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Human Parvovirus B19 Infections among Blood Donors in Selected Blood Centers in Ghana

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Abstract

Background: Blood transfusion is a lifesaving therapy in hospital practice for patients. It is an invaluable human resource for a wide range of medical and surgical conditions, but it is bereft with possible transmission of infection such as Human Parvovirus B19. Human Parvovirus B19 is the only member of the *Parvoviridae* family known to be pathogenic to humans. Infections of Parvovirus B19 occur all year round and can also manifest in all age groups even though a number of the patients show subclinical manifestations. The aim of this research was to determine the sero-prevalence of Human Parvovirus B19 infections among blood donors at selected blood centers in Ghana.

Methods: The research was a cross- sectional study carried out among blood donors who donated blood at the selected blood banks from March 2018 to March 2019. Blood samples were collected from the participants and screened for both IgG and IgM antibodies to Human Parvovirus B19 using ELISA Kits to establish the sero prevalence of the virus among the blood donors. Risk factors associated with the spread of the infections were also determined.

Results: A total of 167 participants took part in the study and majority of them were males (93.7%) and about70.7% of them were between the ages of 30 to 39 years. At the end of the sample analysis, 10 (5.9%) of the participants tested positive for the IgM antibodies (Reactive), indicating active infections whilst 108 (64.7%) were reactive for IgG antibodies, representing those that have been exposed to the virus before. Though none of the study participants have had any blood transfusion before, about 47.3% had however donated blood before. From the demographics data gathered, the study also showed that none of the participants had multiple sex partners, used drugs, smoked or shared needles but about 22.8% had however received hepatitis B vaccination.

Conclusion: The outcome of the study showed that more than 50% of the participants were reactive to the IgG antibodies indicating previous exposure to the virus and 5.9% were reactive for the IgM antibodies an indicator of current and active infection. These findings indicate that it may be very important to include Parvovirus screening in the procedure for blood donation in order to make blood transfusion therapy a safe process.

Keywords: Human Parvovirus B19; Interferon Gemma; Interleukins; Blood Transfusion; Immunoglobulins

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Abbreviation: ELISA: Enzyme Linked Immunosorbent assay; HPV B19: Human Parvovirus B19; IgG: Immunoglobulin G; gM: Immunoglobulin M; TTI: Transfusion Transmitted infection; VP1: Viral protein 1; VP2: Viral protein 2; WHO: World health organization

Background

Blood is a biological active substance and an invaluable human resource for a wide range of medical and surgical conditions [1]. Blood transfusion is therefore a lifesaving therapy in hospital practices for patients, but the blood and its products provided should be as safe as possible and adequate to meet the patient requirements. Without blood, the management of many medical conditions such as trauma, cardiac surgeries, organ transplantation, malaria and obstetric haemorrhage, would have been difficult or nearly impossible [2]. However, this life-saving procedure is often associated with significant clinical risks, which can be broadly classified as infectious or non-infectious complications [3]. Infections of the blood are common and these can be transmitted when the blood of an infected and asymptomatic individual is transfused to a healthy person [4]. Based on this fact, the World Health Organization (WHO) recommends that all blood donations should be screened for infections prior to use [5]. In fact, the main challenges inscribed throughout the history of blood transfusion have been centered on adequacy and safety of the blood supply. Subsequently, the first step for safe blood donation and transfusion depends on building a panel of regular, voluntary, non-remunerated blood donors who may have minimal infectious risks [4, 5]. In fact, the goal of WHO was to obtain all blood supplies from voluntary, non-remunerated donors by 2020 [5]. Although most advanced countries have been able to achieve this goal, the same cannot be said about resource limited countries. As such, most resource limited countries are still confronted with challenges in terms of limited access to efficient blood transfusion or the provision of safe blood particularly with regards to transfusion transmitted infection (TTI) [6,7]. This is because strategies such as conversion from paid to voluntary non-remunerated blood donors, improvements in donor screening, improvement of assays that detect transfusion-transmissible infections in donor blood, regular quality control on blood units, leukoreduction techniques, proper blood management and haemovigilance needed to improve blood supply and minimize transfusion risk are considered to be too expensive to implement in most resource-limited settings. Transfusion transmitted infections therefore remains and some of the infections that have been detected include cytomegalovirus, Epstein Barr virus and Human Parvovirus B19 virus which is a newly recognized agent of blood transfusion transmitted disease [8, 9]. Human Parvovirus B19 is the only member of the *Parvoviridae* family known to be pathogenic to human and is an important pathogen that can cause morbidity and mortality as seen in patients across the globe [10, 11]. The virus is found in the blood and respiratory secretions of infected persons and can be transmitted through respiratory secretions, transplacentally and more importantly by transfusion of blood or blood products [12].

