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HEPATITIS B VIRUS DISTRIBUTION AND ASSOCIATED RISKS FACTORS IN SOME SELECTED RISK GROUPS IN ENUGU METROPOLIS

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Abstract: The Hepatitis B virus (HBV) causes liver infection that can be life–threatening and often leads to chronic liver disease, liver cirrhosis and liver cancer. A cross sectional study was conducted to determine Hepatitis B virus distribution and associated risk factors among some high-risk groups in Enugu Metropolis. A total of 518 subjects were sampled over a period of 2 years, from January 2013 to January 2015. The subjects consist of 233 blood donors, 164 pregnant women, 37 diabetic patients, 62 HIV patients and 22 sickle cell patients. Approximately 5 ml of blood was aseptically collected from each volunteer and analyzed for hepatitis B surface antigen (HBsAg). All HBV positive patients were analyzed for liver enzymes, alanine transaminase (ALT) and aspartate transaminase (AST). Patients co-infected with HIV were analyzed for CD4+ lymphocyte count. Out of the 518 samples, 45 (8.7%) were positive for HBsAg. The prevalence of HBV was highest among HIV patients 16 (25.8%), followed by blood donors 20 (8.6%), pregnant women 9 (5.5%) and 0% for both sickle cell and diabetic patients. Higher prevalence of HBsAg was found among males 25 (10.2%) than females 20 (7.3%). Out of the 45 HBsAg positive patients, 8 (17.8%) had high ALT levels, while 9 (20.0%) had high AST levels. Abnormal levels of transaminases (ALT and AST) were more in HIV patients (43.8%) than in pregnant women (22.2%) and blood donors (10%). All patients co-infected with HIV-HBV had CD4+ lymphocyte count of less than 1000 cells/µl even though they were on combination antiretroviral therapy. The risk factors of HBV in this study were multiple sex partners, age and unemployment (P=0.000), (P=0.045) and (P=0.014) respectively. There was high prevalence of HBV among HIV patients and blood donors and an intermediate prevalence among pregnant women in Enugu Metropolis. A vaccination programme targeting these risk groups is highly recommended.

Keywords: Hepatitis B virus, HBsAg, transaminases (ALT, AST), HIV patients, blood donors, pregnant women.

Introduction

Hepatitis B virus (HBV) is one of the most common and serious viral hepatitis and continue to be a major health problem throughout the world[1-3]. Sub-Saharan Africa, including Nigeria is one of the regions with significant burden of HBV. Countries can be defined as having a high, intermediate or low prevalence HBV infection based on a prevalence of HBsAg carrier of 8% and above, 2-7%, and

less than 2% respectively[4]. In areas of high endemicity such as sub-Saharan Africa, with average carrier rate of 10 – 20% in the general population, transmission during childhood is a significant factor. There is a high level of horizontal transmission at this period in life, which has been attributed to the high rate of playing and sharing of toiletries and sharps including cutting, scraping or scratching objects in the region. As such, HBV infection commonly occurs in childhood in Africa resulting in an increased tendency to chronicity[5]. Equally, studies show that vertical/perinatal transmission is the major route of transmission especially in rural areas. It has been established that chronic infection with HBV occurs in 90% of infants infected at birth, 30% of children infected at 1 – 5 years, and 6% of persons infected above 5 years[6]. The high prevalence of chronic HBV infections in areas of high endemicity makes it a major cause of morbidity and mortality in those areas[1,7-8]. Three major phases: immune tolerant, immune active and inactive carrier phases exist in chronic HBV infection. The immune tolerant and immune active carriers are normally hepatitis B e antigen (HBeAg) positive, while the inactive carriers are HBeAg negative[9]. Chronic hepatitis characterized by negative HBeAg may be associated with elevated aminotransferase and continuous necro-inflammation[10]. Alanine transaminase (ALT) is the most common enzyme used in evaluation of liver disease. Studies have shown that minimal increase in serum ALT is significantly associated with an increased risk of liver-related mortality in the general population[11]. Despite the introduction of birth dose HBV vaccine in 2004 and adoption of a $3 billion Nigeria Strategy on Immunization and PHC System Strengthening, 2018-2028, in Nigeria, recent seroprevalence studies by Ifeorah *et al*.[12,13], Bakarey *et al*.[14], Okoroiwu *et al*.[15], Omatola *et al*.[19], Mustapha *et al*.[17] and Oluremi *et al*.[18,19] on HBV have indicated yet high prevalence of HBV infection in the country. Other studies have equally recorded high prevalence of the disease in different parts of the country[20,21]. Being a blood-borne virus[22], people who require frequent medical care and those constantly exposed to blood such as surgeons[23], blood donors[15,22,24], sickle cell anaemia patients[5] and many more, are at increased risk of HBV infection through exposure to contaminated blood or body fluids[25]. Also individuals with reduced immunity, such as pregnant women[13,26] and the immunocompromised, such as HIV patients[16,21] are also at increased risk of HBV infection, and more worrisomely, co-infected individuals are 17 times more likely to die of liver-related diseases than those infected with only HBV[27]. The WHO Global Hepatitis reports 2017, reported that the 194 Member States of World Health Organization is committed to eliminating viral hepatitis as a health threat by 2030 (defined as a 65% reduction in mortality and a 90% reduction in incidence compared with the 2015 baseline). Prior to that, in May 2016, the World Health Assembly adopted the Global Health Sector Strategy (GHSS) on viral hepatitis for 2016-2021[28]. This study was carried out to determine the prevalence of HBV among some high risk groups in Enugu metropolis of Enugu State, Nigeria. In addition, the transaminases enzymes among HBV positive individuals were analyzed and the CD4+ count and associated risk factors of the HIV positive patients were determined.

