Asian Hematology Research Journal

1(4): 140-152, 2018; Article no.AHRJ.46895

Some Haematological Parameters, Malondialdehyde and Glutathione Peroxidase Status of Diabetic Patients in Sokoto, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors OFU, EO and AAN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AAN, BHA, MHA and BAI managed the analyses of the study. Authors UNJ, AGI and INC managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AHRJ/2018/v1i430094
Editor(s):
(1) Dr. Juan Carlos Troiano, Professor, Department of Medicine and Production of Fauna, School of Veterinary Medicine, University of Buenos Aires, Buenos Aires, Argentina.
Reviewers:
(1) Dennis, Amaechi, Veritas University, Nigeria.
(2) E. Siva Rami Reddy, Tantia University, India.
(3) Aba Patrick Emeka, University of Nigeria, Nigeria.
Complete Peer review History: http://www.sdiarticle3.com/review-history/46895

Original Research Article

Received 09 November 2018
Accepted 27 January 2019
Published 25 February 2019

ABSTRACT

Background: World Health Organization statistics identify 150 million people with diabetes mellitus worldwide and suggest that this figure may double by 2025.
Aim: This research was conducted to determine the status of some haematological parameters and free radicals among diabetic patients attending Specialist Hospital, Sokoto.

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Study Design: This was a case control study involving two groups; 29 controls (non-diabetic) and 58 diabetic subjects.
Methodology: The participants were divided into two groups; 29 controls (non-diabetic) and 58 diabetic subjects. Five milliliters (5 ml) of blood was collected into ethylenediaminetetraacetic acid (EDTA) and plain containers for haematological and free radicals' analysis respectively. The full blood count (FBC) investigation was carried out using automated haematology analyser while malondialdehyde (MDA) and glutathione peroxidase status (GPx) investigations were carried out using chemical and enzymatic methods respectively. The FBC, GPx and MDA status of both control and subjects were compared in this study.
Duration: The study lasted for a period of six months between April to September, 2017.
Results: The results obtained in this study showed a significant increase in WBC, neutrophil, eosinophil and Platelet counts of diabetic subjects ($P \leq 0.05$) when compared with controls, while other FBC parameters, MDA and GPx were not significant ($P > 0.05$). There was significant decrease in the basophil count of the diabetic subjects based on marital status ($P \leq 0.04$), while other parameters measured were not significant ($P > 0.05$). The result also showed a significant increase in the MDA of diabetic subjects based on age ($P \leq 0.05$), while others were not significant ($p > 0.05$). the study also showed no statistical difference in the FBC, MDA and GPx of the smokers and non-smokers ($P > 0.05$)
Conclusion: This research shows that WBC count, neutrophil, eosinophil and platelet count of the diabetic patients increased. There is need for a further research on direct free radical estimation and total antioxidant status in relation to diabetes, which gives more information than the indirect method of estimation. It is recommended that white blood cells and platelet levels should be closely monitored for proper management diabetic patients.

Keywords: Haematological; parameters; free radicals; diabetic; patients; Sokoto.

1. INTRODUCTION

Diabetes mellitus (DM) is a complex metabolic disorder involving many organs and can devastate the lives of affected individuals [1]. It is characterized with chronic high blood glucose that could lead to morbidity and mortality [2]. The number of people suffering from diabetes worldwide is increasing at an alarming rate. It is predicted that about 366 million people are likely to be diabetic by the year 2030 [3].

However, diabetes is mostly classified basically into two major types: Type I Diabetes (T1D) and Type II Diabetes (T2D). Type I diabetes (Insulin dependent) which is due to immune mediated beta-cells destruction, leading to insulin deficiency, while Type II diabetes (Non-insulin dependent) that occur as a result of insulin secretory defect and insulin resistance. About 5-10% of the total cases of diabetes worldwide are due to T1D. T1D is the most common type of diabetes in children and adolescents while T2D is common among young adults [4,5]. At least 90% of adult individuals with diabetes have type II diabetes [6].

