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Studies on Oxidative Stress Markers Parameters of Type 2 Diabetics in Owerri, Imo State

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Abstract **Original Research Article**

Type 2 diabetes mellitus is a chronic metabolic disorder which has emerged as a health challenge globally due to its insidious on set, late recognition and complications. The present study was aimed at evaluation of oxidative stress markers in Owerri. Cross Sectional Studies was conducted at Federal Medical Center and Imo State specialist Hospital, Owerri. A total of three hundred subjects which include each one hundred and fifty type 2 diabetics and apparently control subjects between the ages forty and sixty nine years were recruited. Ten millimeters of venous blood was aseptically collected from thesubjects. Spectrophotometric, nephlometric, enzyme immunoassay were used for determination of these parameters. The data was analyzed using statistical package for social science 20.0. Test with a probability value of $P<0.05$ was considered statistically significant. Result from oxidative stress markers showed statistically significantly higher value ($P=0.001$) in malondialdehyde (2.02 ± 0.32 v 1.76 ± 0.28nmol/l), lower values ($P = 0.0001$) in glutathione peroxidase, superoxide dismutase, vitamin C and Vitamin E (43.22 ± 5.74 v 54.77 ± 7.8iu/l, 171. 62 ± 6.91 v 180.55 ± 7.20u/l, 0.84 ± 0.26 v 1.45 ± 0.32mg/dl, 0.80 ± 0.11 v 1.12 ± 0.17mg/dl) when type 2 diabetics was compared with control subjects The female type 2 diabetics had statistically significantly higher value ($P = 0.001$) in vitamin E when compared with male (0.83 ± 0.89 v 0.77 ± 0.11mg/dl).

Keywords: Type 2 diabetes, chronic metabolic disorder, Diabetes Mellitus.

INTRODUCTION

Diabetes Mellitus is a chronic metabolic disease which is associated with a high incidence rate of morbidity and mortality globally. Type 2 diabetes mellitus is the most prevalent one and makes up 90% of cases of diagnosed diabetes [1]. Type 2 diabetes mellitus formerly known as non-insulin dependent diabetes mellitus or adult-onset diabetes is a metabolic disorder that is characterized by hyperglycaemia due to insulin resistance and relative reduced insulin secretion by beta pancreatic cells [2]. International Diabetic Federation [3] reported that 425 million (8.8%) adults between the ages of twenty to seventy - nine years had diabetes and predicted to rise up to 625 (9.9%) by the year 2045. According to Adeloye *et al.*, [4], the pooled prevalence of type 2 diabetes in Nigeria in 2017 was 5.7%. Also, the prevalence of 5.7% was reported by Uloko *et al.*, [5] as pooled geopolitical prevalence in 2018. In Imo State, the prevalence of type 2 diabetes was 8.7% was which reported among geriatric individuals [6].

Diabetes mellitus is a state of oxidative stress that results in unexpected complications [7]. Oxidative stress secondary to persistent hyperglycaemia and dyslipidemia plays a key role in the pathogenesis of type 2 diabetes mellitus and its complications manifest by excess reactive oxygen species, auto-oxidation of glucose, non-enzymatic protein glycosylation, lipid peroxides formation, impaired activities of antioxidant defense [8]. Lipid peroxidation is an autocatalytic free radical-mediated process where poly-unsaturated fatty acids in cell membranes undergo degradation to form lipid hydroperoxidation and the secondary products which include malondialdehyde, 4-hydroxynonenal, hexanal, propanal [9]. Antioxidants are molecule which inhibits oxidation of other molecules by removing free radicals intermediate such as superoxide anions, hydroxyl radicals, and hydrogen peroxide, protect and prevent to an extent oxidative destruction of cellular tissues [10]. These antioxidants include enzymes such as superoxide dismutase, glutathione perioxidase, catalase, redutase; co enzyme Q10 ; non enzymatic which include low molecular weight compounds such as glutathione, uric acid, bilirubin; minerals such as

selenium, manganese, zinc; vitamins such as A, C and E; carotenoid, flavonoid, and others [11, 12]. Depletion of these antioxidants defense increases the risk of complications in type 2 diabetes mellitus [13].

Malondialdehyde is secondary product of lipid peroxidation and its biomarker is an indicator for free radicals [14]. Superoxidase dismutase is an antioxidant enzyme that catalyses the conversion of superoxide radical to hydrogen peroxide and molecular oxygen [15]. Vitamin E is fat soluble antioxidant that prevents free radicals, reduces cytotoxic effect of oxidized lipoprotein, smooth muscle, proliferation, platelets aggregation and inflammation [11].

