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Journal of Public Health and Epidemiology

### Full Length Research Paper

# Malaria among relatives escorting sick patients during the dry season to Karume Health Centre, Mwanza, Northwestern Tanzania

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Malaria is still a public health problem in the world. It accounted for an estimated 214 million cases and 438,000 deaths in the year 2015. During the dry season, most people are likely to be asymptomatic and therefore fail to be diagnosed with malaria. This study established the proportion of people who came to health facility, escorting sick relatives and had detectable malaria parasites. This was a cross sectional study. All relatives who escorted sick patients to Karume Health Centre between August and December 2013 were screened for malaria using malaria rapid diagnostic test (mRDT) and single round PCR targeting mitochondrial DNA. A total of 400 relatives were screened for malaria using two methods. Prevalence of malaria was 14.5 and 16.8% by mRDT and polymerase chain reaction (PCR), respectively. The prevalence of malaria was higher among febrile patients by methods, mRDT (17.8%) and PCR (17.1%), respectively. The prevalence of asymptomatic malaria was 16.4 and 16.5% by mRDT and PCR, respectively. The overall agreement between the two tests was 87.1% with positive agreement of 63.8% and negative agreement of 91.2%. There were a substantial proportion of patients with malaria who visited the health facilities during the dry season. mRDT and single round PCR targeting mitochondrial DNA had a good agreement and can be used for detection of both symptomatic and asymptomatic malaria. Provider initiated screening can help to improve malaria detection during the dry season, as we move towards reduced malaria prevalence and elimination phase.

**Key words:** Asymptomatic malaria, prevalence of malaria among relatives, mitochondrial DNA, polymerase chain reaction (PCR).

#### INTODUCTION

Malaria is still a public health problem especially in the WHO African region. Form 2015, the African region

accounted for 88% of the 214 million cases that occurred in the world (WHO report, 2015). The trend however

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shows that malaria is declining globally from 262 million cases in 2000 to 214 million cases in 2015 (WHO report, 2015). The decrease is attributed to the use of Insecticide treated bed nets (ITNs), indoor residual spraying and efficacy of artemisinin based combination therapies (ACTs).

In most regions including Tanzania, malaria transmission occurs mainly during the rainy season and is lowest during the dry season (Oesterholt et al., 2006). During the dry season, there are usually few cases of malaria and morbidity and mortality goes down to close absence of the disease depending on endemicity of malaria (Oesterholt et al., 2006; Ardiet et al., 2014). During the dry season therefore, most individuals are likely to be asymptomatic while carrying the parasites.

Asymptomatic patients far outnumber the symptomatic infections (Lindblade et al., 2013; Alves et al., 2002; Lin et al, 2014) and are likely not to come to hospital. These infections may last from weeks to months and to an average of 194 days (Males et al., 2008; Bottius et al., 1996; Felger et al., 2012). It has been reported that proportions of individuals remain with the parasites in blood circulation and can be transmitted at the beginning of the next rainy season.

Studies in Africa have clearly demonstrated that, asymptomatic cases harbour gametocytes and can therefore be a significant transmission reservoir (Dunyo et al., 2006; Bousema et al., 2004). Another challenge during the dry season is the diagnostic method used for screening (Ardiet et al., 2014; Permeger et al., 2006). mRDT is now widely accepted in malaria endemic countries but its sensitivity when parasitaemia is low is a limiting factor. Existence of submicroscopic parasites (Kamugisha et al., 2012; Lindblade et al., 2013) makes the gold standard test microscopy to be of limited value, during dry season when parasites loads are low and patients are asymptomatic. Thus investigating the utility of other sensitive methods such as single round PCR targeting, mitochondrial DNA is important in the era of declining malaria when elimination is becoming an agenda.

In this study we screened people who come to hospital, not seeking treatment for themselves but escorting relatives who are sick. The aim was to see what proportion of these relatives actually reaches a service providing facility and has fever as, malaria infection. This is a form of active case detection (Van Eijk et al, 2016) that is utilizing relatives and siblings at the health facility.

#### **MATERIALS AND METHODS**

#### Study area and design

This was a cross sectional study conducted at Karume Health centre in Mwanza city, Northwestern part of Tanzania. The area is mesoendemic lying in Lake Victoria basin at an altitude of 1140 m above sea level. The basin is endemic for malaria with perennial transmission. The catchment area of Karume health centre is about

40.000 inhabitants.

#### Study participants and recruitments

This study recruited all relatives and fellow siblings who escorted their relatives to receive care at Karume health centre. All relatives who escorted their sick families to the centre during the study period were informed about the study and those who agreed signed an informed consent. Those who consented were interviewed using a structured questionnaire, intending to collect the social demographic characteristics and the use of antimalarial drugs in the past two weeks prior to recruitment. A clinical examination was done to the participants and blood taken for mRDT were dried in blood spot for PCR investigation.

#### Parasitological examination

The presence of malaria parasites was determined using mRDT and PCR targeting parasite mitochondrial DNA. mRDT was performed immediately at Karume health centre using SD Bioline Malaria Ag Pf/Pan mRDT, Korea. The test was done according to manufacturer's instructions. PCR targeting parasite mitochondrial genes was done in Uppsala University in Sweden. The method used was as described previously (Haanshuus et al., 2013). Only a single round PCR was done contrary to the nested PCR usually performed for DNA analysis of other malaria parasite genes. It has been stated previously that, due to high number of mitochondria per parasite, a single round PCR is enough to detect even small amounts of DNA.

#### Data analysis

Data entry, cleaning and analysis was done using SPSS version 17 software. Frequencies and cross tabulation were done to obtain proportions and chi square for associations of the study variables. Due to comparison of two tests without a gold standard, Cohen's kappa test was used. P-value of less than 0.05 at 95% confidence interval was considered significant.

#### **Ethical clearance**

The study was approved by joint CUHAS/Bugando ethics committee. Permission to conduct the study at Karume health centre was sought from other relevant administrative authorities. Participants above 18 years signed an informed consent before participating in this study. For those below 18 years, an informed assent was sought together with informed consent from the guardians/parents. All patients who were diagnosed with malaria were treated with artemether-lumefantrine (ALU) accordingly. Those who had no malaria but were febrile were investigated for the cause of fever and treated.

#### **RESULTS**

#### Social demographic characteristics

A total of 400 relatives of patients who attended Karume health centre were recruited. Females were the majority with 333(83.3%). The youngest participant was 2 years old while the oldest was 90 years with mean age of 30.2. The majority 367(91.8%) of participants were adults

**Table 1.** Socio-demographic characteristics of the study participants.

Variable	Number	Percentage
Age group		
≤5 years	4	1.0
6-10 years	5	1.3
11-18 years	24	6.0
<18 years	367	91
Sex		
Female	333	83.3
Male	67	16.8
Fever		
Yes	152	38.0
No	248	62.0
·	·	·

**Table 2.** Comparison of mRDT and single round PCR targeting mitochondria DNA.

Parameter	mRDT positive	mRDT negative	Total
PCR positive	37	30	67
PCR negative	21	312	333
Total	58	342	400

above 18 years (Table 1).

#### Prevalence of malaria

Among the 400 participants, 152(38%) had fever (body temperature >37.5°C) on the day of recruitment. The overall prevalence of malaria by using mRDT was 58(14.5%). The prevalence was higher among those who were febrile at recruitment 23(17.8%), compared to those who were afebrile (asymptomatic host malaria) 35(16.4%), though the difference was not statistically significant (p-value 0.79) (Table 2). The prevalence by, single round PCR targeting mitochondrial DNA was 67(16.8%), asymptomatic relatives was 16.5% and febrile relatives was >17.1% The prevalence was higher among febrile relatives than those who were afebrile and the difference was statistically significant (p-value 0.022).

#### Comparison of mRDT and mitochondrial DNA tests

When the two diagnostic tests were compared (Table 2) in the absence of a gold standard test, the overall percent agreement between mRDT and PCR was 87.1% and p-value was <0.001. The positive agreement was 63.8% and the negative agreement was 91.2%. There was a

good agreement between the two tests by Cohen's kappa of 0.5.

#### Prevalence of fever with age

When the participants were divided into different age groups, the prevalence of fever declined with age. The prevalence was 100% in the age group <5 years and lowest in the adult group of 18-90 years. The difference was statistically significant (p-value = 0.004).

#### DISCUSSION

#### Prevalence of malaria by mRDT

The overall prevalence of malaria was high considering that, this study was done during the dry season when transmission is low. There have been a different prevalence's of malaria during the dry season but it is known that, a significant proportion of individuals remain with microscopically undetectable parasites in blood which form a reservoir for the next transmission.

