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ORIGINAL ARTICLE

Determination of Some Haematological Parameters and Oxidative Stress Markers in Vesico-Vaginal Fistula Patients Attending Maryam Abacha Women and Children Hospital, Sokoto

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Abstract

Introduction: Vesico-vaginal fistula (VVF) is still a persisting scourge in developing countries with devastating medical and social consequences. The goal of this study was to assess the haematological parameters and oxidative stress markers of VVF patients compared with healthy control female subjects.

Material and Methods: This study was carried out at VVF Centre, Maryam Abacha Women and Children Hospital, Sokoto. This is a descriptive study conducted on 50 VVF patients and 50 controls to determine some haematological parameters and oxidative stress markers. Questionnaire was used to obtain sociodemographic information while laboratory investigations were used to obtain haematological and free radical marker results.

Results: The values obtained from the control and VVF subjects for PCV, RBC, hemoglobin, platelets, lymphocytes, neutrophils, monocytes and basophils were statistically non-significant ($p>0.05$) while WBC ($5.45 \pm 0.31 \times 10^9$/L,$59 \pm 0.27 \times 10^9$/L) and eosinophil ($2.43 \pm 0.33\%$, $4.83 \pm 0.54\%$) were statistically significant ($p<0.05$). For the free radical markers, both Malondialdehyde and glutathione levels were statistically non-significant ($p>0.05$). The correlation of Malondialdehyde and glutathione peroxidase levels with haematological parameters showed that only lymphocyte and monocyte counts were statistically significant respectively.

Conclusion: Prolonged obstructed labour, early marriage, ignorance, lack of knowledge are among the factors responsible for the prevalence of VVF in Sokoto State. White blood cell and eosinophil counts should

be included as part of the laboratory investigations for VVF patients which may serve as indication for asymptomatic bacteriuria, women should not be given out of marriage before they reach age of maturity and Formal education should be made free and mandatory for girls' up to tertiary institution.

Keywords: Vesicovaginal fistula, Malondialdehyde, Glutathione peroxidase, Basophils, Neutrophils.

Introduction

A vesicovaginal fistula is an abnormal connection between the bladder and vagina resulting in incessant urine leakage via the vagina. The most frequent cause of this condition is gynecological and obstetrical damage that arises during surgery (1). About 97% of all VVF cases in developing countries are secondary to prolonged obstructed labour whereas mass of cases manifests after gynaecological or urological surgery in developed nations. Pelvic irradiation, endometriosis, malignancy, anatomical distortion by myomas or ovarian tumors, impaired healing status and infection are other risks factors associated with VVF (2). Raji *et al.* have reported that VVF affects over 2 million women worldwide, with 50,000–100,000 instances thought to occur each year. In Africa, the prevalence is between 2–5/1000 births, and 33,000 new VVF cases are reported there each year. Between 3 and 4 per 1000 deliveries occur in West Africa. In Nigeria, the annual incidence of obstetric fistula is thought to be 2.11 per 1000 live births. The country as a whole was affected, but the northern portion was the worst (3). Unpleasant cultural and religious traditions including child marriage and female circumcision contribute to the prevalence of VVF. Due to the pelvis' incomplete development, child marriage increases an individual's risk of having VVF. However, age, stature, parity, labor duration, malnutrition, chronic anemia, and vitamin D insufficiency could all be contributing factors to 80–90% of VVF associated with extended labor (4).

Materials and Method

Study Population

The population for this study consisted most of the Vesico-vaginal fistula patients that accepted informed consents at Maryam Abacha Women and Children Hospital, Sokoto. These patients are victims of Vesico-vaginal fistula who stayed in the hospital and have not undergone fistula repair. The control subjects consisted of apparently healthy individuals as volunteers among the female staffs and students of Usmanu Danfodiyo University Sokoto (UDUS). In all the subjects and control, informed consent was obtained from them prior to the commencement of the study.