The study was done to evaluate the levels of oxidative stress markers in Type 2 Diabetics in Owerri.

MATERIALS AND METHODS

Study Area

The cross sectional study was conducted at Federal Medical Centre, Owerri and Imo State Specialist Hospital, Owerri.

Study Population

The sample size for the study was calculated using the formula below, according to Aronye [16]. The prevalence rate of type 2 diabetes mellitus in Imo State is 8.7% [6].

$n = z^2p(q)/d^2$

Where

q = 1-P
n = Sample size
p = prevalence of type 2 of diabetes mellitus in Imo State- 8.7%
z = confidence interval 95% - 1.96
d = Degree of accuracy- 0.05
$n = 1.96^2 \times 0.087(1-0.087)/0.05^2 = 3.8416 \times 0.07943/0.0025$
$= 122.057 = 122$
Therefore, the minimum sample size will be 122.

Subjects

Three hundred subjects of both sexes between the ages of fifty to sixty-nine years were recruited for the study. One hundred and fifty type 2 diabetics attending clinic of Federal Medical Centre, Owerri and Imo State Specialist Hospital, Owerri for at least six months were eligible for the study. Also, one hundred and fifty apparently healthy individuals were recruited who came for check up for medical fitness and served as the control subjects.

They were further grouped according to their ages 40-49, 50-59 and 60-69 years, and also according to sex; male (n=75) and female (n=75)

Parameters Studied

The parameters evaluated in this study include malondialdehyde, gluthathione peroxidase, superoxide dismutase vitamin C and vitamin E.

Study Design

This is a cross-sectional study that involved type 2 diabetics and control subjects.

Site of the Study

The analysis was carried out at the chemical pathology laboratory of the Department of Medical laboratory Science of Imo State University, Owerri and Federal Medical Center, Owerri through a letter obtained from the Head of Department through my Supervisor.

Laboratory Procedure

All reagents were commercially purchased and the manufacturer's standard operating procedures were strictly adhered to.

Determination of Glucose

This was carried out by enzymatic oxidase - peroxidase method according to Tietz *et al.*, 2006 [17] as modified by Randox Laboratories, United Kingdom. Catalog number; GL 1021, GL 304, GL366.

Procedure

Three dry cleaned plain test tubes were setup in a jack, labeled test, standard and blank. Then, 20µl of sample and standard wore pipetted into the tubes labeled these and standard respectively. In all the tubes, 2000µl of reagents I was added.The tubes were gently mixed, incubated at 37^{o}C for 10 minutes. The absorbance of sample and standard were read spectrophotometrically at 500nm.

E Determination of Iipid Peroxidation Product - Malondialdehyde

The test carried out by Thiobarbituric acid reactive substance spectrophotometric method, according to Devasgaman *et al.*, 2003 [18].

Determination of Lipid Peroxidation Standard Curve

Procedure

A linear standard curve was prepared by dissolving 24.6mg of tetraethoxypropane in 100mL of deionised water to give a stock solution.

Working standards were prepared by making the following dilution from the stock solution; 1:25, 1:50, 1:75, 1:100, 1:150, 1:200, 1:250, 1;400, 1:500, 1:800, 1:1000 with 0.01N hydrochloric acid in eleven tubes.

Then, 0.5ml of the working solution was added to all the tubes. Also, 2.5ml of 20% trichloroacetic acid was added to each tube. They were gently mixed and

allowed to stand for 10 minutes. In all the tubes, 2.5ml of 0.05M sulphuric acid and 3.0ml of thiobarbituric acid were added respectively. It was allowed to boil for one hour and the tubes were cooled under running tap water. In all tubes, 4.0ml of butan-1-ol was added and it was mixed using a votex mixer and centrifuged for 30 minutes at 2,000rpm.The upper layer containing the thiobarbituric acid reactive substance was carefully separated and it was read spectrophotometrically at 530nm. The standard curve of absorb against malondialdehyde concentration was plotted.

Determination of Lipid Peroxidation Product - Malondialdehyde

The test was carried out by Thiobarbituric acid reactive substance spectrophotometric method, according to Devasgaman *et al.,* [18].

Procedure

In a centrifuge tube, 0.25ml of the sample and 1.25ml of 10% tetrachloroacetic acid was added and was allowed to stay for 10 minutes. Then, 1.25ml of 0.05ml of sulphuric acid and 1.5ml of 0.06% of thiobarbituric acid solution was mixed gently and was placed in boiling water for one hour. The tubes were cooled under running water. Then 2.0ml of butan-1-oll was added. The TBA reactive material was extracted and the absorbance was read at 532nm. The values of the TBA reactive material were extrapolated from the standard curve.