The prevalence of asymptomatic malaria among patients was 16.4%, which was higher than that recently reported in school children in Tanzania (Nzobo et al., 2015). This is almost similar to the previous study in school children in a nearby place, which was 14.3% (Mazigo et al., 2010). In different parts of Africa, the prevalence of asymptomatic malaria varies considerably and prevalence up to 80% was reported in Cameroon (Okell et al., 2012).

#### Comparison of mRDT and mitochondrial DNA

A gold standard method for diagnosing malaria is microscopy. During the dry season and especially in asymptomatic patients, microscopy may be a limited diagnostic test. Existence of sub-microscopic parasites in patients treated effectively with ALU in this area has been shown (Kamugisha et al., 2012), while those in other areas has also been reported. The use of mRDT and PCR during the dry season in this study gave a prevalence that is similar to other places. There was also a good agreement between the two diagnostic tests carried out, and the result is comparable to the findings in other studies.

The single round PCR is also being shown to be effective, in the field for screening of malaria parasites during the dry season. Due to shorter time used and its good agreement with nested PCR in this study and its specificity and sensitivity documented previously (Haanshuus et al., 2013), it is clear that mRDT can be used for screening and diagnostic purposes in endemic countries. Deploying different diagnostic methods for asymptomatic, malaria infection is vital and has been

suggested previously (Bousema et al., 2014).

#### Prevalence of malaria by mRDT with age

This study shows that, by doing active case detection among relatives escorting patients with malaria, it is likely to increase detection of malaria cases. All children aged <5 years, who visited the clinic as siblings to other sick children were also febrile but their fever was not reported. In the higher age group as well, the relatives/siblings also had fever, which was not reported. The patients who were positive for malaria and other diseases such as urinary tract infection and upper respiratory tract infection were treated. Those who received an antimalarial dose might have a reduced risk of coming at a later date, with severe malaria and/or uncomplicated malaria probably associated with other complications such as anaemia.

The main reason for parents coming and complaining or intending to treat only one child could be due to limited resources enough to treat all family members with fever. The limited funding for cost sharing and purchasing drugs are then concentrated to one person, who looks to be more severely ill than others. This study did not look at the factors that were making febrile individuals not to report their illness but recommend such a study in this area. The hidden point may be that, due to limited funding, the treatment given to the sick individual may be divided and utilized by the other sick relatives at home. If this occurs then, failure to complete the required ALU dose and other drugs are likely to occur in both relatives and this may lead to quick building up of drug resistance.

Data from this study shows that mimicking the strategy used by the same health facilities for HIV testing that is known as Provider initiated HIV counselling and testing (PICT), might also work for malaria. Although in PICT for HIV, the testing is for patients coming with other diseases but in lower facilities testing for relatives especially during low transmission, season might be another way of doing Focused Screening and Treatment (FSAT) and hence help in the control against malaria.

#### Conclusion

The prevalence of malaria among relatives visiting the health centre was low, but there were a high percentage of relatives with fever who does not complain about it at health facility. The prevalence among asymptomatic relatives was also low. The children accompanying their family members are also having complaints and malaria. There was good detection of malaria by both mRDT and PCR targeting mitochondrial DNA.

#### **CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

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Full Length Research Paper

# Substance use and factors associated with risky sexual practice in school youth in Asella Town, South-East Ethiopia, 2017

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Globally, risky sexual behavior accounts for large number of opportunities for sexually transmitted infection including human immunodeficiency virus and unintended pregnancy. The study is intended to describe substance use and factors associated with risky sexual practice among school youth. School based cross sectional study was conducted through self-administered questionnaire. Simple random sampling technique was used to select 614. Epi info Version 7 and SPSS version 21 were used. Descriptive statistics and binary multiple logistic regression analysis was done by including variables with p<0.2 in the crude analysis and P-value<0.05 was considered statistically significant. From study participants; 201(33.6%) respondents were sexually active. Among them, 120 (20.07%) had practiced risky sexual intercourse, during sexual intercourse 112 (18.7%) did not use condom consistently. Peer pressure influence to sexual intercourse were 4.4 times more likely having risky sexual practice compared to those who had no influenced by peers pressure [AOR: 4.4 ,95% CI (2.748,6.917)]. Students who drink alcohol were 1.98 more likely to have risky sexual practice than those who did not drink alcohol [AOR=1.98, 95% CI (1.224, 3.190)]. Students who did not discuss openly about sexual and reproductive health issues with parents were 1.86 times more likely to practice risky sexual intercourse compared to their counterparts (AOR:1.86, 95%CI (1.149, 3.009). Conclusively, risky sexual behavior and substance use related issues should be considered in school curriculum. Health authorities should implement youth friendly services in schools and government bodies should incise a strict and sustainable measurement against selling alcohol to under 18 years old.

**Key words:** Risky sexual behavior, substance use, school youth.

#### INTRODUCTION

Risky sexual practice is defined as any human sexual contact which put individual's physical, social and

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psychological health at risk (Malhotra, 2008). It include when unprotected sexual intercourse, early sexual debut and multiple sexual partners occur in a broader context (Blum et al., 2005). The intensity of involvement in sexual risk practice ranges from no sexual relationship to unprotected sexual intercourse with multiple partners and prostitution. Although risky sexual practice does not always indicate a high-risk lifestyle, it often clusters with other risky behaviors, including substance use, violence involvement and poor school performance (Rosenthal, 2012). There are four main types of risks that may arise from sexual activity: unwanted pregnancy, sexually transmitted infection (STI including HIV), physical and psychological injury (National Youth Policy, 2004).

Youth constitutes the population (15 to 24 years of age) and it is a stage in which young people are confronted with some models of major roles that they are supposed to emulate in adult life (National Youth Policy, 2004).. This segment of the population constitutes more than one billion of the world population; one-fifth of the world's population includes youth and young adults, with more than four-fifths in developing countries. Young people constitute one third of the total population in Ethiopia. During the transition from childhood to adulthood, youth establish patterns of behavior and make lifestyle choices that affect both their current and future health (WHO, UNAIDS, UNICEF, 2008).

Youth begin thinking about the future and places more emphasis on goal-setting and self-esteem. However, youth may begin to exhibit more risky sexual behaviors during these ages (McNeely, 2009). It may result from being easily influenced by peers, cultural taboos, inadequate sexual communication, limited support from parents and inappropriate parenting roles (Underwood et al., 2011). Risky sexual practice accounts for all sexually transmitted diseases including HIV/AIDS, psycho social problems such as emotional ability, altered self-esteem, depression, impaired ability to form long term relationship (Malhotra, 2008).

Drug and alcohol use have potential roles in predisposing youths to practice unprotected sex due to urbanization, modernization, exposure to western life style, viewing pornographic materials and other factors associated with early sexual initiation.

Western pornography often preceded sexual initiation and help couple "get into the mood" (Russell et al., 2007). Youths are assets of the society and change agents in filling the gap on whom the future of the country is based. It is clear that this group is on the way of transforming to adulthood, filled with ambition and building their future academic and social career. Neglecting their sexual and reproductive health can lead to social and economic cost, both immediately and in the future. One of the most important commitments a country can make for future economic, social and political progress and stability is to address the sexual and reproductive health needs of this population group (Kilmarx, 2009).

The mission of the ministry of health of Ethiopia is "to

promote good health and reduce illness, ensure access to good and affordable health care and pursue medical excellence". Moreover, the vision of the Ministry Of Education is "building an education and training system which assures quality and equality of education by the year 2020 that aims at producing competent citizens" (Guttmacher, 2011; Sexual Risk Behavior, 2011). Therefore, this study is aimed to describe magnitude of substance use and identify factors associated with risky sexual behaviour in secondary school students which help to provide evidence based valuable information for the decision makers to achieve these mission and vision.

#### **METHODS**

#### Study area and design

School based quantitative cross-sectional study was done in February 2017 in Asella town which is located in Arsi zone of Oromia regional state about 175 km South east Addis Ababa, the capital city of Ethiopia (Table 1). The town has two preparatory, four Governments and four private secondary schools.

#### Population and sample size calculation

School youths in Asella town were the source population and according to Asella town education office, 9806 students were registered within nine to 12 grades in academic year 2016/2017 (Table 2). Sample size for the first objective was calculated using single population proportion formula by considering the assumptions: of total regular students 9806, proportion of risky sexual behavior 61.5% (Dekeke and Sandy, 2014) 95% confidence interval (CI), 5% margin of error, correction formula with 5% non-response rate gives 356 samples. For the second objective of taking substance use as factors for risky sexual practice which gives sample of 410, then the maximum sample was taken with a design effect of two makes final sample size 614.

#### Sampling procedures

The study was conducted in randomly selected (one preparatory and two secondary) schools in Assella town 2017 by lottery method. The first sample was proportionally allocated then representative samples were selected from each stratum through simple random sampling techniques by random table generation using SPSS.