Study Design

This is a cross-sectional study designed among the Vesico-vaginal fistula patients to assess some of the haematological parameters and free radical markers (malondialdehyde and glutathione peroxidase) of the Vesico-vaginal fistula patients' attending Maryam Abacha Women and Children Hospital, Sokoto. Also, apparently healthy subjects without Vesico-vaginal fistula (VVF) were used as controls from staff and students of Usmanu Danfodiyo

University Sokoto (UDUS). Blood sample was collected (from both subject and controls) and tested for complete blood count, malondialdehyde and glutathione peroxidase levels. Results of these parameters were compared with that of control subjects and was analyzed using statistical package for social sciences (SPSS) version 22.

Inclusion Criteria

The following patients who meet the following inclusion criteria were included to participate in the study;

All pre-operated patients with Vesico-vaginal fistula in Maryam Abacha Women and Children Hospital, Sokoto that accepted the inform consent.

Exclusion criteria of Subjects

Patients with co-existing haematological disorders such as patients with sickle cell anaemia, any form of leukemia or other malignancies

Blood Sample Collection and Processing

Five millilitres (5ml) of whole blood were collected from each subject via venepuncture using BD vacutainer system, in which two millilitres (2 ml) were added into K3 EDTA anticoagulated and three millilitres (3 ml) into plain tube under strict aseptic techniques. EDTA anticoagulated sample was used to analyze complete blood count while sample from plain vacutainer blood specimen bottle was allowed to clot at room temperature and later centrifuge at 3000rpm/min for 5 minutes to obtain a clear unhaemolyzed serum. The sera were harvested into sterile serum-separation tubes and rapidly stored at -20°C until assay in batches; for analysis of serum levels of malondialdehyde and glutathione peroxidise. These samples were processed in Pathology Laboratory of Usmanu Danfodiyo University Teaching Hospital (UDUTH).

Laboratory Analysis

Complete Blood Count

Complete blood count was carried out using the five parts automated hematology analyzer (Mythic 22CT, 2008).

Principle

Full blood count was carried out on EDTA anticoagulated samples from the subjects using the 5 parts differential Mythic 22 CT haematology analyser. The analyzers carry out cell counting based on impedance principle developed by Wallace Coulter in 1956. The Coulter counter system is based on the principle of electrical impedance. When the diluent is displaced by blood cells, it causes a measurable change in resistance. The cells are allowed to pass through an aperture through which an electric current is flowing. Cell passing through the aperture displace the diluents; and being bad conductors of electricity, increase the resistance which is counted as a voltage pulse, which are converted to digital recording. The cell suspension is drawn through the aperture with the help of a vacuum pump into a system of tubing.

Procedure

EDTA blood sample was placed on a blood mixer. The mixed sample tube was placed on a tube holder and the blood was aspirated into the analyzer by pressing the aspiration button. The analyzer was then allowed to measure the various parameters and the result was printed out using the inbuilt printer on the analyzer.

Determination of Serum Malondialdehyde (Shah & Walker's 1989)

Serum malondialdehyde level was analyzed using the chemical method (Shah and Walkers' method) spectrophotometrically.

Principle:

Malondialdehyde in serum is to be separated

and determined as conjugate with Thiobarbituric acid, TBA. Serum proteins are precipitated by Trichloric acid, TCA and then removed by centrifugation. The MDA-TBA complex was measured at 534 nm wavelength.

Procedure:

The reaction was performed using 18 x 150 mm Pyrex test tube labeled as test and blank, into which the following reagents were pipetted as follow: One thousand microlitres (1000µl) sample (serum) was added into test tube label test. One thousand microlitres (1000µl) of distilled water was added into test tube label blank. One thousand microlitres (1000µl) of reagent 1 was added into test tube label test and blank. One thousand microlitres (1000µl) of reagent 2 was added into test tube label test and blank and finally One thousand microlitres (1000µl) of reagent 3 was added into test tube label test and blank. The tubes were mixed well and incubated in boiling water bath for 15min., allowed to cool, and then the tubes were left to stand at room temperature for 20 min. Then the tubes were centrifuge at 2000 rpm for 15 min., and then the supernatant layer was read at 534 nm.