Diabetes is the most common cause of chronic kidney disease (CKD) in the Western World, present in nearly two thirds of all patients with renal impairment (7). Anemia is a common complication of CKD, affecting over half of all patients [7,8]. Consequently, diabetes is also the most common cause of renal anemia. However, over-and-above diabetes as simply a cause of renal disease, anemia is also more common in patients with diabetes than those with renal disease of other causes. For example, the Third National Health and Nutrition Examination Survey (NHANES-III), found people in the general population with diabetes were nearly twice as likely to have anaemia, when compared to people without diabetes, but with a similar degree of renal impairment [9]. Anaemia also develops earlier in patients with diabetes than in patients with renal impairment from other causes [10].

Full blood count (FBC) is a common blood test to check a person's general health or to screen for conditions such as anaemia. A full blood count measures the status of a number of different features of the blood, including the amount of haemoglobin in the blood, the number of red blood cells (red cell count), the percentage of blood cells as a proportion of the total blood volume (haematocrit or packed cell volume), the volume of red blood cells (mean cell volume), the average amount of haemoglobin in the red blood cells (known as mean cell haemoglobin), the

number of white blood cells (white cell count), the percentages of the different types of white blood cells (leucocyte differential count), and the number of platelets [11].

Mansi and Lahham revealed that various haematological parameters including packed cell volume (PCV), haemoglobin (Hb), red blood cells (RBC) and white blood cells (WBC) were reported to be altered during the course of diabetes mellitus [12]. Patients with diabetes mellitus show a significant derangement in various haematological paramters [3]. Anaemia is a common finding in patients with diabetes, particularly in those with overt nephropathy or renal impairment [14]. Anaemia has also been shown to be a risk factor for cardiovascular disease in diabetic patients particularly those with chronic kidney disorder. About 27% of diabetics' patients are anemic [15].

Also, Colak reported that diabetes mellitus causes the development of hypochromic anaemia due to a fall in the iron content of the body resulting from oxidative stress associated with the condition [16]. A previous study also showed that the mean values of TRBC, Hb, PCV and mean cell haemoglobin concentration (MCHC) for the diabetic patients were found to be lower than the values of control group, indicating the presence of anemia in the former group [17]. Similarly, a previous report indicated that the occurrence of anemia in diabetes mellitus is due to the increased non-enzymatic glycosylation of RBC membrane proteins, which correlates with hyperglycaemia [14,18]. In red blood cell production, erythropoietin (EPO) is produced by the kidneys. Kidney damage at several levels is a complication of diabetes and one problem often leads to the other. Changes in the kidneys that occur with diabetes range from diabetic nephropathy all the way to chronic kidney disease. Early detection and treatment are essential to prevent or delay disease progression [19].

Chung and colleague, showed that the WBC might play a role in the development and progression of diabetic complications [20]. Studies also show that peripheral WBC count might be associated with type II diabetes coronary artery disease (CAD), stroke, micro and macro vascular complications [21,22]. Studies have shown that differential WBCs especially of eosinophils, neutrophils, and monocytes, participate in the chronic inflammatory process and can lead to CAD through multiple mechanisms that mediate inflammation, cause proteolytic and oxidative damage to the endothelial cells, block the microvasculature, induce hypercoagulability, and promote infarct expansion [23,24,25].

Reactive oxygen species (ROS) is a collective term used for a group of oxidants, which are either free radicals or molecular species capable of generating free radicals. Free radicals may be defined as any chemical species that contains unpaired electrons. Unpaired electrons increase the chemical reactivity of an atom or a molecule. The most important oxygen-containing free radicals in many disease states are hydroxyl radical, superoxide anion radical, hydrogen peroxide, oxygen singlet, hypochlorite, nitric oxide radical, and peroxynitrite radical [26].

Antioxidants can be defined as substances that are able, at relatively low concentrations, to compete with other oxidizable substrates, and thus, to significantly delay or inhibit the oxidation of these substrates. This definition includes the enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase, as well as nonenzymatic compounds such as tocopherol (vitamin E), b-carotene, ascorbic acid (vitamin C), and glutathione, which scavenge the reactive oxygen species [27]. Oxidative stress is due to a disturbance in the balance between the production of ROS and the efficiency of the antioxidant defense. In other words, oxidative stress results if excessive production of ROS overwhelms the antioxidant defence [28].