Materials and Methods

A total of 518 subjects, comprising of 233 blood donors, 162 pregnant women, 37 diabetic patients, 62 HIV patients and 22 sickle cell patients were sampled from January 2013 to January 2015. Blood samples were collected from all the major hospitals in Enugu metropolis including the two teaching hospitals in the state, namely University of Nigeria Teaching Hospital Ituku-Ozalla, Enugu and Parklane Teaching Hospital Enugu. Others were, National Orthopedic Hospital Enugu (a specialist hospital), 82 Division Military Hospital Enugu (a military hospital) and three private hospitals: Prime Care Hospital Trans-Ekulu Enugu, Mother of Christ Hospital Enugu and Kene Hospital Trans-Ekulu Enugu, all in Enugu State, Nigeria.

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Sample Collection: Blood samples (5 ml) was aseptically collected from each volunteer using a sterile syringe and needle from a prominent vein situated at the antecubital fossa of each individual and placed in 5 ml plain bottle. The serum of each sample was separated from the whole blood after clotting and centrifugation and stored at -20°C until tested.

Data Collection and Processing: Questionnaire was administered to the subjects and used to identify social behavioural patterns and demographic characteristics associated with HBV infection.

Confirmation of Status of Patients: Diabetic patients had their diabetic status confirmed by laboratory diagnosis. Fasting blood sugar was estimated for each diabetic patient using Accu check glucometer (Intermed, Vlaeberg, South Africa). The blood samples were collected after over-night fasting. Diabetic condition was inferred if the glucose level obtained was above the upper limit of the normal range (> 120 mg/dl). HIV patients were those confirmed HIV Positive using Determine HIV test strips (Alere Medical, Japan) and Unigold HIV test devices (Trinity Biotech, Bray, Ireland). The genotypes of sickle cell patients were reaffirmed using the SOWEAM electrophoresis method (Soweam Electrophoresis Equipment, UNN). All samples were ran with controls of known AA, AS and SS blood samples. Pregnant women were sampled while attending antenatal clinics. The Blood donors were sampled after undergoing the routine examination required that certify an individual fit to donate blood except for HBV status that is not routinely determined.

Laboratory Testing / Examination: CD4 count was determined using the BD Facscount system with the BD FACSCOUNT CD4/CD3 reagent kit, following the manufacturer's instructions. Briefly, whole blood collected from patients who tested HIV positive were introduced into k_2EDTA vacutainers which were placed on mixer to homogenize. Each reagent tube was vortexed upside down for 5 s and upright for 5 s on a vortex machine then opened with a coring station. The blood was mixed adequately by inverting the vacutainer 5 to 10 times. After mixing, 50 µl of whole blood was dispensed into the reagent tube, capped, vortexed upright for 5 s and incubated for 60 min. After incubation, 50 µl of fixative solution was added into each tube and vortexed. Each tube was then placed on the BD FACSCOUNT system for counting of the CD4 cells.

