentage values represent variables expressed as a proportion over their respective total (n), Obesity is defined as BMI ≥ 30 kg m⁻², Hypertension = blood pressure $\geq 140/90$ mmHg, Diabetes = fasting blood sugar greater or equal to 7.0 mmol L⁻¹, Hypercholesterolaemia = total cholesterol > 5.2 mmol L⁻¹, Hypertriglyceridaemia = triglyceride > 1.8 mmol L⁻¹, Low HDL-Cholesterol = HDL-C < 1.0 mmol L⁻¹, High LDL-cholesterol = LDL-C > 1.8 mmol L⁻¹, and Hyperuricaemia = uric acid > 416.4 μ mol L⁻¹ (for men) and 356.9 μ mol L⁻¹ (for women), oxidative stress = MDA > 0.7 μ mol L⁻¹.

treatment and newly diagnosed AP-naïve patients respectively with an odds ratio of 0.9 but the difference was not statistically significant ($p = 0.837$).

Prevalence of components of MetS

Assessment of the percentage prevalence of the individual components of MetS per the classification criteria for the study population is presented in Table 4. Patients on treatment are more prone to being obese when classified by the NCEP ATP III, IDF and WHO criteria with percentage prevalence of 32.0% ($p = 0.001$), 47.0% ($p < 0.000$) and 40.0% ($p = 0.015$) respectively. Twenty-three percent (23%) of the patients on treatment are more likely to develop significantly raised blood pressure compared to 11.0% of the newly diagnosed patients when classified by the NCEP ATP III and IDF criteria ($p = 0.038$). However, no statistically significant difference was observed when raised blood pressure was assessed in patients on treatment and newly diag-

nosed AP naïve patients using the WHO criteria ($p = 0.308$). No significant difference in the prevalence of raised fasting blood sugar was observed in the study population when evaluated with the three classification criteria and likewise in the prevalence of raised triglyceride levels. The prevalence of low HDL cholesterol in patients on treatment as determined by the NCEP ATP III and WHO criteria showed no statistical significance but when classified by the IDF criteria, 50.0% of the patients on treatment were at risk of developing reduced HDL levels compared to 11.0% in newly diagnosed AP naïve patients and the difference was statistically significant.

Risk factors of MetS

Table 5 shows univariate analysis of the risk factors of MetS evaluated by the three classification criteria for the study population. In using the NCEP ATP III criteria as a dependent variable for the presence

Table 3: Prevalence of MetS and its score among the studied population stratified by treatment

Variables	Total (n=200)	On Treatment (n =100)	Newly Diag- nosed (n= 100)	OR(95% CI)	P value
<i>National Cholesterol Education Programme Adult Treatment Panel III criteria</i>					
MetS	23(11.5%)	21(21.0%)	2(2.0%)	13.0(3.0-57.3)	< 0.000
MetS Score					
0	52(26.0%)	19(19.0%)	33(33.0%)	0.5(0.3-1.0)	0.056
1	81(40.5%)	34(34.0%)	47(47.0%)	0.6(0.3-1.0)	0.061
2	44(22.0%)	26(26.0%)	18(18.0%)	1.8(0.9-3.5)	0.087
≥3	23(11.5%)	21(21.0%)	2(2.0%)	13.0(3.0-57.3)	< 0.000
<i>World Health Organization criteria</i>					
MetS	27(13.5%)	13(13.0%)	14(14.0%)	0.9(0.4-2.1)	0.837
MetS Score					
0	102(51.0%)	42(42.0%)	60(60.0%)	0.5(0.3-0.8)	0.011
1	47(23.5%)	24(24.0%)	23(23.0%)	1.1(0.6-2.0)	0.869
2	22(11.0%)	19(19.0%)	3(3.0%)	7.6(2.2-76.6)	0.000
≥3	19(9.5%)	15(15.0%)	4(4.0%)	4.2(1.4-13.3)	0.008
<i>International Diabetic Federation criteria</i>					
MetS	31(15.5%)	29(29.0%)	2(2.0%)	20.0(2.6-26.7)	<0.000
MetS Score					
0	58(29.0%)	22(22.0%)	36(36.0%)	0.5(0.3-0.9)	0.029
1	70(35.0%)	32(32.0%)	38(38.0%)	0.8(0.4-1.4)	0.374
2	50(25.0%)	26(26.0%)	24(24.0%)	1.1(0.6-2.1)	0.744
≥3	22(11.0%)	20(20.0%)	2(2.0%)	12.3(2.8-54.0)	<0.000