Under normal metabolic conditions, these ROS are eliminated rapidly in normal cells by the antioxidant defense system. However, several studies have shown that high levels of free radicals and the simultaneously declined antioxidant enzyme levels lead to cell damage, inactivation of enzymes, and lipid peroxidation. Accumulated evidence indicates that oxidative stress-activated signaling pathways mediate insulin resistance and β-cell dysfunction. These consequences of oxidative stress can promote the development of diabetes complications. Therefore, oxidative stress, antioxidant defense, cellular redox status should be regarded as the central players in diabetes and its complications [29]. Another study also showed that persistent hyperglycaemia in DM is usually accompanied by increased production of free radicals especially ROS. Weak defence system of the body becomes unable to counteract the enhanced ROS generation and as a result condition of

imbalance between ROS and their protection occurs which leads to domination of the condition of oxidative stress [30,31]. This oxidative stress is known to play a pivotal role in cellular injury from hyperglycemia [32].

Diabetes mellitus and one of its complications, diabetic nephropathy, represent a leading cause of end-stage renal diseases (ESRD) in the developed countries especially United States and Europe [33,34]. Hyperglycaemia of diabetes mellitus and its attendant metabolic syndromes that is insulin resistance, hyperglycaemia, hypertension, dyslipidaemia, obesity, and some social characters of these patients e.g. smoking and the use of xenobiotics, predispose to diabetic nephropathy. These accelerate free radical generation and attenuate the antioxidant defense system creating oxidative stress [35,36,37]. Consequently, increased free radical production and attenuation of antioxidant system is currently receiving the highest attention when discussing diabetic nephropathy [38]. The aim of this study is to determine the status of some haematological parameters (full blood count) MDA and GPx among diabetic patients attending Specialist Hospital, Sokoto. There is paucity of data on haematological parameters and free radical status of diabetic patients in Sokoto, North Western Nigeria. Data generated in this study will help policy makers and management of Specialist Hospital Sokoto on steps to take for effective diagnosis and management of diabetes and associated complications in patients.

2. METHODOLOGY

2.1 Study Area

This study was conducted in the Specialist Hospital, Sokoto. Samples were analyzed in the Haematology and Chemical pathology laboratory departments of Usmanu Danfodiyo University Teaching Hospital (UDUTH). The Specialist Hospital is a secondary health care institution located in Sokoto Metropolis, committed to the provision of quality healthcare services to people in Sokoto State and its surrounding. Sokoto state is located in the northwest of Nigeria, near to the confluence of the Sokoto River and the Rima River. It's located between longitudes 11°30" to 13° 50" East and latitude 4° to 6° North. It has a land area of about 25,973sq kilometers and a density of 170 sq kilometer. The state is in the dry Sahel surrounded by sandy terrain and isolated hills with an average annual temperature of 28.3°C (82.9°F). Sokoto state had a population of 3.6 million as at the 2006 census with projected population of 5,117,511.236 in 2017. The Metropolis is estimated to have a population of 427,760 people, majority of which are Hausa and Fulani while Zabarmawa, Tuareg and other non- indigenous settlers from the neighboring areas form the minority of the population [39].

2.2 Study Population

The study population for this research includes 58 patients with diabetes as subjects and 29 non-diabetic patients who were monitored as controls. Both subject and controls were recruited from the Specialist Hospital, Sokoto, North-Western Nigeria.

2.3 Study Design

This case control study which involves collection of blood samples from diabetic patients and non-diabetic patients and tested for full blood count, MDA and GPx estimation.

2.4 Sample Size Determination

The sample size was determined according to Cochran, [40] using the formula:

n = Minimum sample size
z = Standard normal deviation and probability.
P = Prevalence or proportion of value to be estimated from previous studies.
q = Proportion of failure (= 1 - P)
d = Precision, tolerance limit, the minimum is 0.05. Using prevalence of 3.9% [41]

Therefore,

$$n = \frac{Z^2pq}{d^2}$$

Where,

Z = 95% (1.96)
P = 3.9% (0.039)
q = 1 - 0.039 =0.961
d = 5% (0.05)

Therefore, $n = \frac{(1.96)^2(0.039)\ (0.961)}{0.05^2}$

$n = 58$

2.5 Ethical Consideration

Ethical approval of this study was obtained from the ethical committee of Specialist Hospital, Sokoto.