The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in each of the tested serum sample were determined using EliTech AST/GOT 4+1 SL and Elitech ALT/GPT 4+1 SL respectively (Seppim, Sees, France) according to the manufacturer's instruction. Four volumes of the Reagent 1 (AST: Tris buffer, pH 7.80 (100 mmol/l), L-Aspartate (330 mmol/l), LDH (2000 u/l), MDH (1000 u/l); ALT: Tris buffer, PH 7.50 (120 mmol/l), L-alanine (680 mmol/L), LDH (2000 u/l)) were mixed with 1 volume of reagent 2 (AST: x-ketoglutarate (78 mmol/l), NADH (1.1 mmol/l); ALT: x-ketoglutarate (Lactate dehydrogenase) (97 mmol/l), NADH (1.1 mmol/l)) to make the working reagent for both AST and ALT respectively. After mixing in appropriate proportion (400 µl of working reagent and 40 µl of serum sample), the resultant mixture was incubated for 50 s. The change of absorbance per min during 175 s at wavelength 340 nm was measured using Microlab 300 automated machine.

HBV surface antigen (HBsAg) was detected by ELISA method using Monolisa HBsAg ultra assay (Bio-Rad 3, Marnes-la-coquette, France). The test kit consists of 96 well micro titer plate coated with monoclonal anti-HBs antibodies (mouse), with other required reagents. Test was performed according to the manufacturer's instructions. Briefly, 100 µl each of negative controls, positive controls and serum samples were added to the appropriate wells. Then, 50 µl of conjugate solution (peroxidase conjugated to anti-HBs) was quickly added into all wells and incubated for 1 h and 30 min in a water bath at 37°C. After incubation, the wells were emptied by aspiration and washed 5 times with 300 µl of washing solution (Tris NaCl buffer pH 7.4). A 100 µl of freshly prepared development

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solution was added into each well and incubated in the dark for 30 min at room temperature. At the end of the incubation, 100 µl of stopping solution (1N H_2SO_4) was added to each well, allowed to stay for 4 min and read at 450 nm using the plate reader (Hyperion Diagnostics, Germany). The negative control mean optical density and the cut-off value were calculated. Then the ratio of each sample was calculated. Samples with ratio values < 1 are considered to be negative by Monolisa HBsAg ultra, while samples with ratio values ≥ 1 are considered positive.

Results

Distribution of HBV infection in Different sample population.

Out of the 518 subjects tested for the presence of hepatitis B virus in Enugu, Nigeria, 45 were positive for HBV (Table 1), yielding an overall prevalence of 8.7%. The highest prevalence of HBV was among HIV patients (25.8%; N = 62), followed by Blood donors (8.6%; N = 233) and pregnant women (5.5%; N = 164). Both the sickle cell and diabetic patients showed 0% prevalence for HBV. Distribution according to sex (Table 2) showed that 10.2% (N = 245) of the males tested positive for HBV, while 7.3% (N = 273) of the females tested positive. The highest prevalence of HBV among males was in the age group 26-35 yr (11.7%; N = 197), while among females the highest prevalence was in the 36-45 yr age group (23.1%; N = 13). Overall, the highest prevalence of HBV was found in the age group 36-45 yr (11.1%; N = 45) (Table 2). The age distribution of HBV among HIV patients showed highest prevalence (39.3%; N = 28) among the age group 26- 35 yr, while all the HBV found among pregnant women and blood donors were in the age group 26-35 yr (results not shown).

Among the blood donors that tested positive for HBV, 2 (10.0%; N = 20) had abnormal (raised) levels of transaminases, with 1 patient each having a raised AST and ALT levels respectively. Among the HIV patients that were co-infected with HBV, 7 (43.8%; N = 16) had raised levels of transaminases, with all 7 having raised AST levels, while 5 had raised ALT levels (Table 3). Among the pregnant women that tested positive for HBV, 1 (11.1%; N = 9) had abnormal transaminases and the patient had raised values of both parameters (Table 3). Comparison of the transaminases between the HBV infected and non-infected patients showed that there was a statistical difference in both ALT and AST values between the two groups, with a P value of 0.011 and 0.001 for ALT and AST respectively (Table 4).