Calculation:

The concentration of MDA (nmol/ml) was calculated by using the following formula: Concentration of MDA (nmol/ml

Table 1: Demographic characteristics of study participants.

Parameters	Controls (n=50) N (%)	VVF Subjects (n=50) N (%)	P value	Remark
Age group (years)				
10-20	10 (20%)	16 (32%)	0.044	SS
21-30	30(60%)	25(50%)		
31-40	10(20%)	4(8%)		
>40	Nil	5(10%)		
Marital Status				
Single	30(60%)	Nil	<0.001	SS
Married	20(40%)	34(68%)		
Divorced	Nil	16(32%)		
Age of marriage (years)				
<18	5(10%)	46(92%)	<0.001	SS
18 & above	45(90%)	4(8%)		
Educational Background				
Informal	5(10%)	47(94%)	<0.001	SS
Primary	6(12%)	1(2%)		
Secondary	9(18%)	2(4%)		
Tertiary	30(60%)	Nil		
Tribe				
Hausa-Fulani	41(82%)	42(84%)	0.002	SS
Yoruba	5(18%)	Nil		
Igbo	Nil	Nil		
Others	Nil	8(16%)		

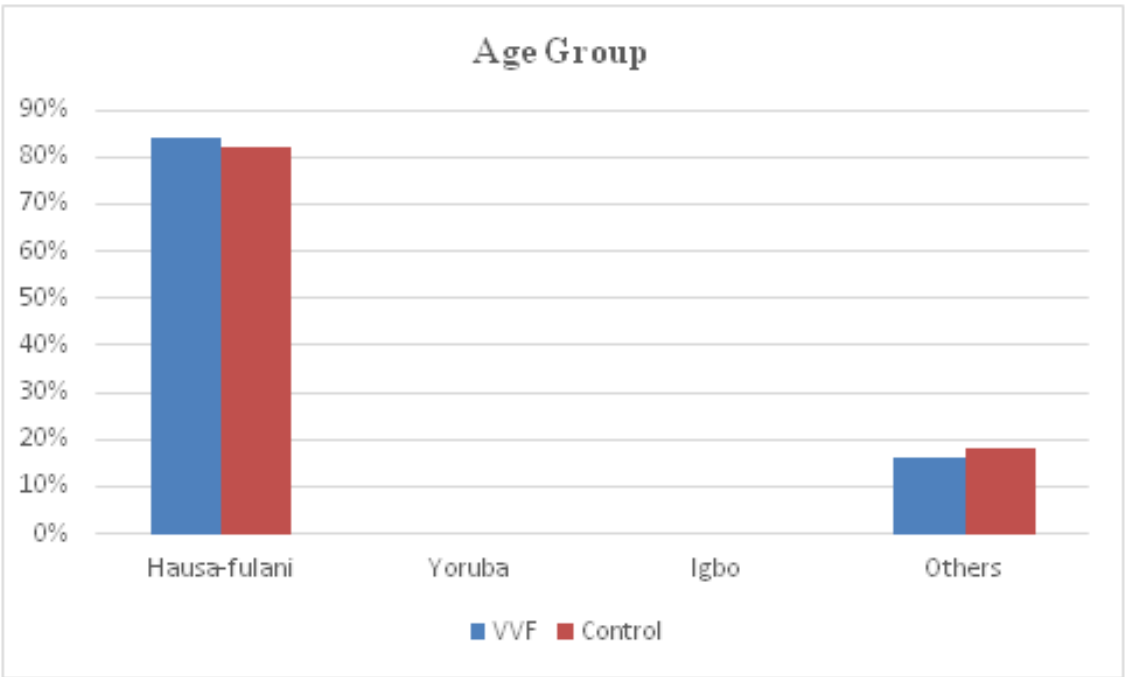

Fig 1: Distribution of Age of the VVF patients and Control