#### Data collection tools and procedures

Structured self-administer questionnaire was prepared in English through reviewing literatures and translated to Amharic and Afanoromo then back to English to ensure consistency. Pre-test was done on 5% of the sample in Dosha secondary school and necessary modifications were made. Three diploma nurses were assigned for facilitation of data collection and training was given for supervisors and facilitators. Data was double-entered to assure data quality.

#### Data management and analysis

The collected data was reviewed and checked for completeness

**Table 1.** Socio-demographic characteristics of school youth in Asella, South-East Ethiopia, 2017.

Variable (n=598)	Frequency (%)		
Age			
15-18	414(69.2)		
19-24	184(30.8)		
Sex			
Male	292(48.8)		
Female	306(51.2)		
Level of education			
Grade 9	166(27.8)		
Grade 10	156(26.1)		
Grade11	135(22.6)		
Grade 12	141(23.6)		
Religion			
Orthodox	356(59.5)		
Muslim	129(21.6)		
Protestant	89(14.9)		
Catholic	24(4)		
Ethnicity			
Oromo	324(54.2)		
Amhara	149(24.9)		
Tigray	27(4.5)		
Gurage	63(10.5)		
Others#	35(5.9)		
Raised by			
Both parents	500(83.6)		
By one parent	67(11.2)		
Other *	31(5.2)		
Currently live with			
Both parents	404(67.6)		
Father	14(2.4)		
Mother	84(14)		
Alone	72(12)		
Others +	24(4)		

Others#; Hadiya, wolayita, Sidama, Adere, Kembata. Others\*;grandmothers,grandfathers, aunt, uncle. Others\*; aunt, uncle, Brothers, sisters.

before data entry then coded and entered into Epi info Version 7 finally exported to SPSS for cleaning and analysis. Descriptive statistics was done. Multivariate binary logistic regression analysis was estimated by taking all explanatory variables with p<0.2 in crude analysis (Table 4). The crude and adjusted odds ratio with 95% CI was computed and P-value<0.05 was considered statistically significant.

The model was built through stepwise logistic regression

technique. Confounders and interaction effect was checked by likelihood ratio test. Multicollinarity was checked by Variance Inflation Factors (VIF). Outliers were checked by standardized residual and influential observations were also checked by dfbeta statistics.

The overall model goodness of fit was checked by Hosmer-Lemeshow and the prediction power by Receiver-Operating Characteristic (ROC). Ethical clearance was obtained from Institutional Review Board (IRB) of Arsi University. Informed oral consent was taken from each participant and all the information gained during data collection was kept confidentially.

#### RESULTS

#### Demographic and socio-economic characteristics

Totally, 598 students participated which makes the response rate 97.4%. Majority respondents (414, 69.2%) were between the age 15 to 18 years old with mean age 17.9±1.7. About half, (306, 51.2%) were females and most of them (356, 59.5 %) were orthodox Christian in religion. More than half (324, 54.2%) were Oromo ethnic group. Currently, 404 (67.6%) of the respondent were living with both parents. Nearly half (245, 45.1%) of student's father educational level were college and above, whereas 35 (6.5%) were unable to read and write. Related to mothers educational level about 135 (23.2%) of student's mothers' were collage level and above but 69 (12.1%) were unable to read and write.

#### Substance use and other behavioral characteristics

Substance abuse in the last 12 month among the respondents were 218 (36.45%) of those; 121 (55.5%) were males, half of them (119, 54.6%) were less than 18 years old. The most common substance abused was alcohol which accounts for 125 (20.9%). That was the second most abused substance which account for 67 (11.2%), mostly used by 34 (50.7%) male participants. Smoking cigarettes (16, 2.6%) and shisha (10, 1.6%) were also used. About 322 (53.8%) had exposure to sexual explicit media, of those, 214 (66.4%) were aged below 18 years old and 162 (50.35) were females. About 212 (35.5%) of their best friend's had sexual history and out of those, 25 (4.2%) practiced sexual intercourse urged with prostitutes.

#### Knowledge related with pregnancy and HIV

Two third (385, 64.4%) of the respondents believed that pregnancy could occur at 14<sup>th</sup> day of menstrual cycle, while 24 (4%) believed it can occur during menstrual period. About half (288, 48.2%) agreed that a girl could be pregnant before her first menstruation. Almost all (587, 98.2%) believed that HIV can be prevented by being faithful and 586 (98%) believed that it can be

Table 2. Parental characteristics of school youth in Asella, south-East Ethiopia, 2017.

Variable (n=598)	Frequency (%)
Father currently alive	
Yes	543(90.8)
No	55(9.2)
Father's educational status	
Can't read and write	35(6.5)
Read and write	92(16.9)
Elementary	55(10.1)
High school	116(21.4)
College and above	245(45.1)
Father's employment status	
Employed	497(91.5)
Unemployed	46(8.5)
Markey and a street of the street	
Mother currently alive Yes	571(95.5)
No	27(4.5)
Mother's educational status	
Can't read and write	69(12.1)
Read and write	143(25)
Elementary	80(14)
High school	144(25.2)
College and above	135(23.7)
College and above	133(23.7)
Mother's employment status	
Employed	336(58.8)
Unemployed	235(41.2)
Parents know where you are	
Yes	519(86.8)
No	79(13.2)
	. 3(13.2)
Parents know who are with you	205(04)
Yes No	365(61)
NO	233()39
Discuss on SRH issues with parents	
Yes	217(36.3)
No	381(63.7)
Family monthly income(ETB)	
0-500	13(2.2)
500-1000	63(10.5)
1000-2000	114(19.1)
2000-3000	80(13.4)
3000-5000	135(22.6)
5000-10000	164(27.4)
>10000	29(4.8)

avoided by avoiding sharing cutting materials. On the other hand, 100 (16.7%) respondents agreed that HIV transmission can be prevented by taking antibiotics prior to sexual intercourse. About 516 (86.3%) of the respondents perceived that HIV transmission can be prevented from mother to child. In this study, 336 (56.2%) believed that a person can have STI without knowing.

#### Sexual characteristics

From 598 respondents, 201 (33.6%) ever had sexual intercourse of which, 120 (59.7%) were less than 18 years of age; the mean age at first sexual intercourse was 16.052±0.804 for male and 15.742±0.905 years for female. Regarding first sexual partner, 177 (88%) was boy/girlfriends while only 12 (6%) was teachers. Concerning multiple sexual partner, 98 (48.7%) and 46 (22.9%) of sexual active participants had two or more life time and within the last 12 months, respectively. From every person who had sexual intercourse less than half 89 (44.3%) used condom in their first sexual intercourse, of those 49 (55.1%) used on their own interest while 15 (16.8%) argued with joint decision. In addition, inconsistent condom user were higher (112, 55.7%) of those, 47 (42%) used condom occasionally but 25 (22.3%) never used condom. Furthermore, 47 (23.4%) faced partner's objection for condom use.

#### Proportion of risky sexual practice

Among the 598 secondary school youth that participated in this study, 120 (20.07%) respondents had practiced sexual intercourse less than 18 years of age with 95% CI (16.8, 23.3); among this, 62 (51.6%) were female. Regarding condom use, 112 (18.7%) did not use condom consistently with 95% CI (15.6, 21.7), more than half (66, 59%) were females. About 46 (7.7%) of sexually active participant had multiple sexual partners in the last 12 months with 95% CI (5.5, 9.8).

#### Factors associated with risky sexual practice

Students who had peer pressure were 4.4 times more likely to have practiced risk sexual behavior than those who had not had peer pressure [AOR: 4.36, 95%CI (2.748, 6.917)]. Students who drink alcohol were almost two times more likely to practice risky sexual behavior than those who did not drink alcohol [AOR=1.98, 95% CI (1.224, 3.190)]. Students who did not discuss openly about sexual and reproductive health issues with parents were 86% more and likely practiced risky sexual intercourse than their counterparts [AOR=1.86, 95% CI (1.149,3.009)]. Students who read/saw sexual explicit media/pornography were 60% [AOR=1.60, 95% CI

(1.010, 2.529)] more likely practiced risky sexual intercourse as compared to those who did not see or read pornography. Even though, it is not statistically significant in multivariate analysis, students who reside with one parent's were two times [AOR=2.03 (95% CI (1.165, 3.542)] more likely practiced risky sexual behavior.