Determination of Glutathione Peroxidase

This test was carried out by erythrocyte glutathione peroxidase activity spectrophotometric method, according to Paglia and Valentine [19] as modified by Randox Laboratories limited. Catalog number: RS 504, RS 505 HG 1539.

Procedure

In a dry cleaned plain test-time, 0.05ml of heparinized whole blood was diluted with 1 ml of the diluting agent. It was mixed, incubated at for 5 minutes at 25%c and 1.0ml of haemoglobin reagent was added. Two dry cleaned plain test tubes were set up, labeled test and blank. Then, 0.05ml of the diluted sample was added to the tube labeled test and 0.05ml of distilled water was added to the tube labeled blank. In all the tubes, 2.5ml of R1 (mixture of RIa and R1b) and 0.1ml of cumen hydroperoxide was added appropriately. The tubes were allowed to stay for one minute. The absorbance was read spectrophotometrically at 340nm and was re-read after one and two minutes. The reagent blank value was subtracted from the sample value. Appropriate calculation was made.

Determination of Superoxide Dismutase

The test was carried out by xanthine oxidese method according to Woolliams *et al.,* [20] as modified by Randox laboratories Limited, United Kingdom. Catalogue number: SD 124, SD 126.

Procedure

A clean dry centrifuge tube was set up in a rack; 0.5ml of heperinised whole blood was added. Ti was centrifuge at 3,000 rpm for ten minutes and the plasma aspiration off. The red cells was washed for four times with 3 ml of 0.9l of sodium chloride solution, centrifuge for ten minutes at 3,00 rpm after each wash. The washed red cell was made up of 2.0ml with cold re-distilled water.

Standard curve was prepared by diluting 5ml of S6 (undiluted standard) to produce standard S5 to S3 with 6ml of sample diluents to produce S2 respectively. Then 0.05ml of diluted washed cells standard and Ransod sample diluents was added to the corresponding labeled tubes in all the tubes, 1.7ml of mixed substrate and 0.025ml of xanthine oxidase was added. The initial absorbance A was read after 30 seconds and the final three minutes against sample diluents at 505 nm, the result was recorded.

Calculation

$$\Delta A\ /\min ofstdorsample = \frac{A_2 - A_1}{3}$$

$$\%inhibition \frac{\Delta Astdorsample}{\Delta S_1/min} x\ 100$$

$$\%\ inhibitionwasobtainedfromthethestandardcurve$$

$$SODunitmlofwjoleblood = SODunitmlfromstdcurvex\ 100$$

Determination of Vitamin C

This method was carried out by 2,4 – dinitrophenylhydrazine spcetrophotometric method ,according to Roe,1960 .

Procedure

A dry cleaned plain test tube was set up in a rack, 1.0ml of plasma, 1.0ml of 10% trichloroacetic acid and 0.5ml of chloroform were added. It was stoppered, shaken vigorously for 15 seconds and centrifuged for 10minutes at 2,000 rpm. It was separated to obtain test supernatant. Three dry cleaned plain test tubes were set up, labeled test, standard and blank. Then, 1.0ml of clear supernatant was added to the tube labeled test and 0.5ml of ascorbic acid standard was added to the tube labeled standard. In the tubes, labeled standard and blank, 0.5ml of 10% trichloroacetic acid was added. In all the tubes, 0.4ml of colour reagent was added. They were stopped, mixed and placed in water bath at 56^0C for 1 hour. It was allowed to cool in the bath for 5 minutes. In all the tubes, 2.0ml of ice cold 85% sulphuric acid was added. It was allowed to stay at room temperature for 30 minutes. The standard was read spectrophmetrically at 570nm against blank.

Determination of Vitamin E

The test was carried out by spectrophtometric micro method according to Quife *et al.,* [21].

Procedures

Three clean dry test tubes were set up, labeled test, standard and blank. Then, 1.5ml of plasma, standard reagent and distilled water was pipetted into corresponding tubes labeled test standard and blank accordingly. In the tubes labeled test and blank, 1.5ml of absolute ethanol was added, while to the tube labeled standard, 1.5ml of distilled water was added. In all the tubes, 1.5 ml of xylene was added, mixed very well and centrifuged for ten minutes. The xylene layer was separated and 1.0ml was pipette to each of the new corresponding tubes. In all the tubes, 1.0ml of alpha, alpha - dipyridyl was pipetted, stoppered, and was well mixed. In a clean dry curvette, 1.5 ml of each of test and standard was added separately and was read against the blank at 460nm. Finally, 0.33ml of ferric chloride was added to all the tubes and the final absorbance was read after 15 minutes at 520nm. The readings were recorded.