2.6 Inclusion and Exclusion Criteria

2.6.1 Inclusion criteria

- Confirmed patients with diabetes of all age group attending Specialist Hospital, Sokoto.
- Diabetic patients who were willing to give a written informed consent.

2.6.2 Exclusion criteria

- Non-diabetic patients.
- Diabetic patients who refused to give a written informed consent.
- Diabetic patients not attending Specialist Hospital, Sokoto.

2.7 Informed Consent

Written informed consent was obtained from the patients or guardian of all study participants.

2.8 Collection and Method of Analysis

Five milliliters (5.0 ml) of blood was collected from each participant. Immediately, 2.0 millilitres was transformed into EDTA bottle and the remaining 3.0 millilitres into a plain container. The EDTA anticoagulated sample was used for full blood count estimation while sample from the plain tubes was allowed to clot to obtain the serum for glutathione and malondialdehyde estimation. These samples were tested in Haematology and Chemical pathology Laboratory departments of Usmanu Danfodiyo University Teaching Hospital (UDUTH).

Full Blood Count was carried out using the five-part automated haematology analyser (Mythic 22CT, 2008). Glutathione Peroxidase Activity was assayed using Cayman's Glutathione Peroxidase Assay Kit [42] and Serum Malondialdehyde was determined using chemical method [43]

2.9 Data Analysis

Data obtained were entered into a statistical package (such as SPSS version 22) on a computer to define the nature of the distribution of data for each group. Statistical differences of data were analyzed using series of statistical analyses such as Mean, standard deviation, t-test, Analysis of variance (ANOVA) was used to compare the mean ±SD of the full blood count and free radicals of diabetic patients and the normal control subjects depending on the nature and distribution of data. Probability test was carried out to determine the level of significance. And a p-value of ≤ 0.05 was considered statistically significant.

3. RESULTS

A total of 58 diabetic patients and 29 non-diabetic (controls) were recruited for the study. The diabetics comprised of 37 (63.79%) males and 21(36.21%) females with overall mean age of 49.21 ± 11.37 and were tested for FBC, MDA and GPx.

Table 1 shows the FBC, MDA and GPx Status among Diabetic Subjects and Controls. There was a statistical increase in in WBC, Neutrophil, Eosinophil and Platelet count parameters between the diabetic subjects and controls ($P \leq 0.05$). There was no statistical difference in GPx, MDA and other FBC parameters between the diabetic subjects and controls ($P > 0.05$).

Table 2 shows the FBC, MDA and GPx of Diabetic Patients in the Study Groups Separated by Gender (Male and Female). There was no statistically significant difference in the FBC, MDA and GPx parameters between the diabetic patients and controls ($P > 0.05$).

Table 3 shows the FBC, MDA and GPx of Diabetic Patients in the Study Groups Separated by Marital Status; (Married and Single). There was a statistical increase in the basophil count among the single diabetic subjects. ($P \leq 0.05$). There was no statistical difference in GPx, MDA and other FBC parameters among the diabetic subjects ($P > 0.05$).

Table 4 shows the FBC, MDA and GPx of Diabetic Patients in the Study Groups Separated by Age Groups. There was statistically significant difference in the MDA among the diabetic patients in various age groups ($P \geq 0.05$).

Table 5 shows the FBC, MDA and GPx of diabetic patients in the study groups separated by smoking. There was no statistically significant difference in the FBC, MDA and GPx parameters among the diabetic patients ($P > 0.05$).

4. DISCUSSION

In this present study, we observed that the RBC, Hb and also PCV values as indices of anaemia were lower among diabetic subjects compared to non-diabetic controls, though they were not significant. This research is at variance with the previous findings which says that anaemia is prevalent among diabetic's patients and may also be significant in determining the outcome of heart failure and hypoxia-induced organ damage in patients with diabetes [44]. Oyedemi and colleagues also found that anaemia is a common finding in patients with diabetes, particularly in those with overt nephropathy or renal impairment [14]. Anaemia is associated with an increased risk of diabetic complications including nephropathy, retinopathy and macrovascular disease. Similarly, a previous study observed that the mean values of TRBC, Hb, PCV and MCHC for the diabetic patients are lower than the values of control group, indicating the presence of anemia in the former group [17]. The observed decrease in the RBC, Hb and PCV values of the diabetic patients could be attributed as a result of poor dieting and low standard of living by the diabetic patients.