Infections caused by Human Parvovirus B19 occurs all year round and affect all ages, many of whom show subclinical manifestations [13]. It has also been shown that close to a third or a sixth of blood donors have the B19 antibodies but may be asymptomatic [14]. Acute infection is associated with a viremic phase which is followed shortly by an IgM antibody production that occurs between 10 to 14 days post infection. After this there is the production of IgG4 against the viral capsid, then a decline in viraemia with the production of IgM. After a few months IgM declines but the IgG persists longer to convey immunity against reinfection [15]. Indeed, Parvovirus B19 is emerging as a common viral infection with a serious threat of getting transmitted via blood transfusion. However, current blood banking practice does not require routine screening for human Parvovirus B19 prior to blood donation and transfusion. It is therefore important to determine the prevalence of Parvovirus B19 antibodies among blood donors as this study seeks to do.

Methods

Aim, design and setting of the study

The aim of this study was to determine the sero-prevalence of Human Parvovirus B19 infections among blood donors. The study was a cross-sectional study conducted at the Bolgatanga regional hospital blood bank and the Accra Area blood centre, Korle-bu from March 2018 to May 2019.

Characteristics of participants

The study involved one hundred and sixty-seven (167) healthy males and females who visited the blood bank to donate blood voluntarily or for a relative (family replacement) and passed both the medical and laboratory examination prior to donation.

Data collection procedure

Sampling method

All donors who met the inclusion criteria and gave their informed consent were used for the study. Each participant was taken through pre-donation counselling and then interviewed using a well- structured questionnaire.

Questionnaire Administration

A structured questionnaire was administered to the participants. Information obtained included socio-demographic characteristics, clinical data and risk factors which were taken in reference to human Parvovirus B19 infection.

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Specimen collection and testing

After the recruitment, 5ml of blood samples were taken from the participants, spun and plasma separated for use in the testing. If samples could not be analyzed straight away, they were frozen at -80^{0}C to be worked on later. The participant's sera were tested for Human Parvovirus B19 IgG and IgM antibodies using the enzyme-linked immunosorbent assay (ELISA) test. This was based upon the use of micro titer strip wells precoated with Parvovirus B19 antigens (conformational epitopes of VP-2 and linear epitopes of specific part of VP-1). These were used in binding antibodies in the specimen. The manufacturers' instructions were followed in all steps. The ELISA microwell plate reader was used in taking readings at a wavelength of 450nm.

Assay procedure for IgG

All reagents and chemicals were brought to room temperature and the working solution was diluted in the following manner: 10µL of sample + 1ml of sample diluent were mixed well before pipetting. The required number of micro titer strips or wells were selected and inserted into the holder and samples dispensed into them. The wells were covered with foil and incubated for 60 minutes at 37^{0}C. The contents were briskly shaken. The wells were rinsed 5times with diluted wash solution (300ul per well). Striking of the wells sharply was done on absorbent paper to remove residual droplets. Enzyme Conjugate were dispensed into each well and incubated for 30 minutes at room temperature (20^{0}C to 25^{0}C). The contents of the wells were shaken. 100µL of substrate solution was then added into all wells. They were incubated for exactly 15minutes at room temperature (20^{0}C to 25^{0}C) in the dark. The enzymatic reaction was stopped by adding 100µL of stop solution to each well. The optical density was read at 450/620nm with a micro titer plate reader within 30 minutes after adding the stop solution.

Interpretation of Results

Samples were considered POSITIVE if the absorbance value was 20% above cut-off value. Samples were considered NEGATIVE if the absorbance value was 15% below cut-off value. Samples with an absorbance value of 20% below the cut-off value were considered to be in GREY ZONE. Such tests were repeated 2-4 weeks later with a fresh sample. If the second test was again in grey zone, the sample was considered NEGATIVE.