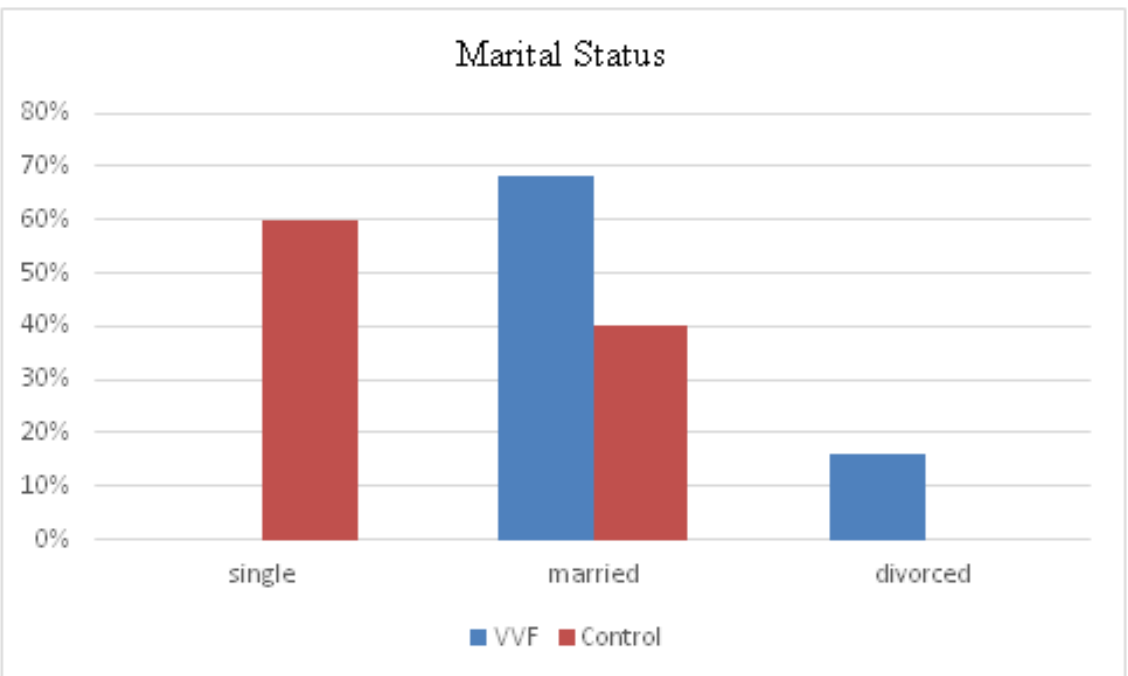

Fig 3: Age of marriage of both the VVF and Control participants

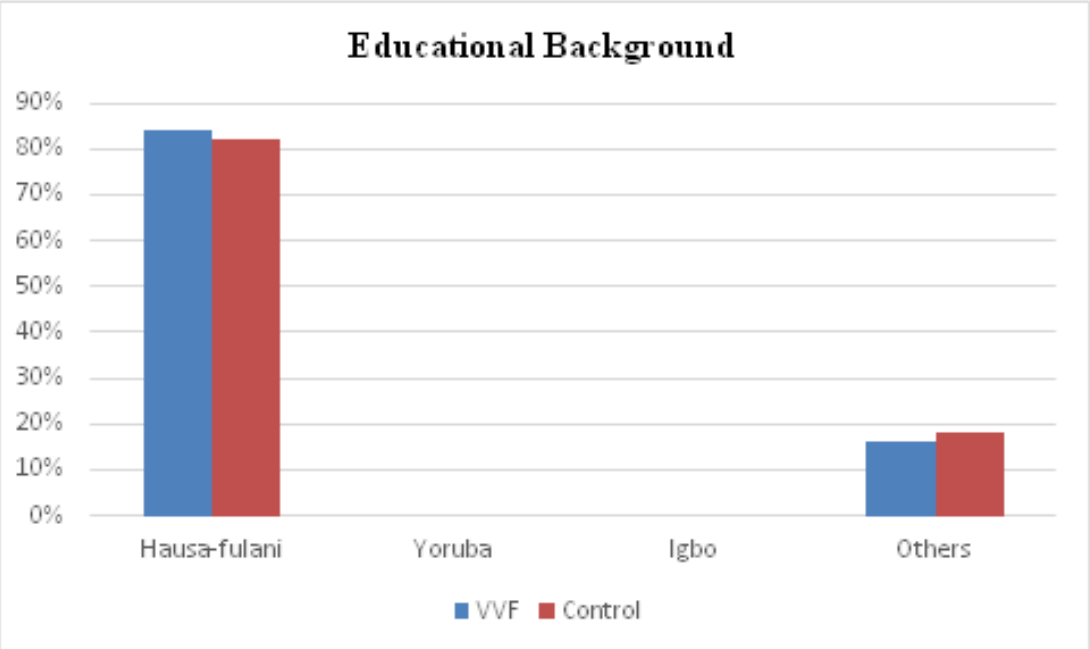

Fig 4: Educational Background of the VVF patients and Control

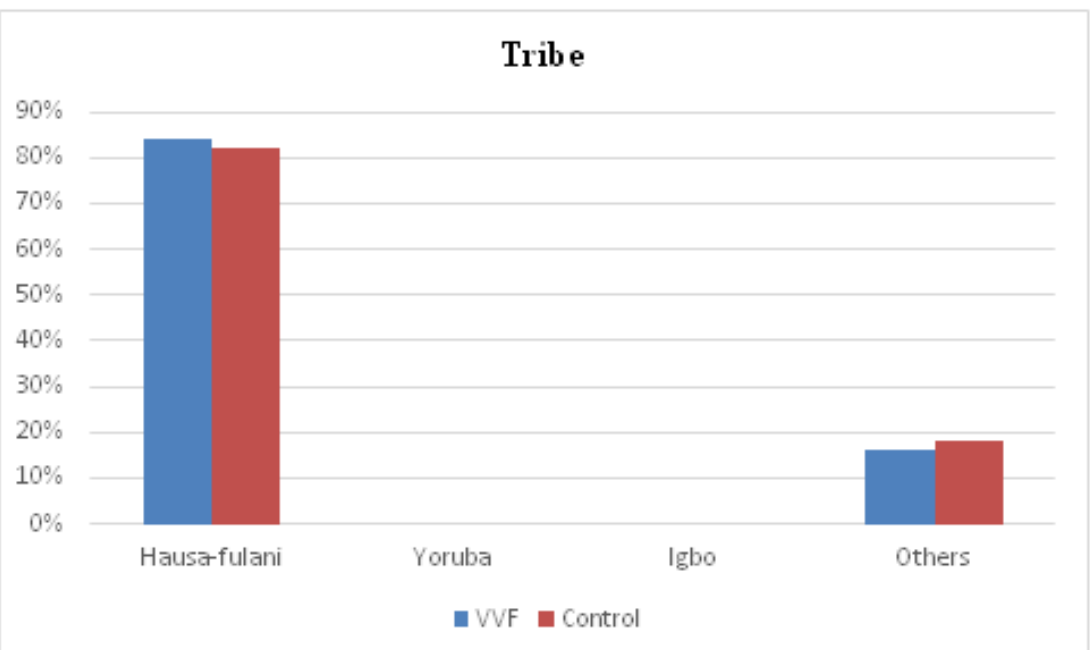

Fig 5: Tribe of both the VVF and control participants

Table 2: Shows The Mean ± SEM Of Age And Haematological Parameters Of Controls Subjects And Vesico-vaginal Fistula Subjects.