The result also showed that the WBC, neutrophil, eosinophil and PLT counts were significantly higher among diabetic subjects compared to non-diabetics. However, a positive correlation was observed between raised TWBC, PLT and diabetes. In this present study, we observed that WBC count, neutrophil, eosinophil and PLTcounts were significantly higher among diabetic subjects compared to non-diabetics ($P \leq 0.05$). Our finding is in agreement with previous reports which suggest that platelet counts are higher and contribute to vascular events in patients with insulin resistance [45]. Diabetes mellitus is a metabolic syndrome. Previous report shows that PLT and TWBC counts are higher in patients with T1D than without the metabolic syndrome and that the rise is in a "dose-dependent" fashion. Increase in PLT and TWBC with increasing blood glucose in patients with T1D could be a result of a stress response. WBC counts correlated positively with platelet counts, which may suggest that a shared mechanism drives both the elevated platelet and WBC counts in patients with this syndrome [46]. Clinically elevated platelet counts are frequently seen in diabetics with a long duration of disease. Elevated platelet levels as well as platelet dysfunction could be injurious to the microcirculation and enhance the risk for vascular complications. Previous report seems to suggest the possibility that elevated platelet count could be used as a prognostic indicator of future diabetic complications [47]. Similarly, previous report has shown that raised PLT values are commonly seen in inflammatory and infectious diseases. Reactive thrombocytosis has also been reported among diabetic patients particularly those with poor diabetic control associated with raised blood sugar level [48]. However, the observed increase in WBC, neutrophil, eosinophil and PLT counts could be attributed as a result of poor health status of the diabetic patients in the locality.

There was a decrease in GPx levels among diabetic patients in comparison with the controls according to our study, though not statistically significant. This decrease among diabetic subjects could be attributed to increased oxidative stress as evidenced by lipid peroxidation. The antioxidant decrease reflects the war of antioxidants against oxidative stress to minimize the oxidative damage. When the total antioxidant status is high and enough to combat the oxidative stress the MDA levels are in the normal limits and vice versa. However, our finding is in variance with a study from South Karnataka on individual antioxidants among diabetics that reported a significant decrease of erythrocyte reduced Glutathione (GSH) whereas oxidized Glutathione (GST) levels are slightly elevated among the diabetics. Similar study from Hungary also reports both increase of few and decrease of other few individual antioxidant enzymes [49]. A recent study also reported decreased total antioxidant status in comparison to Diabetic patients with neuropathy by using the Cayman kit [50]. Duman and collaegues, from Turkey have observed significant decrease of antioxidants among the diabetic population [51]. Several studies have revealed lowered antioxidant and enhanced peroxidative status in diabetes condition [52]. There is still a need for further research in the field of free radicals and antioxidants. The prime aim should be in elucidating the underlying mechanisms by which free radicals bring about the pathogenesis. This would help expand the scope of treatment options.

Table 1. FBC, MDA and GPx parameters in study groups

Parameters	Control (29)	Diabetic subjects(58)	t-value	p-value
WBCC ($\times10^9$/l)	5.28±1.51	6.67±1.89	3.47	0.01
RBCC ($\times10^6$/µl)	4.26±0.77	4.12±0.60	0.42	0.08
Haemoglobin (g/dl)	12.47±2.15	11.81±1.74	-1.53	0.13
PCV ($\times10^9$/l)	35.75±6.37	34.00±4.77	-1.43	0.16
Monocyte ($\times10^9$/l)	8.04±5.14	5.90±3.75	-2.22	0.29
Neutrophil ($\times10^9$/l)	44.80±10.34	50.44±9.19	2.60	0.01
Lymphocyte ($\times10^9$/l)	44.67±8.86	43.46±8.36	-0.62	0.54
Eosinophil ($\times10^9$/l)	0.41±0.16	1.79±0.50	-1.95	0.05
Basophil ($\times10^9$/l)	0.41±0.16	0.49±0.24	1.62	0.11
Platelet ($\times10^9$/l)	263.90±71.00	297.53±79.14	-3.34	0.02
GPx (nmol/mn/ml)	14.15±12.00	10.90±7.51	-1.51	0.14
MDA (µmol/l)	25.22±8.63	28.72±11.17	1.48	0.14