Assay procedure for IgM

All reagents were brought to the room temperature (20-25^{0}C) before starting the test. The reagents were mixed gently prior to use without inducing foaming. A clean, disposable tip was used for dispensing each control and sample.100µl controls were dispensed together with diluted samples into their respective wells. They were incubated for 1 hour at 370C. After incubation, each well was washed three times with 300µl of washing solution.100µl Parvovirus B19 anti-IgM conjugate were dispensed into all wells except for the blank well. It was incubated at room temperature for 30 minutes. Each well was washed three times after incubation. This was done with 300µl of washing solution. About 100µl of TMB substrate solution was dispensed into the wells. This was incubated for 15 minutes under room temperature in the dark. A 100µl stop solution was dispensed into the wells. Measurement of optical density was carried out at 450nm wavelength.

Interpretation of results

Samples were considered POSITIVE if the absorbance value was>10% of cutoff value. Samples were considered NEGATIVE if the absorbance value was <10% of cut-off value. Samples with an absorbance value of 10% below the cut-off value were considered to be in GREY ZONE. Such tests were repeated 2-4 weeks later with a fresh sample. If the second test was again in grey zone, the sample was considered NEGATIVE

Data analysis

The data obtained from the study was analyzed using SPSS 20 and the results are presented in tables below. The prevalence for each viral infection was calculated and expressed as percentages. Correlation analysis was also conducted to find the relationship between variables as well as a Pearson chi-square analysis. The relationship between the socio-demographic characteristics and clinical manifestations were determined. P value of <0.05 was considered significant.

Results

Demographic Characteristics of Participants

A total of 167 participants were enrolled for the study. The outcome of the data analysis shows that majority (93.7%) of the participants were males, and more than half (70.7%) were between the ages of 30 and 39 years. It was also seen that majority were educated as most of them had completed secondary education and nearly half (43.1%) have had tertiary education. Also, the majority (65.9%) were married with 3.6% widowed (table 1).

Medical and lifestyle characteristics of the participants

The risk factors associated with the Parvovirus were also studied and findings showed that none of the participants had had blood transfusion before. However, about 79 (47.3%) of them have given or donated blood before and out of these, about 7 people representing 8.86% had donated blood in less than a year, about 26.58% between 1 to 5 years and about 59.49% for more than 10 years ago. The results also showed that only 2 (1.2%) of the participants had undergone surgery

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in the past and only 38 (22.8%) of them have received the hepatitis B vaccine. Again, it was seen that none of the participants had multiple sex partners and majority (98.8%) used condoms during sexual contact. Also, none of the participants abused drugs, shared needles, or smoked but 49 (29.3%) of them have tasted alcoholic beverages before but do not drink regularly (table 2).

Prevalence of Parvovirus B19 IgG and IgM among age groups and education

The IgG and IgM levels of B19 antibodies of the participants were analyzed to determine infectivity and the results showed that 10 (6%) of the participants were reactive for IgM whilst the majority (94%) were non-reactive for IgM. With respect to IgG, it was seen that 108 (64.7%) of the participants were reactive while 59 representing 35.3% were non-reactive (table 3).

Prevalence of Parvovirus B19 IgG and IgM antibodies according to history of blood transfusion and donation

The outcome of the study showed that out of the participants who had donated blood, 1.8% were reactive and 4.19% of them who had not donated blood were also found to be reactive for IgM. For those who had not donated blood before, 5.99% were reactive for IgG (table 4).

Prevalence of Parvovirus B19 IgG and IgM according to Medical and Lifestyle Characteristics

The medical and lifestyle practices of the participants were also analyzed in relation to Parvovirus B19 reactivity. These medical and lifestyle practices included surgery, hepatitis B vaccination, having multiple sex partners, condom use, use of drugs, sharing of needles, smoking and alcohol use (table 5).

Table 1: Demographic characteristics of participants.