#### DISCUSSION

# Substance use and magnitude of risky sexual behavior

Even though it is possible to prevent STIs including HIV/AIDS and unplanned pregnancy through effective strategies; significant numbers of students acquire different problems due to risky sexual behavior which include: early sexual initiation, low contraceptive use rates, multiple sex partners and poor sexual negotiation skills on condom use (Malhotra, 2008; Blum et al., 2005; Rosenthal, 2012). Worldwide, young people begin sexual activity relatively earlier; similarly, one fifth of school youth in Assella town had practiced risky sexual behavior with age less than 18 years in line with study in Northwest Ethiopia (20.4%) (Bizu et al., 2015), but lower than studies conducted in Addis Ababa (26.7%) (Gizaw et al., 2014), Haramaya (25.3%) (Shore and Shunu, 2017) and Benishangul Gumuz (24.1%) (Agajie et al., 2017). On other hand higher than studies in southern Ethiopia (17.9%) (Daka and Shaweno, 2014), North West, Ethiopia (13.7%) (Dadi and Teklu, 2014) and Jiga (16%) (Kassa et al., 2016). This discrepancy might be due to biological, socio-cultural and behavioral difference between study areas that influences human sexuality (Table 3).

Having more than one sexual partner's is one of the risky sexual behaviors, which is common among youths. Among sexually active respondents 98 (49%) ever had sex with multiple sexual partners and about guarter 46 (22.9%) multiple sexual partners within the previous 12 months. The finding is in line with studies in Debre-Brehan, and Hosanna (53.4%, 47.6%) (Muluken and Maereg 2012; Likawunt and Mulugeta, 2013), but higher than other studies in Ugandan (35%) (Agardh et al., 2011), Addis Ababa (37.5%) (Nigatu and Seman (2011) and Gondar and Behar-Dar (37.5%) (Wasie et al., 2012). The disparity might be due to behavioral, cultural and socio-economical variation between settings that influence human sexuality. Beside this, knowledge and experience on related health issue might preserve them from risky practice

Even though consistent and correct use of condom is one of the effective strategies to prevent STIs including HIV/AIDS most youth did not practiced it properly. Similarly in this study less than half (44.3) of the sexually active participant had used condom consistently

**Table 3.** Sexual and other behavioral characteristics of school youth in Asella, South-East, Ethiopia, 2017.

Variable	Frequency (%)
Peer pressure(n=598)	
Yes	259(43.3)
No	339(56.7)
Ever seen/read pornography	
Yes	322(53.8)
No	276(46.2)
Ever had sexual intercourse	
Yes	201(33.6)
No	397(66.4)
Age at first sex (n=201)	
< 18 years	120(59.7)
> = 18years	81(40.3)
Relationship of first sexual partner	
Boy/girlfriend	177(88)
Teacher	12(6)
Others	12(6)
Initiations of first sex	
Own will	178(88.5)
Forced	7(3.5)
For Money	3(1.5)
Materials/Gifts	13(6.5)
lifetime sexual partners	
One	103(51.2)
Two and More	98(48.7)
Sexual partners in the last 12 month	
One	155(77.1)
Two and more	46(22.9)
Ever had sex for cash or gift	
Yes	15(7.5)
No	186(92.5)
Condom used for first sex	
Yes	89(44.3)
No	112(55.7)
Suggestion for condom	
Myself	49(55.1)
My partner	25(28.1)
Joint decision	15(16.8)
Use condom at last sexual intercourse	
Yes	139(69.2)
No	62(30.8)

Table 3. Contd.

Consistence condom use	
Yes	89(44.3)
No	112(55.7)
Partner's objection on condom use	
Yes	47(23.4)
No	154(76.6)
Opinion for partner's objection	
Do sex not to miss partner	15(32)
Insist on using condom	5(10.6)
I will provide condom	11(23.4)
No sex without condom	16(34)

<sup>\*</sup>Others persons: Relatives, married persons.

whereas; about 22.3% never used condom during sexual intercourse. The result is similar with the study done in Haramaya (20.4%) whereas, the finding is lower than for other studies in Nigeria, Ugandan, Debre-Brehan, Jimma, Addis Ababa, Hawassa and Hosanna (65, 35, 53.4 69.1, 71.5, 58, 80%) (Federal Democratic Republic of Ethiopia Population Census Commission, 2008; Muluken and Maereg, 2012; Likawunt and Mulugeta, 2013; Agardh et al., 2011; Nigatu and Seman, 2011; Wasie et al., 2012; Berhan et al., 2011; Daniyam et al., 2010; Tura et al., 2012), respectively.

This discrepancy might be due to difference in sociocultural and behavioral characteristics that influence condom use, in addition availability and accessibility of condom might vary across setting. About 4.2% of sexually active male participants had sex with prostitutes and 7.5% of sexually active participant had sex for the exchange of cash, favors or gift which is similar with the report of Central Statistical Agency [Ethiopia] and ICF International (2012), report that transaction sex among men for the exchange of money was 5% (Central Statistical Agency [Ethiopia] and ICF International, 2012), addition to demographic, socio-economic and behavioral factors, service availability and cultural of the community might contribute for this variation. In this study, substance use in the last 12 months was 36.5% with alcohol been the most commonly used substance (20.9), and among alcohol user, 35.2% had risk sexual practice which is lower than the study in Pawe Benishangul Gumuz (38.7%) (Agajie et al., 2015) but higher than that of Haramaya 32.1% by Shore and Shunu (2017).

#### Factors associated with risky sexual practice

Decisions of sexual activity often occur simultaneously and substance use increases the probability to initiate sexual activities and had been globally associated with youth risky sexual behaviors (Bizu et al., 2015). In-line with other studies (Abosetugn et al., 2015; Gizaw et al., 2014), this study identify alcohol conception as factor for risky sexual behavior. This could be because risk perception ability decreases with alcohol consumption; those who use alcohol were more likely to practice risky sexual behaviors. Beside, this availability and accessibility of alcohol in local places for school youths might vary across setting.

Similar with studies conducted in Jimma, Addis Ababa, Haramaya and Humera (Tura et al., 2012; Nigatu and Seman, 2011; Shore and Shunu, 2017; Dadi and Teklu, 2014) exposure to sexual explicit media/pornographic was predictor that increases risky sexual practice. However other study in Addis Ababa fails to indicate statistical significant effect (Gizaw et al., 2014). Contradicting to these findings, other study in Haramaya indicate watching sex film as protective for risky sexual behavior Dingeta et al. (2012). The discrepancy might be due to positive and negative influence of watching sex film. Some of them may get experience on how to prevent risky sexual practice whereas other groups may be liable and need to enjoy what they observe in film.

Decision-making for sexuality in adolescent is a dynamic process that can be underestimated or overestimated by external force in which peer pressure is considered to have a significant influence on youth's sexual practices (McNeely and Blanchard, 2009). Survey conducted in Asia and Africa indicated that, young women and men are under strong social and peer pressures to engaged in risky sexual behavior (Blum et al., 2005) which is the same with studies conducted in Ghana and different parts of Ethiopia (Bizu et al., 2015; Dadi and Teklu, 2014; Dekeke and Sandy, 2014; Oluwatoyin and Oyetunde, 2014; UNAIDS, UNICEF, 2007; Cherie and Berhane, 2012; Shore and Shunu, 2017). This study revealed peer pressure as predictors

**Table 4.** Bivariate and multiple logistic regression analyses for risky sexual practice among school youth in Asella, South-East, Ethiopia, 2017.

Variable (n=598)	Risky p	oractice	COR (95%CI)	AOR(95%CI)	P- value
· · · · · · · · · · · · · · · · · · ·	Yes	No	· ·	· ·	
Sex					
Male	58	234	1	1	
Female	62	244	1.025(0.687,1.530)	1.060(0.700,1.581)	0.809
Level of education					
Grade 9	31	135	0.695(0.403,1.201)	0.722(0.415,1.256)	0.249
Grade 10	27	129	0.634(0.361,1.114)	0.648(0.365,1.149)	0.137
Grade 11	27	108	0.757(0.429,1.338)	0.774(0.434,1.381)	0.386
Grade 12	35	106	1	1	
Raised with					
Both parents	97	403	1	1	
By one parent	22	45	2.031(1.165,3.542)*	1.613(0.880,2.956)	0.122
Other peoples	1	30	0.138(0.019,1.028)	0.118(0.015,1.001)	0.052
Currently living with					
Both parents	85	317	1	1	
By one parents	18	82	0.819(0.466,1.438)	0.857(0.484,1.516)	0.596
Alone	16	56	1.066(0.582,1.951)	1.066(0.548,2.077)	0.850
Others	1	24	0.162(0.022,1.218)	0.167(0.022,1.274)	0.084
Family discussion on SRH					
Yes	31	186	1		
No	89	292	1.829(1.168,2.863)*	1.859(1.149,3.009)**	0.012
Parents know where and when you are					
Yes	74	291	1.034(0.685,1.560)	1.003(0.629,1.599)	0.989
No	46	187	1		
Ever seen/read pornographic					
Yes	82	240	2.14(1.400,3.271)*	1.599(1.010,2.529)**	0.032
No	38	238	1	1	
Peer pressure to had sexual intercourse					
Yes	89	170	5.202(3.318,8.154)*	4.360(2.748,6.917)**	0.0001
No	31	308	1	1	
Alcohol drinking					
Yes	44	81	2.838(1.825,4.413)*	1.976(1.224,3.190)**	0.005
No	76	397	1	1	

for risky sexual practice. This might be due to the fact that youths are eager to share their day to day life experience and they take majority of their time with their friends. However, study in Addis Ababa revealed that peer pressure had no statistical significant effect on risky sexual practice (Gizaw et al., 2014). This discrepancy might be due to negative and positive influence of peer

pressure as well as self efficacy of the study subject to ward external force.