The $CD4^+$ count of the HIV patients infected with HBV showed that they all had a count lower than 1000 cells/µl, while 4 had counts of less than 200 cells/µl, with all 4 being below 100 cells/µl. All the 4 patients with $CD4^+$ counts less than 100 cells/µl had raised levels of one or both transaminases. Out of the 16 patients, only 1 patient was yet to be introduced to antiretroviral (ARV) drugs and she had the highest count of 999 cells/µl (Table 5). Table 6 shows that there was a statistical difference (P = < 0.001) between the $CD4^+$ count of HIV/HBV positive patients and the HIV/HBV negative patients. The risk behaviour pattern showed that having multiple sex partners was highly associated with HBV positivity with a P value of < 0.001 (Table 7). The demographic characteristic showed that being married, unemployment and age were all associated with HBV positivity with P values of <0.001, 0.014 and 0.045 respectively. Only educational status showed no association with HBV positivity (Table 8).

Discussion

This study shows that the prevalence of Hepatitis B virus in Enugu metropolis is high at 8.7%. An endemicity of >8% HBsAg, usually associated with high level of chronic liver disease as earlier stated, is common in Sub-saharan African countries like Nigeria[4], and a systematic review and meta-analysis on the prevalence of HBV in Nigeria from 2000-2013 has shown a pool prevalence of 13.6%[23]. This signifies a substantial health burden to the States and the country as a whole. The 8.7% HBsAg seroprevalence recorded in this study, however, is lower than the 12.2% national prevalence estimated from apparently healthy

Nigeria general population by Olayinka *et al*.[29] and 12.0%[30] in Benue state population. The prevalence obtained is very close to the 8.3% that was reported in Nnewi, Anambra State, a bordering state in the same zone[8]. Also, the HBsAg prevalence rate in this study is slightly higher than 7.3% obtained from asymptotic and low income community in Ibadan[31]. Sub-group prevalence analysis on high risk population groups in this study shows that the highest prevalence of HBsAg was among HIV patients (25.8%; N = 62), followed by Blood donors (8.6%; N = 233) and pregnant women (5.5%; N = 164), while both the sickle cell and diabetic patients showed 0% prevalence for HBsAg. The prevalence rate of 25.8% of HBsAg among HIV/HBV co-infected patients in this study is lower than 30.6% obtained by Ogwu-Richard *et al*.[32] and 37% obtained by Olawumi *et al*.[33], but slightly higher than 20.6% recorded by Forbi *et al*.[34]. However, lower prevalence of HBsAg of 3.5%[16], 10.3%[12,35], 8.0%[36], 7.9%[37], 6.02%[38], 6.43%[39], 8.5%[27], 6.6-6.7%[40,41], 7.8%[42] and 2.2%[43] among HIV positive patients have been reported in various studies carried out in different States across the country. From the forgoing, HBV/HIV coinfection among HIV patients in these studies reaffirmed the high prevalence of HBV among HIV patients and thus is the major cause of liver-related diseases and deaths among these patients, and therefore the need for HBV/HIV combination antiretroviral therapy no matter the stage of the disease[28]. Furthermore, the 8.6% HBsAg prevalence among Blood donors recorded in this study is lower than 19.6%[44], 31.71%[45], 10%[24], and 9.8%[22] reported from studies carried out at different blood donaton units in the country. Jeremiah *et al*.[46] reported the same 8.6% HBsAg prevalence among blood donors in the semi-arid part of the country, while Okoroiwu *et al*.[15] after retrospective study (from 2005-2016) to determine the trend of HBsAg among blood donors record a gradual decrease in HBsAg prevalence, with an overall HBsAg prevalence of 4.1%. The blood donors in this study constitute mostly relatives of patients requiring blood transfusions in the hospitals studied. It is therefore not surprising that the prevalence in this group is very close to the high prevalence in the total study population. Thus, the level of prevalence in blood donors highlights the need for proper and adequate screening of blood before transfusion. The discrepancies in the prevalence of HBsAg can be attributed to differences in the target groups studied, screening methods, as well as differences in the level of health care facilities, integration of HBV vaccination and cultural differences. Several reasons could be contributing to the high endemicity of HBV in the country including inaccessibility of HBV vaccine and/or negative attitudes towards HBV vaccination by the general public and lack of well-organized and coordinated preventive and control measures. The 5.5% prevalence of HBsAg among pregnant women is lower than 6.7% in Gamawa, Bauchi[17], 16.5% in Osogbo[47], 7.3% in Lagos[48] and 8.3% in Southwestern Nigeria[49]. However, Ifeorah *et al*.[13] recorded same 5.5% HBsAg among pregnant women in Southwestern Nigeria. The absence of HBsAg in diabetic and sickle cell population studied may not be unconnected with the low number of individuals in these target groups. Unfortunately, Jibrin *et al*.[5] and Emechebe *et al*.[50] reported 17.3% and 8.1% HBsAg prevalence, respectively, among children with sickle cell anaemia. Also, Ndako *et al*.[51] reported 13.3% HBsAg among type 2 diabetic patients. Additionally, Iroezindu *et al*.[52] in their studies, have revealed that HBV in diabetic patients causes Impaired Fasting Glucose (IFG). The higher HBV prevalence among young adults in this study is a support of the role of sex in HBV transmissions, as this group is known to be more sexually active, usually < 39 years. This is in agreement with the findings of other studies[35, 39, 49]. It also gives credence to the risk analysis in this study that found having multiple sex partners to be highly associated with HBV infection, lending a voice to the call for discretion in sexual relationships. The results of this study show that males had higher prevalence of infection than females. This may be connected with the fact that men are more