	Characteristics	Frequency	Percentage (%)
Gender	Male	156	93.7
	Female	11	6.6
Age	18 – 19 years	23	13.8
	20 – 29years	26	15.6
	30 – 39years	118	70.7
Education	None	13	7.8
	Primary	15	9
	Secondary	65	38.9
	Tertiary	72	43.1
	Quaternary	2	1.2
Marriage	Single	51	30.5
	Married	110	65.9
	Widow	6	3.6
Occupation	Self employed	34	20.4
	Civil servant	11	6.6
	Driver	2	1.2
	Farmer	8	4.8
	Health worker	9	5.4
	Student	43	25.7
	Other	60	35.9

Table 2: Medical and lifestyle characteristics of the participants

	Characteristics	Frequency	Percentage (%)
Blood transfusion	Yes	0	0
	No	167	100
Blood Donation	Yes	79	47.3
	No	88	52.7
Donation period	Less than 1 year	7	8.86
	1 – 5 years	21	26.58
	6 – 10 years	4	5.07
	More than 10years	47	59.49
	Total	79	100
Surgery	Yes	2	1.2
	No	165	98.8
Hepatitis B vaccine	Yes	38	22.8
	No	129	77.2
Multiple sex partners	Yes	0	0
	No	167	100
Condom use	Yes	165	98.8
	No	2	1.2
Drug use	Yes	0	0
	No	167	100
Sharing needles	Yes	0	0
	No	167	100
Smoking	Yes	0	0
	No	167	100
Alcohol use	Yes	49	29.3
	No	118	70.7

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Table 3: Reactivity and prevalence of IgG and IgM antibodies.

		IgM		IgG	
		Reactive	**Nonreactive**	**Reactive**	**Non-reactive**
Sex	Male	9 (5.39%)	147	104	52
			-88.02%	-62.23%	-31.14%
	Female	1 (0.59%)	10 (5.98%)	4 (2.44%)	7 (4.19%)
	18 – 19	1 (0.59%)	22 (13.17%)	12 (7.19%)	11 (6.59%)
Age	20 – 29	1(0.59%)	25 (14.97%)	16 (9.58%)	10 (5.98%)
	30 – 39	8 (4.80%)	110	80 (47.90%)	38
			-65.87%		-22.75%
	None	0	13 (7.78%)	12 (7.18%)	1 (0.59%)
	Primary	2 (1.20%)	13 (7.78%)	14 (8.38%)	1(0.59%)
Education	Secondary	3 (1.80%)	62 (37.12%)	43 (25.75%)	22
					-13.17%
	Tertiary	5 (2.99%)	67 (40.12%)	37 (22.16%)	35 (21%)
	Quaternary	0	2 (1.20%)	2 (1.20%)	0

Table 4: Prevalence of IgG and IgM antibodies according to blood transfusion and donation.

		IgM		IgG	
		Reactive	Nonreactive	Reactive	Nonreactive
Transfused	Yes	0	0	0	0
	No	10 (5.99%)	157 (94.01%)	108 (64.67%)	59 (35.33%)
Blood donation	Yes	3 (1.80%)	76 (45.51%)	50 (29.94%)	29 (17.37%)
	No	7 (4.19%)	81 (48.50%)	58 (34.73%)	30 (17.96%)
Donation period	Less than 1 year	0	7 (4.19%)	3 (1.80%)	4 (2.4%)
	1 – 5 years	0	21 (12.57%)	12 (7.19%)	9 (5.39%)
	6 – 10 years	0	4 (2.40%)	2 (1.20%)	2 (1.20%)
	Can't remember	10 (5.99%)	125 (74.85%)	91 (54.49%)	44 (26.35%)