A wide range of studies carried out across the world indicate that strict parental monitoring and open discussion with their child's on sexual issues is positively associated with reduced adolescent health risk, delayed sexual intercourse, fewer sexual partners and safe

sexual intercourse (Agajie et al., 2015).

Similarly, in this study, the odds of risky sexual practice were almost two times higher in those who did not discuss openly about SRH issues with parents compared to their counterparts which is supported by different researches elsewhere (Abosetugn et.al. 2015; Biddlecom et al., 2009; Shore and Shunu, 2017; Agajie et al., 2015; Kassa et.al., 2016; Ameh, 2013).

Therefore, parental control and monitoring helps to decrease probability of risky practice among youth through a protective influence and by educating their children about sexuality.

#### Strengths and limitations of the study

The study utilized primary data from real life of students that gave insight about risky sexual practice in school youth. The response rate of the study was high that decrease non response bias. The study was based on self-report, which is subject to errors and social desirability biases. In addition, the study arise very sensitive and private issues so possibility of underestimation cannot be ruled out. Since the study was on high school youth the results may not represent out of school youth. Due to cross-sectional nature of the study design it is impossible to determine the causal effect relationship.

#### CONCLUSION AND RECOMMENDATIONS

Considerable proportion of school youths were involved in risky sexual practice like early sexual initiation, having multiple sexual partners and inconsistence condom use which predispose them to sexual related health (SRH) problems. Peer pressure, alcohol drinking, no discussion about SRH issues and exposed to sexual explicit media/pornographic were identified as factors that increase the odds of practicing risky sexual practice. Ministry of education (MOE) should consider risky sexual behavior and substance use related issues in school curriculum. Health authorities including Ministry of Health (MOH) should implement youth friendly services in schools and government bodies should incise a strict and sustainable measurement against selling alcohol less than 18 years old.

Asella town health office should introduce effective education on SRH issues like substance use and risky sexual behaviors including sex at early age, unprotected sexual intercourse and multiple sexual partners in collaboration with Asella town education office. Parents should be encouraged to discuss openly about SRH issues with their children including risky sexual behavior, sexual explicit media and substance use. Since it assesses individual behavior other researcher should conducted a study with mixed study design.

#### **CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

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# Journal of Public Health and Epidemiology

#### Full Length Research Paper

# The characteristics of benign prostatic hyperplasia (BPH) in Rumah Sakit Umum Haji Medan

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Benign prostatic hyperplasia (BPH) is an enlarged prostate gland caused by progressive hyperplasia of glandular cells or stoma cells from prostate tissues. BPH is a common problem that increases in people from 40 years old. The symptom is lower urinary tract symptoms (LUTS) that consist of the followings: Strenuous urination, frequent urination, urinary hesitancy and retention. This study aims to know the characteristic of BPH patients in RSU Haji Medan from January to December 2015. Research design of this study is descriptive studies. The population of the study consists of patients who were diagnosed with BPH in RSU Haji Medan from January to December 2015. Total sampling method was used. Research data were retrieved by taking the patients' entire medical record with BPH in RSU Haji Medan from January to December 2015. 84 persons were diagnosed with BPH. The highest age group of the population is 60 to 70 years (34 people (40.5%). The most common complaint of the patients with BPH is the inability to urinate (42 people (50.0%). Common volume size of prostate is 40 to 60 g (people (38.1%). The most common therapy is transurethral resection of the prostate (TURP) (people (76.2%). The highest co morbidity is hypertension (20 people (23.8%). The patients that had BPH most in RSU Haji Medan were 60 to 70 years old; the chief complaint is not being able to urinate; common size of prostate volume is 40 to 60 g; the most common therapy that is used is TURP, and the highest co morbidity is hypertension.

**Key words:** Benign prostatic hyperplasia (BPH), older group, transurethral resection of the prostate (TURP), hypertension.

#### INTRODUCTION

Benign prostatic hyperplasia (BPH) is a benign enlargement of the prostate gland due to progressive glandular cells or cells of the prostate tissue stoma. BPH is the most common disorder experienced by men of over 40 years old (Purnomo, 2011; Arif, 2007; Glina and Felipe, 2013).

BPH would be a clinical condition if there are varieties

of symptoms in patients. These symptoms are known as lower urinary tract symptoms (LUTS) consisting of urinary symptoms (voiding symptoms) as follows: strenuous, repetitive urination, weak emission of urine and imperfect emptying of the bladder. It is also accompanied by symptoms of urinary retention (storage symptoms) urgency, frequency and nocturia (Arif, 2007).

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Although it in the urinary tract; he or she would often expel urine especially at night, and sometimes the urine cannot be held back. Disruption of the flow of urine would cause bacteria to easily replicate and stick to urotelium (Purnomo, 2011).

Factors affecting BPH is the background conditions of patients such as age, family history, obesity, increased blood cholesterol, a diet high in animal fat, exercise, smoking, alcohol, diabetes mellitus and sexual activity. The influence of age and prostate volume on symptom scores shows that there is a significant relationship between BPH and age (p <0.0001) (Presti, 2016).

Complications that occur in patients with BPH if not treated include trabeculation that is, the thickening of detrusor fiber due to very high intra- vesical pressure caused by obstruction and also the mucosa jar that comes out between detrusor fiber. If the saturation is enlarged, it would become a diverticle. Another complication is the formation of bladder stones from the accumulation of urine residue in jars. In Surakarta, there are 14 out of 15 BPH patients with a prostate volume of more than 20 cc that have residual urine, one of the factors that cause the formation of stones in the bladder (Presti et al., 2008). The world's estimated number of BPH patients is as much as 30 million. In the United States, there are more than 50% ofmen aged 60 to 70 years experiencing BPH, and between the ages of 70 to 90 (90%) experience symptoms of BPH (Presti et al., 2008).

In Asia, the prevalence of BPH increases from the age group 40 to 49 years, 50 to 59 years and 60 to 69 years (18%, 29% and 40% respectively). In Indonesia alone, there has been no definite incidence; but it was prevalent at two major hospitals in Jakarta, the RSCM and Sources Sane (1994-1997), with 1040 cases (Association of Indonesia's Urologists, 2009).

Patients with mild symptoms (for example, IPSS <7) should be counselled to modify their lifestyle and be watchful. Patients with mild and bothersome symptoms should undergo further assessment. Treatment options for patients with bothersome, moderate (for example, IPSS 8 to 18) and severe (for example, IPSS 19 to 35) symptoms of BPH include to be watchful and modification of lifestyle, as well as medical, minimally invasive or surgical therapies (Rizki, 2010). The same thing also happened in RSU Haji. The standard of treatment performed is dependent on the severity of the disease and most patients receive surgical treatment.

Based on literature studies conducted, researchers found no studies examining the characteristics of benign prostatic hyperplasia patients in Medan. Therefore, the researchers of this work were interested in conducting research on the characteristics of BPH patients in RSU Haji Medan from January to December 2015.

#### **METHODOLOGY**

This research is a descriptive study that aims to determine the characteristics of patients with BPH in RSU Haji Medan, from January to December 2015.

#### Time and place of research

This study was conducted from April to December 2016 in RSU Haji Medan with BPH sample data.

#### Population and sample

The population of the study was all patients with BPH diagnosed in RSU Haji Medan, 2015. Research samples were data from patients with BPH; the data were taken from medical record in Haji Hospital Medan from January to December 2015. Total sampling was done for the patients with BPH in RSU Haji Medan from January to December 2015.

#### Inclusion criteria

- (1) Patients diagnosed with BPH in medical records at RSU Haji Medan from January to December 2015.
- (2) Complete medical record data (age, main complaint, the size of the prostate volume of ultrasound results, the therapy carried and co morbidities).

#### **Exclusion criteria**

- (1) Patients with clinical and ultrasound results tending towards prostate cancer.
- (2) Having done surgery for BPH.

#### Data collection technique

Data collection was performed by taking secondary data of sufferers of BPH obtained from medical records of RSU Haji Medan, from January to December 2015.