Calculation

$$\frac{readingoftestat\ 520nm - rreadingoftestat\ 460x0.29x10}{readingofstandardat\ 520nm}$$

Statistical Analysis

Data was analyzed using software, statistical package for social science (SPSS), version 20.0. The results were expressed as mean and standard derivation (mean±SD). Comparison of differences in the mean values between the groups were assessed using one way analysis of variance (Anova) and Independent student T – test. Variable between the age groups was assessed using Post Hoc test.

RESULTS

Table-1: Comparison of the Mean Values of Oxidative Stress Markers In Type 2 Diabetes Mellitus and Control Subjects of The Study Population

Parameters	Type 2 diabetes mellitus subjects (n = 150) mean ± SD	Control subjects (n = 150) mean± SD	T-test	P. value
Malondialdehyde (nmol/L)	2.02 ±0.32*	1.76 ±0.28	7.451	0.0001
Glutathione peroxidase(iu/L)	44.13 ±5.74*	54.77 ±7.80	13.520	0.0001
Superoxide dismutase (u/mL)	171.62± 6.91*	180.55± 7.20	10.952	0.0001
Vitamin C (mg/dL)	0.84 ±0.26*	1.45 ±0.32	17.931	0.0001
Vitamin E (mg/dL)	0.80 ±0.11*	1.12 ±0.17	19.616	0.0001

KEY

n: Number of subjects in each group

***:** statistically significant when compared with type 2 diabetic group (P<0.05).

Table-1 shows the mean values of some of the oxidative stress markers; malondialdehyde, glutathione peroxidase, superoxide dismutase, vitamin C and vitamin E in type 2 diabetics and control subjects. From the study, the mean value of malondialdehyde (2.02 ± 0.32) in type 2 diabetics was higher which was statistically significant (P = 0.0001) when compared with the mean value (1.76 ± 0.08) of the control subjects.

Inversely, the mean value of enzymatic antioxidant; glutathione perioxidase (44.13 ± 5.74) was lower in type 2 diabetics which was statistically significant (P=0.0001) when compared with the mean value (54.77 ± 7.80) of the control subjects.

Also, the mean value of superoxide dismutase (171.62 ±6.91) was lower in type 2 diabetics which was statistically significantly (P = 0.001) when compared with the mean value (180.55 ± 7.20) of the controlled subject.

Also, the mean value of superoxide dismutase (171.62 ±6.91) was lower which was statistically significantly (P = 0.001) when compared with the mean value (180.55 ± 7.20) of the controlled subject.

The mean value of non enzymatic antioxidant; vitamin C (0.84 ± 0.26) was lower in type 2 diabetics which was statistically significant (P = 0.001) when compared with the mean value (1.45 ± 0.32) in the control subjects.

Also, the mean value of non-enzymatic antioxidants vitamin E (0.80± 0.11) was lowed in type 2 diabetics which was statistically significant (P=0.001) when compared with the mean value (1.12±0.17) of the control subjects.

Table-2: The Mean Values of Measured Oxidative Stress Markers in Type 2 Diabetes Mellitus Subjects in Relation to Age of the Study Population.

Parameters	40-49 Years (n=50) mean ± SD	50-59 Years (n=50) Mean ± SD	60-69 (n = 50) mean ± SD	F-value	P-value	P-value a vs b	P-value b vs c
Malondialdehyde(nmol/L)	169.83 ±26.4	143.64 ±22.7*	143.48 ±21.9	20.332	0.0001	0.0001	0.999
Glutathione	1.98 ±0.34	1.99 ±0.25	2.08 ±0.35	1.607	0.204	0.967	0.336
peroxidase(iu/L)	43.22 ±5.7	45.52 ±6.22	43.64 ±4.90	2.317	0.102	0.111	0.227
Superoxide dismutase	174.04± 8.41	172.84± 7.79	169.28± 4.61	3.008	0.056	0.824	0.189
Vitamin C (mg/dL)	0.80 ±0.26	0.88 ±0.29	0.82 ±0.25	1.499	0.227	0.230	0.403
Vitamin E (mg/dl)	0.70± 0.09	0.81±0.11*	0.80±0.11*	8.71	0.0001	0.001	0.876

KEY

n: Number of subjects in each group
p - value: P- value across all type 2 diabetics age groups
p valuea vs b: P-value of comparison between the age group of 40-49 and 50-59 years in type 2 diabetics
P valueb vs c: P-value of comparison between the age group of 50-59 and 60-69 years
***:** statistically significant when compared between the ages of 40-49 years (P<0.05).
****:** statistically significant when compared between the ages of 50-59 years (P<0.05).