Parameters	Controls (n=50)	VVF (n=50)	p-value	Remark
PCV (%)	33.37 ± 0.94	33.7 ± 0.54	0.760	NSS
RBC	3.97 ± 0.12	4.26 ± 0.11	0.092	NSS
Hb (g/dL)	11. 82 ± 0.32	11.97 ± 0.19	0.678	NSS
WBC (10^9/L)	5.45 ± 0.31	6.59 ± 0.27	0.008	SS
PLT(10^9/L)	248.65 ± 16.02	249.22 ± 11.32	0.977	NSS
LYMP (%)	39.99 ± 1.56	46.44 ± 1.48	0.098	NSS
Neutrophil (%)	42.45 ± 1.94	39.09 ± 1.04	0.180	NSS
Monocytes (%)	5.73 ± 0.52	6.89 ± 0.61	0.152	NSS
Basophil (%)	0.38 ± 0.03	0.40 ± 0.02	0.656	NSS
Eosinophil (%)	2.43 ± 0.33	4.83 ± 0.54	<0.001	SS

Hb=hemoglobin, LYMP= lymphocytes, NSS= non-statistically significance, PCV= packed cell volume, PLT= platelets, RBC= red blood cell, SS= statistically significance, VVF= Vesico-vaginal fistula, WBC= white blood cells.

Table 3: Shows the Mean ± SEM of MDA and Glutathione of controls subjects and Vesico-vaginal fistula subjects.

Parameters	Controls (n=50)	VVF (n=50)	p-value	Remark
MDA	0.0002 ± 0.0002	0.0002 ± 0.0001	0.374	NSS
GPx	15.99 ± 2.97	9.78 ± 1.28	0.64	NSS

GPx= glutathione peroxidase, MDA= Malondialdehyde, NSS= non-statistically significance, and SS= statistically significance,

Table 4: Correlation of Malondialdehyde and Haematological Parameters Of Vesico-Vagina Fistula Subjects

Parameters	Correlation co-efficient (r)	P value	Remark
PCV (%)	0.052	0.717	NSS
RBC (10^{12}/L)	-0.089	0.541	NSS
HB(g/dL)	-0.056	0.699	NSS
WBC (10^{9}/ L)	-0.018	0.90	NSS
PLT (10^{9}/ L)	-0.314	0.851	NSS
LYM (%)	0.27	0.026	SS
NEUTROPHIL (%)	-0.015	0.917	NSS
MONOCYTE (%)	-0.141	0.329	NSS
BASOPHIL (%)	0.66	0.647	NSS
EOSINOPHIL (%)	0.180	0.210	NSS

Hb=hemoglobin, LYMP= lymphocytes, NSS= non-statistically significance, PCV= packed cell volume, PLT= platelets, RBC= red blood cell, SS= statistically significance, WBC= white blood cells.

Table 5: Correlation of Glutathione Peroxidase and haematological parameters of Vesico-Vagina Fistula Subjects

Parameters	Correlation co-efficient (r)	P-value	Remark
PCV (%)	0.109	0.450	NSS
RBC	0.015	0.916	NSS
HB (g/dL)	0.136	0.348	NSS
WBC (10^9/ L)	-0.207	0.149	NSS
PLT (10^9/ L)	-0.003	0.983	NSS
LYPM (%)	0.027	0.851	NSS
NEUTRO (%)	-0.127	0.378	NSS
MONO (%)	0.416	0.003	SS
BASO (%)	-0.124	0.390	NSS
EOSINOPHIL (%)	-0.240	-0.094	NSS

GPx= glutathione peroxidase, Hb=hemoglobin, LYMP= lymphocytes, MDA= Malondialdehyde, NSS= non-statistically significance, PCV= packed cell volume, PLT= platelets, RBC= red blood cell, SS= statistically significance, VVF= Vesico-vaginal fistula, WBC= white blood cells.