#### Data processing

The data processing was done through the following stages:

- (1) Editing
- (2) Coding
- (3) Data entry
- (4) Cleaning
- (5) Tabulation

#### Data analysis

All data collected were processed and prepared in a frequency distribution table. Statistical test was done using computer application.

**Table 1.** Distribution of patients with benign prostatic hyperplasia in RSU Haji Medan based on their age.

Age (years)	Frequency	Proportion (%)
40-50	1	1.2
50-60	9	10.7
60-70	34	40.5
70-80	32	38.1
>80	8	9.5
Total	84	100

**Table 2.** Distribution of patients with benign prostatic hyperplasia in RSU Haji Medan based on main complaints.

Main complaint	Frequency	Proportion (%)
Being unable to urinate	42	50.0
Having difficulties to urinate	16	19.0
Painful urinate	15	17.9
Bleeding urinate	5	6.0
Lower abdominal pain, Weak emission of urine	3	3.6
Low back pain	3	3.6
Total	84	100

**Table 3.** Distribution of patients with benign prostatic hyperplasia in RSU Haji medan based on the volume size of their prostate through ultrasound.

Prostate volume size (g)	Frequency	Proportion (%)
20-40	30	35.7
40-60	32	38.1
60-80	10	11.9
>80	12	14.3
Total	84	100

**Table 4.** Distribution of patients with benign prostatic hyperplasia in RSU Haji Medan based on type of therapy.

Therapy type	Frequency	Proportion (%)
TURP	64	76.2
Medicaments	16	19.0
Open prostatectomy	4	4.8
Total	84	100

#### **RESULTS**

In Table 1, it is found that the highest numbers of people

**Table 5.** Distribution of patients with benign prostatic hyperplasia in RSU Haji Medan based on comorbidity.

Comorbidity	Frequency	Proportion (%)
Hypertension	20	23.8
None	19	22.6
Vesicolithiasis	14	16.7
Urethral Stricture	6	7.1
Inguinal Hernia	5	6.0
Nefrolithiasis	4	4.8
UTI	4	4.8
Urinary Retention	4	4.8
Dyspepsia	2	2.4
DM	2	2.4
Dyspepsia + Headache	1	1.2
Nefrolithiasis + Vesicolithiasis	1	1.2
UTI + Hypertension	1	1.2
Ureterolithiasis	1	1.2
Total	84	100

with BPH disease are those in the age group of 60 to 70 years (34 people (40.5%), followed by the age group of 70 to 80 years (32 people (38.1%)), the age group of 50 to 60 years (9 people (10.7%)), and the age group of 80 and above years (8 (9.5%)); the lowest age group is 40 to 50 years (1 person (1.2%)). In Table 2, it is found that the complaints most perceived by BPH patients include: inability to urinate (42 people (50.0%), difficulties in urinating (16 people (19.0%)), painful urination (15 people (17.9%)), bleeding urination (5 people (6.0%)); the lowest include: lower abdominal pain, weak urine emission and back pain (3 persons each (3.6%)). In Table 3, it is found that the prostate volume size observed by the sonogram is 40 to 60 (32 people (38.1%)), followed by 20 to 40 g (30 people (35.7%)), and 80 g (12 people (14.3%)); the lowest is 60 to 80 g (10 people (11.9%)). In Table 4, it is found that the therapies conducted most are: TURP (64 people (76.2%), followed by medicament (16 people (19.0%); the lowest is open prostatectomy (4 people (4.8%). In Table 5 it is found that the mostly found co morbidity is hypertension in as many as 20 people (23.8%).

#### **DISCUSSION**

From previous research findings, it is known that the highest numbers of people with BPH disease are those in the age group of 60 to 70 years (34 people (40.5%). Amalia (2010) also found that BPH patients are mostly within the age group of 60 to 69 years (44.2% (23)).

people) (Amalia, 2010). Lower urinary tract symptoms (LUTS) represent one of the most common clinical complaints in adult men. The prevalence of LUTS increases with age (Gravas et al., 2015; Fjellestad-Paulsen, 1993; Rembratt et al., 2004).

The major complaint found is not being able to urinate (42 people (50.0%). The obstruction caused by BPH is not only caused by the presence of prostate masses that clog the posterior urethra, but also due to the existing smooth muscle tone in the prostate stoma, prostatic capsule and smooth muscle of the bladder neck. If these conditions continue to occur then it is possible that there would be clogging of the lumen of the urethra, making the patient unable to urinate and will ultimately result in hydroureter, hydronephrosis, and consequently renal failure (Purnomo, 2011).

The prostate volume size is mostly 40 to 60 grams (32 people (38.1%)). The increasing size of the prostate volume depends on the underlying cause. One of them is the abnormal growth (hyperplasia) of the prostate that may be caused by local growth factors or the abnormal growth factor receptors, leading to increased proliferation or decreased cell death (apoptosis) (Tanagho, 2008).

The most widely performed type of therapy is TURP (64 people (76.2%). This result is supported by Roar (2015) study which showed that most patients with BPH underwent the TURP therapy and most frequently at the age of 73 to 77 years (24.6%) (Roar, 2015). Monopolar TURP remains the golden standard treatment for patients with bothersome, moderate or severe LUTS who request active treatment or who either fail or do not want medical therapy (Roar, 2015; Nickel et al., 2010; Hindley et al., 2001; Madersbacher et al., 2000; Roehrborn et al., 2007; Vesely et al., 2006; Fowler et al., 2005).

Bipolar TURP has evolved as an equivalent alternative to monopolar technique. Recent reports suggest bipolar resection is associated with a reduction in the risk of dilutional hyponatremia (TUR syndrome), improvements in intraoperative visibility and may result in shorter catheterization times (Nickel et al., 2010). The most comorbidity is hypertension (20 people (23.8%). This is supported by Kuspriyanti (2015) research which showed that most of BPH patients having elevated blood pressure in hypertension group amounted to 46.52% (Kuspriyanti, 2015).

From this study we can conclude that the highest number of people with BPH disease is in the age group of 60-70 years (34 people (40.5%); the major complaint is inability to urinate (42 people (50.0%); the prostate volume size is mostly 40 to 60 grams (32 people (38.1%)); the most widely performed type of therapy is TURP (64 people (76.2%)); the most co morbidity is hypertension (20 people (23.8%)).

From this study we expect that the society would now

know about the disease founnd in the prostate gland, particularly BPH. This disease has some symptoms such as being unable to urinate, halting urination or weak emission of urine, painful urination, bleeding urination, and even lower back pain. This disease sometimes also has similar symptoms with other diseases, namely Urinary Tract Stones, in which the patients often complain of halting and bleeding urination.

It is expected that BPH patients conduct further investigation to know the development of the disease such as knowing the size of the prostate volume and whether it is malignant or not, in order for them to prevent its recurrence.

#### **CONFLICT OF INTERESTS**

The author has not declared any conflict of interests.

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Journal of Public Health and Epidemiology

Full Length Research Paper

# Effects of some micronutrients on mice infected with *Plasmodium berghei*

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Malaria is a disease which is prevalent in tropical regions; however, it is preventable and curable with the use of certain micronutrients thereby reducing the life threatening disease. The effect of micronutrients (vitamin C, calcium, iron and magnesium) on mice infected with Plasmodium berghei NK-65 species was evaluated using standard methods. The percentage suppression and the parasitemia levels were counted daily to evaluate the effect of the micronutrients given intraperitoneally to the infected mice. The results show that vitamin C and calcium caused a significant decrease in the parasitemia count from 529 to 425/field and from 533 to 441/field respectively, while iron and magnesium caused significant increase in parasitemia level from 516 to 592/field and 528 to 709/field respectively. The packed cell volume increased in the mice treated with calcium, iron and vitamin C while there was significant decrease in the mice treated with magnesium. Red blood cell increased in the mice treated with iron. The white blood cell was reduced in the mice treated with iron and vitamin C but no significant change in the white blood cell of the mice treated with calcium but there was increase in the white blood cell of the mice treated with magnesium. The biochemical components of the blood from treated mice revealed that sodium significantly increased in the mice treated with the micronutrients with the highest sodium recorded in the mice treated with Iron. Therefore, foods or drugs that are rich in iron and magnesium should not be taken when treating malaria as they will aid parasite multiplication.

**Key words**: Micronutrients, *Plasmodium*, parasitemia, mice, blood.

#### INTRODUCTION

Malaria is a preventable and curable disease, yet it remains a devastating tropical disease, with high infection and mortality statistics. Malaria, a life threatening disease is caused by a parasitic infection of the red blood cells by *Plasmodium* parasites transmitted through a bite of the female *Anopheles* mosquitoes. This disease is prevalent

in tropical and subtropical regions and is mostly associated with poverty. Clinical symptoms of malaria include headache, fever, chills and vomiting which are usually mild but if not treated immediately could lead to delirium, metabolic acidosis, cerebral malaria and multiorgan system failure (lyiola et al., 2011). World Health

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Organization (WHO) estimates that each year, more than 200 million people are infected with malaria worldwide (WHO, 2016). There were 214 million cases of malaria worldwide in 2015; 90% of which occurred in Sub Saharan Africa. Out of these, 438,000 resulted in death globally of which 78% were children under the age of five (WHO, 2015).