OR = odds ratio, CI = confidence interval, MetS = Metabolic syndrome

or absence of MetS: treatment (cOR = 13.0, p = 0.001), obesity-WC¹ (cOR = 3.2, p = 0.011), obesity -WC² (cOR = 2.6, p = 0.037), obesity BMI (cOR = 1.8, p = 0.026), raised BP¹ (cOR = 3.1, p = 0.020), hyperuricaemia (cOR = 5.1, p = 0.015), hypercholesterolaemia (cOR = 2.5, p = 0.048) and raised TG (cOR = 3.1, p = 0.012) turned out to be significant risk variables and predictors of MetS. However, when the risk variables were further assessed with adjustment for age (Table 6), only treatment (adjOR = 6.8, p = 0.015), obesity-WC¹ (adjOR = 2.5, p = 0.048), obesity BMI (adjOR = 4.8, p = 0.009) and raised TG (adjOR = 4.4, p = 0.004) turned out as true significant predictor variables for MetS when classified by the NCEP ATP III criteria.

When the IDF criteria was applied, female gender (cOR = 2.5, p = 0.041), treatment (cOR = 20.0, p < 0.001), obesity-WC¹ (cOR = 3.8, p = 0.001), obesity

-WC² (cOR = 3.6, p = 0.001) and reduced HDL-C² (cOR = 2.6, p = 0.016) were significant risk variables and predictors of MetS with the exception of oxidative stress (cOR = 0.2, p = 0.034) which appeared to confer some level of protection against the development of MetS. Upon adjusting for age, the above mentioned risk variables turned out as true significant predictors of MetS in addition to obesity BMI (adjOR = 3.1, p = 0.045) with adjusted odds ratio of 2.9, 9.5, 3.3, 3.2 and 2.3 respectively. Furthermore, oxidative stress turned to be a significant protector from the development of MetS (Tables 5 & 6). No statistically significant differences were observed in the risk variables when classified by the WHO criteria in both univariate analyses and age-adjusted analyses (Tables 5 & 6).

Table 4: Prevalence of MetS components among the study populations stratified by treatment

Variables	Total (n = 200)	On Treatment (n = 100)	Newly Diagnosed (n = 100)	P value
OBESITY				
NCEP ATP III Criteria				
> 102 cm (men)/> 88 (women)	43 (21.5%)	32 (32.0%)	11 (11.0%)	0.001
IDF Criteria				
> 90 (men)/> 80 (women)	64 (32.0)	47 (47.0%)	17 (17.0%)	< 0.000
WHO Criteria				
> 0.9 (men)/> 0.85 (women)	63 (31.5)	40 (40.0%)	23 (23.0%)	0.015
BLOOD PRESSURE				
NCEP ATP III/IDF Criteria				
≥ 130/85	34 (17.0)	23 (23.0%)	11 (11.0%)	0.038
WHO Criteria				
≥ 140/90	28 (14.0%)	17 (17.0%)	11 (11.0%)	0.308
FASTING BLOOD SUGAR				
NCEP ATP III/WHO Criteria				
≥ 6.1	55 (27.5%)	29 (29.0%)	26 (26.0%)	0.752
IDF Criteria				
≥ 5.6	57 (28.5%)	31 (31.0%)	26 (26.0%)	0.531
TRIGLYCERIDE				
≥ 1.7	58 (29.0%)	28 (28.0%)	30 (30.0%)	0.876
HDL-C				
NCEP ATP III/WHO Criteria				
< 0.9 (men)/< 1.0 (women)	36 (18.0%)	23 (23.0%)	13 (13.0%)	0.098
IDF Criteria				
< 1.03 (men)/< 1.29 (women)	61 (30.5%)	50 (50.0%)	11 (11.0%)	< 0.000