Table 5: IgG and IgM results according to medical and lifestyle characteristics

		IgM		IgG	
		Reactive	**Nonreactive**	**Reactive**	**Nonreactive**
Surgery	Yes	0	2 (1.20%)	1 (0.60%)	1 (0.60%)
	No	10 (5.99%)	155 (92.81%)	107 (64.07%)	58 (34.73%)
Hepatitis B vaccine	Yes	1 (0.60%)	37 (22.16%)	20 (11.98%)	18 (10.78%)
	No	9 (5.39%)	120 (71.86%)	88 (52.69%)	41 (24.55%)
Multiple sex partner	Yes	0	0	0	0
	No	10 (5.99%)	157 (94.01%)	108 (64.67%)	59 (35.33%)
Condom use	Yes	10 (5.99%)	155 (92.81%)	106 (63.47%)	59 (35.33%)
	No	0	2 (1.20%)	2 (1.20%)	0
Drug use	Yes	0	0	0	0
	No	10 (5.99%)	157 (94.01%)	108 (64.67%)	59 (35.33%)
Sharing needle	Yes	0	0	0	0
	No	10 (5.99%)	157 (94.01%)	108 (64.67%)	59 (35.33%)
Smoking	Yes	0	0	0	0
	No	10 (5.99%)	157 (94.01%)	108 (64.67%)	59 (35.33%)
Alcohol	Yes	7 (4.19%)	42 (25.15%)	29 (17.37%)	20 (11.98%)
	No	3 (1.80%)	115 (92.81%)	79 (47.31%)	39 (23.35%)

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Table 6: Correlation analysis of IgG and IgM and risk factors.

	Sex	Age	Blood donation	Donation period	Hepatitis B vaccine	Alcohol use	IgM	IgG
Sex	1							
Age	-1.42	1						
Blood donation	0.058	1.67**	1					
Donation period	-0.072	-1.43	0.38**	1				
Hepatitis B vaccine	0.029	0.111	0.087	0.128	1			
Alcohol	0.012	-1.3	0.101	0.188*	0.058	1		
IgM	-0.035	-0.035	-0.087	-0.117	-0.077	0.225**	1	
IgG	0.157*	-0.114	-0.027	-0.121	-0.137	-0.074	0.028	1

Correlation analysis

Correlation analysis was conducted to determine the extent of the relationship between IgG and IgM reactivity and the various risk factors. The results revealed that there was a negative relationship for all the risk indices with respect to IgM. Only alcohol use had a positive correlation with IgM. Also, there was a 22.5% relationship between alcohol use and IgM reactivity which was significant at 95% confidence interval (table 6). The study also found negative relationships between age, blood donation, donation period, hepatitis B vaccination and alcohol use with IgG but a positive relationship with gender.

Discussion

Data for human Parvovirus B19 infection are critical in health policy formulation but are not readily available in Africa. The current study was to determine the sero-prevalence of Human Parvovirus B19 as an indicator of recent (IgM) or previous (IgG) infections among blood donors at two blood centers in Ghana. In the study, majority of the participants were males which could be attributed to the fact that blood donation is mostly done by males since women generally have low hemoglobin levels due to physiological factors and are therefore mostly disqualified as blood donors [7, 16]. It was also seen from the demographic data that the majority of the participants were within the age range of 30-39 years, which was consistent with other studies [17]. Again, it was found that more than 50% of the participants had completed secondary education similar to a study carried out in Malawi [18]. Also, more than 50% of the participants were married, which conforms with an earlier study by Nagalo where more than 52% of the people who showed up for blood donation were married [19]. Furthermore, the medical and lifestyle practices including blood transfusion history, surgery, vaccination, condom use, drug use, needle sharing, smoking and alcohol use were studied and from the outcome, only 22% of the participants had received hepatitis B vaccination. With regards to the prevalence of Parvovirus B19 IgG and IgM antibodies, the study outcome showed an infection rate of 64.63% in the case of IgG and 5.98% in the case of IgM respectively (table3). These findings are consistent with other studies carried out by other authors. For example, Keikha et al., (2006) reported 10.3% IgM antibodies and 21.8% IgG antibodies in their study. Similarly, in a study carried out in Iran, Kaur and Basu (2005) also reported that 30 to 60% of their study participants had B19 antibodies[14, 20, 21]. For the IgM reactive participants, males were about 5 times reactive as compared to females which can be attributed to the fact that the study had more male participants. Again, the age group that was more reactive was the ages between 30 and 39 years and they accounted for 4.49%. For educational background, the study showed that participants with tertiary education had the highest reactivity, followed by secondary, primary, and quaternary and none with the lowest.