Plasmodium berghei infection of laboratory mouse strains is frequently used in research as a model for human malaria because of its similarity to the Plasmodium species which cause human malaria. P. berghei has a very similar life-cycle to the species that infect humans, and it causes disease in mice which have signs similar to those seen in human malaria. More importantly, P. berghei can be genetically manipulated more easily than the species which infect humans, making it a useful model for research into Plasmodium genetics (David et al., 2004).

Micronutrients are vitamins and minerals needed by man in small quantities for good health. They include vitamin C (ascorbic acid), iron, magnesium, calcium, etc (Sies et al., 2015).

Iron is involved in numerous biological processes. It is the most important transition metal in all living organisms (Conti et al., 2010). Vitamin C is a cofactor in many enzymatic reactions, including several collagen synthesis reactions that, when dysfunctional, cause the most severe symptoms of scurvy. In animals, these reactions are especially important in wound-healing and in preventing bleeding from capillaries (Murray et al., 2013). Magnesium (Mg) is the second-most abundant cation in cellular systems. It exerts a large variety of biological functions, ranging from structural roles by forming complex with negatively charged groups such as phosphates in nucleic acids, control role in enzyme activation or inhibition, and regulatory roles by modulating cell proliferation, cell cycle progression and differentiation (Tam et al., 2003).

#### **MATERIALS AND METHODS**

#### Parasites strain

*In vivo* antimalarial testing in mice was done using chloroquine sensitive strain of *P. berghei* (donated by the Animal Unit of the Institute for Advanced Medical Research and Training, College of Medicine, UCH, Ibadan). The parasites stock was maintained by continuous re-infection in the mice.

#### Dosage calculations

According to the Organization of Economic Corporation and Development's (OECD) guidelines, dosage of drug (mg) should be constituted in an appropriate volume not usually exceeding 10 ml/kg (1 ml/100 g) body weight of experimental animals (mice and

rats) for non-aqueous solvent in oral route of administration (OECD, 2001). Mice collected were between 20 to 25 g, hence the dosage of drug according to standard should not exceed 0.2 ml

Dosage (mg) = [Average weight of mice (g)  $\times$  Dose (mg)] / 1000 g

#### Infection of mice

The parasitized erythrocytes for each test were collected from an infected donor mouse with rising parasitemia of 20 to 30%. The mice were sacrificed by head blow, and blood was collected in a Petri dish with an anticoagulant (0.5% trisodium citrate) by severing the jugular vein. The blood was then diluted with physiological saline (0.9%) in proportion of 1:4. Each mouse in every group was then inoculated with 0.2 mL of blood containing about  $10^7\ P$ . berghei infected erythrocytes on day 0 through intra peritoneal route. This was prepared by calculating the percentage of parasitemia of donor mouse and diluting the blood with physiological saline so that 0.2 ml of diluted blood contained 1 x  $10^7$  infected erythrocytes.

#### Suppression

The mice were divided into five groups in which three mice are in each group (A, B, C, D and E (control)) with groups A to D receiving 10 mg/kg of Calcium, Magnesium, Iron, and Vitamin C respectively. The animals in the control group (E) received placebo (saline water). The antimalarial activity of the micronutrients was determined using the Peter's 4-day suppressive test (David et al., 2004).

# Determination of mean weight and temperature changes of the mice

The weights of the mice were measured daily using a sensitive weighing balance to monitor the change in weight of mice. Rectal temperature was also measured with a digital thermometer before infection, and then daily. All the control groups and malaria-infected mice were observed visually throughout the experiment for behavioural changes which include diarrhoea, lethargy, reduced activities, sleeplessness and loss of appetite.

#### Parasitemia count

On Day 4 after infection, a thin smear of blood film was taken from the peripheral blood of the tail of each mouse in the test and control groups. The smears were fixed with methanol and then stained with Giemsa stain. Thereafter, each stained slide was microscopically examined under oil immersion of 1000 magnification power (100x) to evaluate the mean percentage (%) of parasitemia and suppression of each fraction in comparison with control group. The mean parasitaemia was calculated and expressed as follows:

% Parasitemia = 
$$\frac{\text{Total number of Parasitized red blood cells}}{\text{Total number of Red blood cells}} \times 100$$

Percentage parasitaemia suppression was calculated according to the following formula:

**Table 1.** Effect of the micronutrients on parasitemia count in infected mice.

S/N	Micronutrient	Before treatment	During treatment	After treatment	% Suppression/increase
1	Vitamin C	529/field	468/field	425/field	20
2	Iron	516/field	553/field	592/field	15
3	Calcium	533/field	492/field	441/field	17
4	Magnesium	528/field	640/field	709/field	34

#### Preparation of inoculum

Donor *P. berghei* infected mice were anesthetized and the blood was collected by heart puncture. Heparinized blood is taken from a donor mouse, diluted with 5 ml of phosphate buffer solution (PBS) and adjusted to pH 7.2 so that each 0.2 ml contained approximately  $1\times10^7$  infected red cells (David et al., 2004). An aliquot of 0.2 ml of this suspension was injected intra-peritoneally into the experimental mice and treated with micronutrients orally into experimental groups.

#### Determination of mean survival time

Mortality was monitored daily and the number of days from the time of inoculation of the parasite up to death was recorded for each mouse in all groups throughout the follow up period.

The mean survival time (MST) for each group was calculated as follows:

$$MST = \frac{Sum \text{ of survival time of all mice in a group (days)}}{Total \text{ number of mice in that group}}$$

#### **Biochemical tests**

Biochemical tests such as determination of bicarbonate, creatinine, calcium, uric acid and urea level were carried out following the standard method of Baker et al. (2014).

#### Liver functioning tests

Liver functioning test (LFT) such as the total bilirubin, serum total protein, serum albumin, serum globulin and alkaline phosphate were done according to the method of Cheesbrough (2014) to ascertain the physiological status of the liver.

#### Haematological tests

Haematological tests such as packed cell volume (PCV), hemoglobin (HB), red blood cells (RBC), erythrocyte sedimentation rate (ESR) and white blood cells (WBC) differential count were done following the standard method of Cheesbrough (2014).

#### Statistical analysis

Statistical data are presented as mean  $\pm$  SE (standard error). Significance of difference between different treatment groups was tested using one-way analysis of variance (ANOVA) and significant results were compared with Duncan's multiple range tests using SPSS window 10 version 21 software. For all the tests, the significance was determined at the level of P<0.05.

#### **RESULTS**

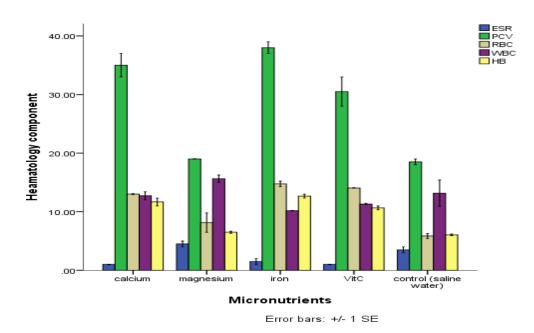
Effect of the micronutrients on the parasitemia count per field showed that magnesium has the highest increase in the number of parasites in the blood from 528 to 709/field. The administration of iron also caused an increase in the parasitemia count in the infected mice. However, administration of vitamin C and calcium caused a reduction in the parasitemia count as shown in Table 1.

Figure 1 reveals the effect of micronutrients on the haematological parameters. It revealed that erythrocyte sedimentation rate significantly (p<0.05) reduced in the mice treated with the micronutrients compared with the control. Also, the packed cell volume increased in the mice treated with calcium, iron and vitamin C while there was no significant (p<0.05) increase in the mice treated with magnesium. Red blood cell increased in the mice treated with micronutrients and those treated with Iron. The white blood cell counts reduced in the mice treated with iron and vitamin C but no significant change in the white blood cell of the mice treated with calcium but there was increase in the white blood cell of the mice treated with magnesium. There was significant (p<0.05) increase in the haemoglobin of all the mice treated with micronutrients compared with the control group.