NCEP ATP III = National Cholesterol Education Program, Adult Treatment Panel III, IDF = International Diabetes Federation, WHO = World Health Organization, HDL-C = High Density Lipoprotein Cholesterol.

DISCUSSION

Prevalence of MetS and Associated Disorders

The prevalence of MetS in psychiatric patients on AP medication calculated in this study using the IDF criteria was 29% compared to 2% in AP-naïve patients. This prevalence rate is slightly higher than the 20 – 25% prevalence rate of MetS in the world's population reported in the IDF consensus statement (Alberti *et al.*, 2006). In using the NCEP ATP III criteria, the prevalence of MetS in patients on AP medication was 21%. This finding is close to the MetS prevalence rate of approximately 22% estimated in the United States using the NCEP ATP III criteria (Ford *et al.*, 2002). The WHO criteria on the

other hand gave a lower rate of MetS in patients on AP medication (13.0%) and a higher rate in the AP-naïve patients (14%) when generally compared to prevalence rates in patients on AP medication and AP-naïve patients respectively determined by the IDF and NCEP ATP III criteria.

A number of publications have mentioned the potential relationship between antipsychotic drugs and hyperglycaemia (Sernyak *et al.*, 2002; Taylor *et al.*, 2005). Ryan *et al.*, (2003) in their study found that first episode and drug naïve patients may show insulin resistance which complicates the underlying mechanisms in this regard. Fasting blood glucose

Table 5: Univariate analysis of risk factors for MetS among the study population (N = 200)

Variables	NCEP ATP III		WHO		IDF	
	cOR(95% CI)	P value	cOR(95% CI)	P value	cOR(95% CI)	P value
Female	2.0(0.7-5.3)	0.168	1.7(0.7-4.0)	0.263	2.5(1.0-6.2)	0.041
Treatment	13.0(3.0-57.2)	0.001	0.9(0.4-2.1)	0.836	20.0(4.6-86.6)	0.000
Obesity-WC ¹	3.2(1.3-8.0)	0.011	1.0(0.4-2.7)	0.976	3.8(1.7-8.6)	0.001
Obesity-WC ²	2.6(1.1-6.1)	0.037	0.9(0.4-2.1)	0.732	3.6(1.6-7.9)	0.001
Obesity-WHR	1.4(0.6-3.6)	0.443	1.6(0.7-3.8)	0.264	1.8(0.8-4.0)	0.152
Obesity-BMI	1.8(1.2-9.6)	0.026	0.6(0.1-2.6)	0.478	2.1(0.8-6.0)	0.143
Raised BP ¹	3.1(1.2-8.0)	0.020	1.5(0.5-4.0)	0.440	1.9(0.8-4.7)	0.161
Raised BP ²	1.9(0.6-5.5)	0.262	1.5(0.5-4.3)	0.469	1.6(0.6-4.3)	0.353
Raised FBS ¹	1.5(0.6-3.7)	0.408	1.4(0.5-3.3)	0.467	0.9(0.4-2.2)	0.818
Raised FBS ²	1.4(0.6-3.5)	0.479	1.3(0.5-3.1)	0.551	0.9(0.4-2.0)	0.718
Hyperuricaemia	5.1(1.4-19.1)	0.015	0.6(0.1-5.1)	0.663	2.2(0.5-8.6)	0.277
Hypercholesterolaemia	2.5(1.0-6.3)	0.048	1.2(0.5-3.2)	0.647	1.8(0.8-4.2)	0.161
Raised TG	3.1(1.3-7.5)	0.012	1.8(0.8-4.3)	0.152	1.4(0.6-3.2)	0.388
Reduced HDL-C ¹	1.7(0.6-4.7)	0.288	1.4(0.5-3.7)	0.540	1.8(0.7-4.3)	0.223
Reduced HDL-C ²	1.7(0.7-4.1)	0.236	1.0(0.4-2.5)	0.921	2.6(1.2-5.7)	0.016
High LDL-C	2.1(0.5-9.6)	0.320	0.8(0.3-2.3)	0.702	1.3(0.4-4.1)	0.610
Oxidative stress	0.6(0.1-5.7)	0.689	0.8(0.1-6.9)	0.818	0.2(0.0-0.9)	0.034