Table 1 above shows the FBC , MDA and GPx parameters among diabetic subjects and controls. There was a statistical increase in WBC, Neutrophil, Eosinophil and Platelet count parameters between the diabetic subjects and controls (P ≤ 0.05)There was no statistical difference in GPx, MDA and other FBC parameters between the diabetic subjects and controls (P> 0.05)

Table 2. FBC, MDA and GPx parameters among diabetic subjects by gender

Parameters	Male 37(63.79%)	Female 21(36.21%)	t-value	p-value
WBCC ($\times10^9$/l)	6.82±2.17	6.40±1.22	-0.81	0.41
RBCC ($\times10^6$/µl)	4.29±0.62	4.36±0.55	0.43	0.67
Haemoglobin (g/dl)	11.64±1.85	12.10±1.51	0.96	0.34
PCV ($\times10^9$/l)	33.45±5.30	34.98±3.55	1.18	0.24
Monocyte ($\times10^9$/l)	5.61±3.55	6.39±4.09	0.76	0.78
Neutrophil ($\times10^9$/l)	51.50±10.13	48.57±7.08	-1.17	0.25
Lymphocyte ($\times10^9$/l)	42.20±8.76	45.69±7.28	1.54	0.12
Eosinophil ($\times10^9$/l)	1.77±0.41	1.82±0.65	0.35	0.73
Basophil (×109/l)	0.49±0.26	0.49±0.21	-0.02	0.98
Platelet ($\times10^9$/l)	195.73±70.04	228.00±91.14	1.53	0.13
GPx (nmol/mn/ml)	10.75±7.06	11.16±8.43	0.19	0.85
MDA (µmol/l)	29.77±10.84	26.86±11.78	-0.96	0.34

Table 2 above shows the FBC, MDA and GPx of diabetic patients in the study groups separated by gender; (male and female) There was no statistically significant difference in the FBC, MDA and GPx parameters between the diabetic patients and controls across gender (P> 0.05)

Table 3. FBC, MDA and GPx parameters among diabetic subjects by marital status

Parameters	Married 54(93.10%)	Single 4(6.90%)	t-value	p-value
WBCC ($\times10^9$/l)	6.64±1.91	7.10±1.53	-0.47	0.64
RBCC ($\times10^6$/µl)	3.94±0.86	4.35±0.58	1.36	0.18
Haemoglobin (g/dl)	10.88±1.11	11.88±1.76	1.12	0.27
PCV ($\times10^9$/l)	33.98±4.90	34.30±2.85	-0.13	0.90
Monocyte ($\times10^9$/l)	6.06±3.83	3.63±0.57	1.26	0.21
Neutrophil ($\times10^9$/l)	51.02±9.18	42.70±5.23	1.78	0.08
Lymphocyte ($\times10^9$/l)	43.12±8.28	48.15±9.29	-1.17	0.25
Eosinophil ($\times10^9$/l)	1.77±0.51	2.05±0.21	-1.06	0.29
Basophil ($\times10^9$/l)	0.47±0.24	0.73±0.25	-2.04	0.04
Platelet ($\times10^9$/l)	211.54±80.13	153.50±37.19	1.43	0.16
GPx(nmol/mn/ml)	10.91±7.74	10.82±3.82	0.02	0.98
MDA(µmol/l)	28.86±11.13	26.85±13.32	0.35	0.73

Table 3 above shows the FBC, MDA and GPx of diabetic patients in the study groups separated by marital status; (married and single). There was a statistical increase in the basophil count among the single diabetic subjects (P ≤ 0.05). There was no statistical difference in GPx, MDA and other FBC parameters among the diabetic subjects (P> 0.05)