outgoing and more active both physically, resulting in higher accidents, and socially leading to more sexual activities. Other studies have reported similar findings[36,39,48,49]. Furthermore, this study, the HBV infected patients had higher raised transaminases compared to uninfected individuals, a confirmation of the effect of the virus on liver. The study also found that the highest rise of tramsaminases was on HIV infected individuals. This is in agreement with the finding that HIV exacerbates the effect of HBV on the liver[33,52,53]. Although there was no attempt in this study to check for further liver complications such as liver cirrhosis and HCC, it is well documented that HIV accelerates the progression of liver to these complications once it is involved. The low CD4+ count found in co-infected patients, even though they are on ART, also is in support of the fact that HBV accelerates the development of Acquired Immunodeficiency Syndrome (AIDS) in HIV infected individuals. This is also in agreement with studies by Olawumi *et al*.[33], Forbi *et al*.[34], Ogwu-Richard *et al*.[32] and Tremeau-Bravard *et al*.[37], where $CD4^+$ counts of mono HBV infected patients or HIV/HBV co-infected patients are lower than the normal range. The risk behaviour pattern showed that multiple sex partners were highly associated with HBV positivity. This is different with findings by Olayinka *et al*.[29], Bakarey and Olaniyan[31], and Mbaawuaga *et al*.[21]. However, Olayinka *et al*.[29] stated that there was a significantly high proportion of HBsAg susceptible persons among unmarried participants (divorced, single, widowed) in the general population studied than the married ones, although there was no statistical difference from the analysis. In addition, the demographic characteristic in this studies showed that being married, unemployment and age were all associated with HBV positivity which are different from the finding by Eke *et al*.[8], but similar to the findings by Mbaawuaga *et al*.[21] in which there was a high statistically significant relationship between the age of respondents and HBV prevalence among Benue people.

Conclusion

This study has reported that there is a high prevalence of HBsAg among some high risk population group (HIV patients, blood donors and pregnant women), but 0% HBsAg among diabetics and sicklers attending major referral hospitals in Enugu, Southeast Nigeria. The blood donors in this study constitute mostly relatives of patients requiring blood transfusions in the hospitals studied and together with the level of prevalence in blood donors highlights the need for proper and adequate screening of blood before transfusion.

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Table 1: Prevalence of HBV amongst various population groups sampled.

Group Analyzed	No Sampled	No. HBV positive	Percentage
Sickle cell patients	22	0	0%
HIV patients	62	16	25.8%
Pregnant women	164	9	5.5%
Blood Donors	233	20	8.6%
Diabetics	37	0	0%
Total	518	45	8.7%

Table 2: Prevalence of HBV infection in relation to gender and age groups.