Obesity-WC¹ = NCEP ATP III criteria, Obesity-WC² = IDF criteria, Raised BP¹ = NCEP ATP III/IDF criteria, Raised BP² = WHO criteria, Raised FBS¹ = NCEP ATP III/WHO criteria, Raised FBS² = IDF criteria, Reduced HDL-C¹ = NCEP ATP III/WHO criteria, Reduced HDL-C² = IDF criteria, cOR = crude odds ratio, CI = confidence interval.

showed no statistical significance when compared in the two study groups but as a mandatory component in the WHO definition, the absence of a statistically significant difference could be a major reason in the inability of the WHO criteria to well define the presence of MetS in the study population. Furthermore, glucose dysregulation in patients on AP in this study might not be explained exclusively on the individual drugs themselves but the contribution of these drugs for other components of MetS including cardiovascular risks and dyslipidaemia might certainly contribute to such predisposition as related in the study of Jin *et al.*, (2004). The relationship between age and MetS has long been documented. Ford *et al.*, (2002) and Alexander *et al.*, (2003) reported increases in the prevalence of MetS with increase in age. A high prevalence rate of MetS was observed in patients on AP medication who were

significantly older when compared to the AP-naïve patients.

Co-morbidities

There is growing evidence that severe mental illnesses are associated with significant physical comorbidities (Davidson *et al.*, 2001; Mitchell and Malone, 2006) that may lead to increased risk of premature mortality in many psychiatric patients (Dembling *et al.*, 1999; Saha *et al.*, 2007). Weight gain is an established side effect of most of the antipsychotic drugs (including typical and atypical antipsychotics) which association is well documented for the first generation (typical) antipsychotics and more recently newer or second generation (atypical) antipsychotic drugs (Klett and Caffey, 1960; Taylor and McAskill, 2000). Excessive weight gain has many adverse clinical consequences includ-

Table 6: Age adjusted odds ratio of the risk factors for MetS among the study population (N = 200)