Table 4. FBC, MDA and GPx parameters among diabetic subjects by age groups

Parameters	Diabetic patients	f-value	p-value
WBCC (×10^9/l)			
20-29	7.76±0.49		
30-39	7.35±2.65		
40-49	6.90±2.51	0.97	0.43
50-59	5.99±0.97		
60-79	6.51±1.66		
70-80	7.16±1.05		
RBCC (×10^6/µl)			
20-29	3.97±1.15		
30-39	4.52±0.53		
40-49	4.52±0.46	1.06	0.39
50-59	4.37±0.44		
60-70	4.22±0.75		
70-80	3.84±0.83		
Haemoglobin (g/dl)			
20-29	12.20±1.93		
30-39	12.06±2.09		
40-49	12.30±1.57	1.02	0.41
50-59	11.88±1.22		
60-69	10.94±2.38		
70-80	10.93±0.87		
PCV (×10^9/l)			
20-29	35.23±4.24		
30-39	35.56±3.25		
40-49	33.99±5.25	2.22	0.06
50-59	35.54±2.74		
60-69	31.60±5.58		
70-80	28.30±8.00		
Monocyte (×10^9/l)			
20-29	5.46±3.24		
30-39	4.12±1.43		
40-49	5.66±2.95	0.84	0.52
50-59	6.78±4.37		
60-69	6.70±5.07		
70-80	3.90±0.20		
Neutrophil (×10^9/l)			
20-29	45.66±2.74		
30-39	51.33±12.65		
40-49	49.07±6.03	0.90	0.48
50-59	48.93±10.02		
60-69	55.08±8.03		
70-80	51.73±14.60		
Lymphocyte (×10^9/l)			
20-29	46.86±2.49		
30-39	45.88±11.24		
40-49	44.72±7.24	0.89	0.49
50-59	43.75±9.13		
60-69	39.80±7.00		
70-80	39.00±8.05		
Eosinophil (×10^9/l)			
20-29	1.80±0.30		
30-39	1.90±0.56		
40-49	1.78±0.66	0.27	0.93

Parameters	Diabetic patients	f-value	p-value
50-59	1.83±0.45		
60-69	1.64±0.44		
70-80	1.83±1.52		
Basophil (×10^9/l)			
20-29	0.63±0.32		
30-39	0.43±0.21		
40-49	0.44±0.20	1.14	0.35
50-59	0.44±0.24		
60-69	0.58±0.31		
70-80	0.66±0.15		
Platelet (×10^9/l)			
20-29	168.00±55.75		
30-39	231.12±101.13		
40-49	215.73±93.94	0.52	0.76
50-59	212.72±67.66		
60-69	190.09±77.33		
70-80	176.00±22.52		
GPx (nmol/mn/ml)			
20-29	13.58±1.47		
30-39	11.59±4.07		
40-49	11.54±6.19	0.30	0.90
50-59	10.03±9.61		
60-69	8.34±8.99		
70-80	8.70±6.59		
MDA			
20-29	26.50±7.38		
30-39	35.28±14.58		
40-49	27.28±11.96	2.29	0.05
50-59	30.30±9.48		
60-69	23.14±6.64		
70-80 (µmol/l)	31.94±19.13		

Table 4 above shows the FBC, MDA and GPx of diabetic patients in the study groups separated by age groups. There was statistically significant difference in the MDA among the diabetic patients in various age groups (P≤ 0.05)

Table 5. FBC, MDA and GPx parameters among diabetic subjects by smoking

Parameters	Non-Smokers 52(89.66%)	Smokers 6(10.34%)	t-value	p-value
WBCC (×10^9/l)	6.75±1.93	5.98±1.34	0.94	0.35
RBCC (×10^6/µl)	4.31±0.59	4.39±0.56	-0.33	0.75
Haemoglobin (g/dl)	11.80±1.79	11.85±1.24	-0.06	0.95
PCV (×10^9/l)	34.22±4.55	32.13±6.57	1.02	0.31
Monocyte (×10^9/l)	5.68±3.42	7.72±6.02	-1.27	0.21
Neutrophil (×10^9/l)	50.94±9.44	46.15±5.33	1.21	0.23
Lymphocyte (×10^9/l)	43.23±8.59	45.52±6.15	-0.63	0.53
Eosinophil (×10^9/l)	1.78±0.52	1.83±0.39	-0.21	0.83
Basophil (×10^9/l)	0.50±0.24	0.33±0.10	1.70	0.09
Platelet (×10^9/l)	210.53±80.43	181.50±67.04	0.84	0.40
GPx (nmol/mn/ml)	10.87±7.57	11.09±7.58	-0.06	0.94
MDA (µmol/l)	28.70±11.57	28.85±7.50	-0.03	0.97