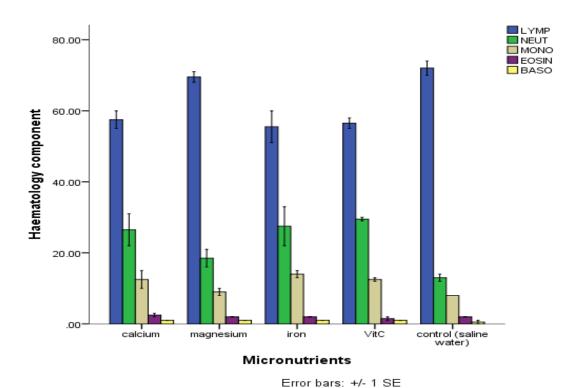
Figure 2 reveals the effect of micronutrients on white blood cell differential count as all the micronutrient used (Ca, Mg, Fe and Vitamin C) caused a decrease in the lymphocyte count in the experimental mice. While the control group recorded lymphocyte count of 75%, the group treated with iron recorded a lymphocyte count of 55%. The micronutrients also caused an increase in the neutrophils to 25% when compared to the count in the control (10.2%). The same pattern was observed for monocytes. There was however no significant (p<0.05) increase in the eosinophils and basophils.

Figure 3 shows the effects of micronutrients on the biochemical components of the blood. The result reveals that sodium significantly (p<0.05) increased in the mice treated with micronutrients and highest sodium was observed in the mice treated with iron at a value of 150 mg.

There was no significant change in the potassium of the treated mice. The chlorine decreased in the mice treated micronutrients with the most significant decrease recorded in the mice treated with vitamin C and iron; also, there was no significant change in the urea of the mice



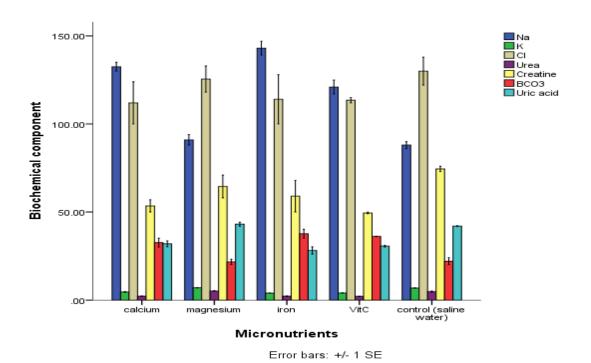
**Figure 1.** Effect of micronutrients on the biochemical parameters of infected mice. ESR = Erythrocytes sedimentation rate; PCV = Packed cell volume; RBC = red blood cell count; WBC = White blood cell count; HB = Haemoglobin.



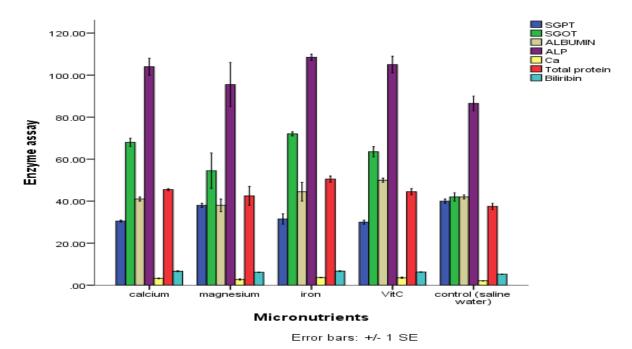
**Figure 2.** Effect of micronutrients on the WBC differential parameters of infected mice. LYMP = Lymphocytes; NEUT = Neutrophills; MONO = Monocytes; EOSN = Eosinophills; BASO = Basophill.

treated with micronutrients. The creatine in the blood component of the mice treated with micronutrients decreased, whereas the bicarbonate increased. A

decrease in the uric acid was recorded in the mice treated with the micronutrients except in the mice treated with magnesium where a slight increase in the uric acids



**Figure 3.** Effect of micronutrients on the haematological parameters of infected mice. Na = sodium (mmole/L), K = potassium (mmole/L), BCO<sub>3</sub> = bicarbonate (mmole/L).



**Figure 4.** The effect of micronutrients on the liver functioning parameters of infected mice. SGPT = Serum glutamic pyruvic transaminase; SGOT = Serum glutamic-oxaloacetic transaminase; ALP = Alkaline phosphate; Ca = Calcium.

#### was recorded.

Effects of the micronutrients on the function of liver in the mice are shown in Figure 4. The result reveals that while there was slight increase in the serum glutamic pyruvic transaminase of the mice treated with magnesium, there was significant (p<0.05) decrease in the mice treated with calcium, iron and vitamin C. There was an increase in the serum glutamic oxaloacetic

transaminase in all the mice treated with micronutrients except in the mice treated with magnesium for which was recorded a decrease in serum glutamic oxaloacetic transaminase recording a value of 55 mg/ml compared with iron which had 75 mg/ml. The albumin was increased in the mice treated with iron, calcium and vitamin C while there was a slight decrease in the albumin of the mice treated with magnesium. There was an increase in the alanine aminotransferase of the micronutrients treated mice, but the bilirubin level did not increase significantly when compared with the control.

#### DISCUSSION

The effect of the micronutrients on the parasitemia count per field revealed that magnesium caused the highest increase in the number of parasites in the blood which is a clear indication that magnesium containing compounds should not be taken when treating malaria. Although there is no written document on the use of magnesium containing compounds when treating malaria, compounds such as magnesium trisilicate prolong malaria infection and should not be taken along some antimalarial drugs as it may cause complications (WHO, 2016).

The administration of iron also caused an increase in the parasitemia count in the infected mice. Iron (Fe) is one of the essential nutrients required by Plasmodium for proliferation and lysing of red blood cell which often lead to anaemia (Ohnishi and Ohnishi, 2011; Sies et al., 2015). Although the main role of iron-containing proteins are; transfer of electrons, transport and storage of oxygen, iron is a necessary trace element found in nearly all living organisms. Iron-containing enzymes and proteins, often containing heme prosthetic groups, participate in many biological oxidations and in transport, hence it is advisable for everyone, most especially the pregnant women not be given forlates during the antenatal to reduce malaria burden (WHO, 2016), rather they should be encouraged to take foods containing iron such as beans, nuts, fish, liver and sea foods.

The effect of micronutrients on the haematological parameters which showed the erythrocyte sedimentation rate to be significantly reduced in the mice treated with the micronutrients compared with the control has shown that the presence of calcium can increase ESR value. Also, the packed cell volume increased in the mice treated with calcium, iron and Vitamin C while there was no significant increase in the mice treated with magnesium. Red blood cell increased in the mice treated with iron than in other groups. This may be due to the fact that presence of iron normally aids the proliferation and production of red blood cells. The white blood cell was reduced in the mice treated with iron and vitamin C, two micronutrients that have been known to boost immunity of animals against parasites (Cheesbrough, 2014).

Evaluation of the effect of the micronutrients assayed for in the white blood cell differential count revealed that all the micronutrient used (Ca, Mg, Fe and vitamin C) caused a decrease in the lymphocyte count in the experimental mice, which is an indication that these micronutrients actually boost the immunity of the mice. Equally, the complementary effects of iron on the animals can be seen in the lymphocyte count as well as the neutrophils which are well reduced when compared with the control.

The effects of micronutrients on the biochemical components of the blood from treated mice have shown that micronutrients caused an increase in sodium level. This may result from the fact that the calcium, iron and vitamin C particularly increased sodium well above the values obtained in the control group. Urea, creatine and bicarbonate are end products that results from metabolized nutrients from the body of an animal (Brooks et al., 2014). Therefore, the ability of some of these micronutrients to reduce the parasitemia count in these mice might be by increased metabolic activity including generation of energy to combat the *Plasmodium* parasites.

There was no significant change in the urea of the micronutrient treated mice which may be because there was no damage to the kidney. The creatinine in the blood component of the mice treated with micronutrients decreased whereas the bicarbonate increased. A decrease in the uric acid was recorded in the mice treated with the micronutrients except in the mice treated with magnesium where a slight increase in the uric acids was recorded.

Effects of the micronutrients on the liver functioning tests of the treated mice revealed that there was slight increase in the serum glutamic pyruvic transaminase of the mice treated with magnesium while there was significant decrease in the mice treated with calcium, iron and vitamin C. This may be because magnesium is not easily excreted from the body of animals by the liver when taken in excess, hence the need to always check their level in water and foods. There was an increase in the serum glutamic oxaloacetic transaminase in all the mice treated with micronutrients except in the mice treated with magnesium for which was recorded a decrease in serum glutamic oxalo-acetic transaminase. This result is an indication of good working condition of the liver (Momoh et al., 2013).

#### Conclusion

The results obtained in this research therefore have shown that iron and magnesium taken either as drugs or food supplement causes increase in parasitemia level thereby aggravating the malaria and complicating it. On the other-hand, vitamin C and calcium, when taken in malaria cases can help fight the malaria parasite and

reduce the parasitemia level. People in malaria endemic area should be given orientation on the type of food to be consumed while treating malaria.

#### **CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

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