Variables	ATP III		WHO		IDF	
	aOR(95% CI)	P value	aOR(95% CI)	P value	aOR(95% CI)	P value
Female	1.9(0.7-5.5)	0.216	1.6(0.7-4.0)	0.292	2.9(1.0-7.8)	0.041
Treatment	6.8(1.5-31.4)	0.015	0.5(0.2-1.3)	0.137	9.5(2.1-43.2)	0.003
Obesity WC ¹	2.5(1.0-6.8)	0.048	0.8(0.3-2.3)	0.697	3.3(1.3-8.5)	0.014
Obesity WC ²	2.0(0.8-5.3)	0.140	0.7(0.3-1.8)	0.451	3.2(1.3-8.0)	0.011
Obesity WHR	1.0(0.3-2.7)	0.940	1.4(0.6-3.3)	0.463	1.2(0.5-3.1)	0.720
Obesity BMI	4.8(1.5-15.4)	0.009	0.6(0.1-2.7)	0.500	3.1(1.0-10.0)	0.045
Raised BP ¹	1.9(0.6-5.5)	0.250	1.1(0.4-3.1)	0.860	0.9(0.3-2.7)	0.803
Raised BP ²	1.0(0.3-3.6)	0.960	1.1(0.4-3.5)	0.815	0.7(0.2-2.6)	0.632
Raised FBS ¹	1.9(0.7-5.2)	0.223	1.5(0.6-3.7)	0.354	1.1(0.4-2.9)	0.889
Raised FBS ²	1.8(0.7-5.1)	0.238	1.5(0.6-3.6)	0.399	1.1(0.4-2.9)	0.914
Hyperuricaemia	3.2(0.8-13.6)	0.108	0.4(0.1-3.6)	0.437	1.0(0.2-4.7)	0.984
Hypercholesterolaemia	2.0(0.7-5.3)	0.183	1.0(0.4-2.7)	0.957	1.3(0.5-3.3)	0.641
Raised TG	4.4(1.6-12.1)	0.004	2.0(0.8-4.7)	0.114	1.8(0.7-4.7)	0.223
Reduced HDL ¹	1.9(0.6-5.6)	0.273	1.4(0.5-3.7)	0.554	2.0(0.7-5.9)	0.184
Reduced HDL ²	1.3(0.5-3.4)	0.600	0.9(0.4-2.2)	0.785	2.3(1.0-5.6)	0.043
High LDL-C	1.9(0.4-5.6)	0.422	0.7(0.3-2.2)	0.582	1.1(0.3-3.8)	0.921
Oxidative stress	0.6(0.1-6.0)	0.655	0.8(0.1-7.2)	0.834	0.1(0.0-0.7)	0.018

aOR = adjusted odds ratio

ing predisposition to a number of physical illnesses like cardiovascular disorders, diabetes, stroke, osteoarthritis and sleep apnoea in addition to low self esteem, decreased quality of life and reduced adherence to treatment. Clinical manifestations of increased lipid profiles are also among the main causes of morbidity and mortality for cardiovascular diseases with increases in serum cholesterol being considered as a major risk factor for such diseases (Austin *et al.*, 1998). A Finish study reported that individuals treated with antipsychotic medications were three times more likely to have high cholesterol or high triglyceride than those who were not taking the drugs (Saari *et al.*, 2004).

Patients on AP medication from this study were three times more likely to have high cholesterol, high LDL-cholesterol and low HDL-cholesterol;

two and half times more likely to be obese and twelve times more likely to indulge in alcoholism. These findings explain the high prevalence rate of MetS in patients on AP medication and their significant risk of developing cardiovascular disease (CVD) compared to the AP naïve patients as determined with the IDF and NCEP ATP III criteria which could likely be worsened if the patient indulges in alcoholism. Alexander *et al.*, (2009) in a study on the prevalence of MetS among Australians with severe mental illness found no difference in the prevalence of MetS between the gender and Mackin *et al.*, (2007) in their study also found that differences in smoking behaviour did not account for excess metabolic and cardiovascular risk in people with mental illness who smoked compared with controls. Female gender, smoking, hypertension, diabetes and hypertriglyceridaemia did not show

significant differences in patients on treatment with AP compared to AP-naïve patients in this study. Furthermore, diabetes did not present as a significant physical co-morbidity which reason could further explain the inability of the WHO criteria to define MetS in this study group as it mandates individuals to be diabetic.

Risk factors of MetS

The outcome measures of the logistic regression shows the capability of AP medication to contribute to excess physical morbidity aside controlling the symptoms of psychiatric illness. Glucose dysregulation could not be explained exclusively by the individual drugs of AP medication but the contribution of the drugs for other components of MetS including cardiovascular risks and dyslipidaemia certainly contribute to such predisposition. Weight gain, especially visceral adiposity, as measured by waist circumference (IDF and ATP III criteria), BMI and dyslipidaemia pose attendant risks for the physical health of the patients.

CONCLUSION

This study demonstrates a high prevalence of MetS (>20%) and the risk of CVD among Ghanaian psychiatric patients on antipsychotic medication. This therefore necessitates baseline and periodic medical evaluations as standard components in the on-going assessment and treatment plans for the patients.

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