Table 5 above shows the FBC, MDA and GPx) of diabetic patients in the study groups separated by smoking. There was no statistically significant difference in the FBC, GPx and MDA parameters among the diabetic patients (P> 0.05)

Key: PCV- Pack cell volume, MDA- Malondialdehyde, GPx- Glutathione peroxidase, RBCC- Red blood cell count, WBCC-White blood cell count, µMol/l- Micromole per litre, nmol/mn/ml- Number of mole per minute per milliliter

There were no statistically significant differences between the FBC values based on the gender of subjects (p = 0.13). Our findings are consistent with previous report which indicated that the RBC and hematocrit concentrations were similar in male and female diabetic patients [53]. However, a previous report showed that there also seems to be a connection between raised platelet number and female gender [47]. However, the result also showed no statistical difference in the GPx and MDA levels based on the gender of subjects (P>0.05).

The result of the present study showed that there is a statistically significant decrease in the basophil count based on marital status of the subjects (P≤ 0.05). This decrease in the basophil count of the married subjects when compared with controls could be attributed as a result of depression in the bone marrow or other conditions such as hypersensitivity or immune-suppressant therapy. However, the result also indicated no statistically significant differences in the other FBC, GPx and MDA values based on the marital status of the subjects. This study also indicated a lower RBC, PCV, Hb and also WBC count among the married subjects compared to the singles. This could be attributed due to the youthful age of the singles (as they are found to be within the age range of 29-34) as they mostly engage in exercises and hence, enhances erythropoises.

The result of the present study showed that there is a statistically significant increase in the MDA levels of the diabetic subjects, based on their age groups (P≤ 0.05). The elevated level of MDA based on the age groups could be attributed as a result dried foods consumption and exposure chemicals such as pesticides and environmental pollution. The result also showed that there were no statistically significant differences in between the FBC and GPx values based in the age groups of the subjects. There is age-related decrease in activity of the GPx antioxidant, though no statistical difference is attained. However, this is in consistent with consistent with the hypothesis that increased free radical damage contributes to aging [54]. A recent study provides evidence that lower levels of Se may contribute to inflammation and mortality in older women [55]. However, few studies have examined the activity of GPx, which depends on Se for its activity, in adults older than 65 years, and fewer studies have examined GPx activity in adults older than 65 years with comorbid illness and disability. This population best reflects the accumulation of free radical damage that results in age-associated conditions, in keeping with the free radical theory of aging [54]. We hypothesized that GPx activity varies by age and would be lower in older compared to younger adults.

The result of the present study showed no statistically significant difference in the FBC, GPx and MDA level of the diabetic subjects based on cigarette smoking (P>0.05). The study however, showed almost similar result in all the parameters and this may be as a result of lower number of cigarette smoking diabetic patients in the study. By reduction in number of cigarette smokers as evidence in the study, cigarette smoking increases the risk of diabetic nephropathy, retinopathy and neuropathy probably via its metabolic effects in combination with increased inflammation and endothelial dysfunction. This association is strongest in type 1 diabetic patients. The increased risk for macro-vascular complications, coronary heart disease (CHD), stroke and peripheral vascular disease is most pronounced in type 2 diabetic patients. The development of type 2 diabetes is another possible consequence of cigarette smoking, besides the better known increased risk for cardiovascular disease [56].

5. CONCLUSION

In this research we found that, some haematological parameters such as WBC count, neutrophil, eosinophil and platelet counts were higher in diabetic subjects, while other FBC parameters, MDA and GPx were not affected. Basophil was also affected based on marital status. And MDA is affected based on age group, while other socio-demographic factors have no effect on the FBC, MDA and GPx parameters of the diabetic patients and controls.

6. RECOMMENDATIONS

There is need for a further research on direct free radical estimation and total antioxidant status in relation to diabetes, which gives more information than the indirect method of estimation. It is recommended that white blood cells and platelet levels should be closely monitored when treating diabetic patients. It is recommended that, FBC and free radical status of the diabetic patients should be made a routine test for proper management of diabetes.

CONSENT

As per international standard or university standard, patient's written informed consent was obtained before participating in the study.

ETHICAL APPROVAL

Ethical approval was obtained from Ethics committee of Specialist Hospital, Sokoto, Nigeria with the following reference number: SHS/SUB/133/VOL 1.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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