Discussion

Vesico-vaginal fistula among women continues to be a major public health problem in Nigeria. From this study, majority of VVF patients were within 21-30 years of age which accounts for 50% of patient while 30%, 8% and 10% were within the age range of 10-20 years, 31- 40 years and >40 years respectively. The age-related differences from the study are in agreement with previous studies of VVF patients within 20-29 years of age in Port Harcourt of 52.5% (5); Sagamu (58.3%) (6); Abakaliki (90.3%) (7). It is However in disagreement with previous study which showed high frequency of VVF in the age group of 15-20 years in Ekiti (40%) (8). The reason for this high frequency within this age, may be due to high reproductive rate within this particular group. Also, women at this group do not usually attend ante-natal care because they may have their deliveries more than once by themselves and felt it will be unnecessary to attend antenatal care.

In this study, the marital status differences were found to be statistically significant ($p<0.05$), for which 68% of the VVF patients are married while 32% are divorced. This is supported by previous studies of Daru *et al.* (9) that showed similar findings. This may be due to the early marriage been practiced in most places of Northern Nigerian.

In this study the age of marriage was statistically significant, 92% of the VVF patients married at an age less than 18 years. This is similar to the study that says most of the vesico-vaginal fistula patients in Northern Nigeria had early marriage; 52.3% of Maiduguri fistula patients got married by 15 years of age (9); 93.6% of Sokoto (10) patients were married before or at 18 years of age, and 81.5 % of Kano patients (11). This is because early marriage is commonly practiced in the Northern part of Nigeria, and sometimes girls are given out in marriage before or shortly after attaining menarche, where 39.1% of the patients were married before attaining menarche. The study also shows that majority of the victims (94%) have no formal education which agrees with a study that says the non-literate patients accounted for 75-81% in Nigeria (12). This can be as a result of ignorance which is rampant among these patients as majority of them are from rural areas. The study shows that majority of the patients are Hausa-Fulani ethnic group (84%) and the remaining 16% are from other tribes such as; Nupe, Arawas, Zabarmawas among others, this is in agreement with the study by Hassan and Ekele, (13) which was conducted in the same area. This is because the major tribe in Sokoto is Hausa-Fulani.

Hematological analyses of the subjects and the controls in this study showed that there was significantly higher white cell count (WBC) in the mean values of the VVF subjects (6.59 ± 0.27) when compared with the controls (5.45 ± 0.31). The p value of the WBC of the VVF subjects when compared with the control was significantly lower.

The elevated WBC (leucocytosis) in this study might be as a result of asymptomatic bacteriuria that most women with VVF suffered from as research showed that 76.1% of the VVF patients suffered from asymptomatic bacteriuria (14). Also, the study found a significant eosinophilia in the patients as compared to the control. Although no published articles to support this study. No significant changes were observed in the PCV, Platelets, Lymphocytes, neutrophil, monocytes, basophil, RBC and HB. The elevation in white cell counts may be as a result of necrosis which is the basis behind Vesico-vaginal fistula formation.

This study showed that the malondialdehyde level of the VVF patientswas statistically in-significant when compared with the control subjects.. Also, glutathione peroxidase level of the VVF subjects was statistically in-significant when compared with controls . This may be because the VVF patients are already on medications prior to the study as a reason for no significant differences.

A significant positive correlation was found to exist between lymphocytes of the VVF patients and malondialdehyde level. Since free radicals are necessary for maintaining optimal immune function, the proliferation of T-lymphocytes is a pivotal event in cell-mediated immunity and it too requires the action of free radicals. Also, B-lymphocytes have functional NADPH oxidase, which also works by producing free radicals (14). A significant positive correlation was also found to exist between glutathione peroxidase and monocytes among the VVF patients. Monocyte plays a vital role in inflammation which could result in the production of excessive number of free radicals. Glutathione peroxidase is necessary for increasing the immune response, controlling inflammation and reducing tissue damage (14).

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