Again, for IgG, male participants were more reactive and accounted for 62.23% in comparison with females who recorded 2.40% in keeping with the variation between the two sexes in the study. The study also showed that participants who were below 19 years recorded the least reactivity. For education, the majority reactive group was recorded by those with secondary education (25.75%), followed by tertiary (22.16%), primary (8.38%) and those with no education recorded 7.18%. The least reactive group was recorded by participants with quaternary education (1.2%). These findings are consistent with the findings of Kishore *et al., (*2010*)* who conducted a study on HPVB19 prevalence among blood donors in India and showed higher prevalence among illiterates and low amount among educated group [22]. From table 4, it is seen that 108 (64.67%) of the participants who had not had a blood transfusion were reactive for IgG and for participants who had donated blood, the study showed that 50 representing 29.94% were reactive for IgG and those who have not donated blood before, 58 (34.73%) were reactive for IgG. Also, a significantly higher rate of IgM antibodies was reported by participants who had donated blood in the past five years. This indicates the

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need for routine screening of blood for Parvovirus B19 as a policy in blood transfusion particularly for those donating for immune compromised individuals. Furthermore, with respect to IgM, the study showed that 10 (5.99%) of the participants who had not have surgery before were reactive and for those who have had hepatitis B vaccination, only 1 (0.60%) was found to be reactive and 9 (5.39%) who have not had a hepatitis B vaccination were found to be reactive. The study also revealed that only 5.99% of the participants with a single sex partner and used condoms were reactive. None of the participants who used drugs, shared needles or smoked, and 5.99% of those in that category were found to be reactive. For the participants who consumed alcohol, 7 (4.19%) were found to be reactive while 3 (1.80%) of those who did not consume alcohol were found to be reactive. When medical and lifestyle characteristics were related to IgG reactivity, majority (107 (64.07%)) of the participants who had not had surgery before were found to be reactive. Only 1 (0.60%) respondent who had undergone surgery before was found to be reactive. It was also seen that 20 (11.98%) of the participants who had received the hepatitis B vaccine were reactive while 88 (52.69%) who had not received the vaccine were reactive. It was also seen that 108 (64.67%) of participants who did not have multiple sex partners, used drugs, shared needles or smoked were IgG reactive. For those who used condoms, 106 representing 63.47% were reactive while 2 (1.20) of the participants who did not use condoms were reactive. Furthermore, 79 (47.31%) of participants who did not consume alcohol were found to be reactive whilst 29 participants representing 17.37% of those who consumed alcohol were found to be IgG reactive. When the prevalence of HPV B19 IgG and IgM were analyzed among age groups, it was seen that seropositivity increased with age which was consistent with the work of Girei in Jos, Nigeria and those reported in other countries [23,24,25]. They found that as age increased, the seropositivity of Parvovirus B19 increased. Gilbert et al (2005) however, found an inverse relationship between seroprevalence of B19 and age [26].

Conclusions

The findings in this study showed the presence of Human Parvovirus B19 infection among the study population with sero-prevalence comparable to the rates found in various countries around the world. This study also showed the effect of formal education, occupation and marriage on seropositivity of individuals to Human Parvovirus B19 and indicates the relevance of considering them as risk factors on the seroprevalence of Human Parvovirus B19. It must be emphasized that, the prevalence of 64.7% obtained for the IgG showed that majority of the participants have been exposed to HPV B19 infection and 6% IgM positivity also shows the virus is active among a relatively good number of the study population. This means Human Parvovirus B19 still poses as a public health problem in relation to blood transfusion.

Declarations

Ethics approval and consent materials

Ethical clearance was obtained from the ethical and protocol review committee of the School of Biomedical and Allied Health Science (SBAHS-MLS/10636611/SA/2018-2019) as well as the institutional ethical committee of The National blood bank (NBSGRD/190907/01) and Bolgatanga regional hospital. All the study details were explained to the donors in vernacular language and informed consent were obtained before the commencement of the study.

Consent for publication: Not applicable

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author's contribution

SAB participated in the design, co-supervised the research, and drafted the manuscript. BTM participated in the design and co-supervised the work proof reading of the manuscript. LKA participated in the design of the study and carried out the experimental work. DNOA carried out the data analysis and editing of the manuscript. LA participated in the supervision of the work and proof reading of the manuscript. All authors read and approved the final manuscript.

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