Age Group	No Sampled	Male No Tested	Male No positive (%)	Female No Tested	Female No positive (%)	Total positive (%)
16-25	19	9	0 (0.0)	10	0 (0.0)	0 (0.0)
26-35	426	197	23 (11.7)	189	17 (9.0)	40 (9.4)
36-45	45	27	2 (7.4)	13	3 (23.1)	5 (11.1)
46 and above	28	14	0 (0.0)	14	0 (0.0)	0 (0.0)
Total	518	247	25 (10.1)	226	20 (8.8)	45 (8.7)

Table 3: AST and ALT levels of Blood Donors, HIV patients and pregnant women infected with Hepatitis B virus.

Group	No Tested	No with raised transaminases (%)	No with Raised AST Value	No with Raised ALT value
Blood donors	20	2 (10.0)	1	1
HIV patients	16	7 (43.8)	7	5
Pregnant women	9	1 (11.1)	1	1

Normal Ranges
AST – 8-40 (U/L)
ALT – 5-40 (U/L)
BD = Blood Donors
HIV = Human immunodeficiency virus
ANC = Antenatal clinic
AST= Aspartate aminotransferase
ALT= Alanine aminotransferase

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Table 4: T –test comparing ALT and AST levels of HBV positive individuals in sample population with HBV negative.

Statistical Parameters	HBV positive		HBV negative	
	ALT	AST	ALT	AST
Mean	30.48	37.70	14.71	15.24
Std. Deviation	38.332	39.840	7.776	6.562
P value	0.011	0.001		
P < 0.05				

Table 5: CD4 + T- Cell counts of patients concomitantly infected with HIV and HBV.

SEX	ALT (U/L)	AST (U/L)	CD4+CELL COUNT	ARV DRUG ADMINISTERED
F	9	15	511	Yes
M	19	27	367	Yes
F	6	17	398	Yes
M	18	23	428	Yes
F	49	59	494	Yes
M	18	30	370	Yes
M	19	23	240	Yes
M	9	15	240	Yes
F	29	34	689	Yes
F	19	29	999	No
F	36	96	39	Yes
F	17	60	33	Yes
M	45	92	295	Yes
M	78	112	35	Yes
M	79	72	62	Yes
M	142	118	385	Yes

Normal CD4 + T- Cell count is > 1000 Cells/µl,
AST = 8 – 40 U/l,
ALT = 5 – 40 U/l
Antiretroviral drug (ARV) used – non-nucleoside and nucleoside reverse transcriptase inhibitor namely Stavudine, Lamivudine and Nevirapine.

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Table 6: T-test comparing CD4 counts in HBV/HIV positive and HBV/HIV negative

Statistical Parameters	HBV/HIV positive CD4	HBV/HIV negative CD4
Mean	368.21	1362.40
Std. Deviation	264.200	273.085
P value	< 0.001	

P< 0.05

Table 7: Risk behaviour patterns of HIV patients in relation to HBV infection

Risk variables	Total number	Number HBV positive (%)	Poisson chi square test P value
Injection drug user			
Yes	3	0 (0.0)	0.338
No	59	16 (27.1)	
Multiple sex partner			
Yes	10	7 (70.0)	< 0.001
No	52	9 (17.3)	
STDS			
Yes	8	3 (37.5)	0.280
No	54	13 (24.1)	
Tattoo			
Yes	1	0 (0.0)	0.586
No	61	16 (25.8)	
Transfusion			
Yes	11	2 (18.2)	0.700
No	51	14 (27.5)	
Hepatitis vaccine			
Yes	13	1 (7.7)	0.149
No	49	15 (30.6)	

P < 0.050

Table 8: Association of demographical characteristics of HIV patients to HBV positivity.

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Demographic Characteristic	Total Number N = 62	Number HIV positive N = 16	Poisson chi square Test p value
Marital status			
Single	13	3 (23.1)	< 0.001
Married	49	13 (26.5)	
Education			
None	2	0 (0.0)	0.816
Primary	22	7 (31.8)	
Secondary	18	5 (27.8)	
Tertiary	20	4 (20.0)	
Occupation			
Self employed	7	2 (28.6)	0.014
Civil servant	32	4 (12.5)	
Unemployed	23	10 (43.5)	
Age			
16 – 25	5	0 (0.0)	0.045
26 – 35	24	11 (45.8)	
36 – 45	21	5 (23.8)	
46 and above	12	0 (0.0)	

P < 0.050