The results showed progressive increase in the mean value of malondialdehyde across all age groups (1.98 ± 0.34; 1.99 ± 0.25; 2.08 ± 0.35) which was not statistically significant (p=1.607). There was increase in the mean value between the ages of 50-59 years (1.99± 0.25) which was not statistically significant(p = 0.204) when compared with the ages of 40-49 years (1.98 ± 0.34) and also 60-69 years (2.08 ± 0.35) which was not statistically significant (p = 0.967) when compared with the ages of 50-59 years (1.99 ± 0.25).

There was non progressive non statistically (p = 0.204) increase in the mean value of glutathione peroxidase in all the age groups (43.22 ± 5.7; 45.52 ± 6.22; 43.64 ± 4.90).The mean value of glutathione peroxidase between the ages of 50 - 59 years (45.52 ± 6.22) was increased which was not statistically significant (P = 0.111) when compared with the age between 40-49 years (43.22 ± 5.7). While, between the ages of 60-69 years (43.64 ± 4.90), there was decrease which was not statistically significant (P = 0.227) when compared with the age between 50 - 59 years (45.52 ± 6.22).

Result showed that there was progressive decrease in the mean value of superoxide dismutase across all age groups (43.64 ± 4.90; 172.84±7.79; 169.28±4.61) which was not statistically significant (p = 0.056). the mean value between the ages 50-59 years (172.84±7.79) was decreased which was not statistically significant (p = 0.824) when compared with the mean value of ages between 40-49 years (174.04±8.41). also there was a decrease in the mean value of ages between 60-69 years (169.28±4.61) which was not statistically significant (p=0.189) when compared with the mean value of ages between 50-59 years (172.84±7.79).

There was no significant difference (P=0.227) in the mean value of vitamin C across all age groups (0.80 ± 0.20; 0.88 ± 0.29; 0.82 ± 0.25). Also, between the ages of 40 - 49 years (0.80 ± 0.20) and 50 - 59 years (0.88 ± 0.29), there was no significant difference (p = 0.230). Similarly, between 50 - 60 years and 60 - 69 years (0.82 ± 0.25), there was no significant difference (P=0.403).

There was non progressive increase in the mean value of Vitamin E across all age groups (0.70±0.09; 0.81±0.11; 0.80 ±0.11) which was statistically significant (p = 0.0001). The mean value between the ages of 50-59 years (0.81±0.11) was increased but was statistically significant (p = 0.01) when compared with the mean value of ages between 40-49 years (0.70±0.09). The mean value between the ages of 60-69 years (0.80 ±0.11) was increased, but was not statistically significant (p=0.876) when compared with the mean value between the ages of 50-59 years (0.81±0.11).

DISCUSSION

Higher level of malondialdehyde was observed in type 2 diabetics when compared with the control subjects. Malondialdehyde is a biomarker of lipid peroxidation and oxidative stress metabolic disturbances contributes to oxidative stress which results from an imbalance between oxidants such as reactive oxygen species; reactive nitrogen species and antioxidant defend system. Diabetics undergo oxidative stress due to prolonged exposure to hyperglycaemia which causes tissue damage through increased intracellular formation of advanced glycation end products, activation of protein kinase C isoforms, polyol pathway, glucose oxidation and overeactivity of hexosamine pathway. The free radicals formed impairs membrane function in type 2 diabetics by decreasing membrane fluidity, increases glycation of proteins, enhances oxidation of low density lipoprotein which can lead to macrovascular and microvascular complications. The present study agrees with the

previous studies. Aouacheri *et al*., [22] reported higher level of malandialdehyde in type 2 diabetes due to increased lipid peroxidation in cell membrane. This study is consistent with the work of Nakhjavan *et al.*, [23] and Khemka *et al.,* [24] that reported that type 2 diabetes was independent associated with increase level of lipid peroxidation, oxidation of low density lipoprotein formation of atherosclerosis risk and cardiovascular diseases. This is in accordance with the study conducted by Hamaira *et al.,* [25] that showed high level of malondialdehyde in type 2 diabetes due to weak enzymatic and non-enzymatic antioxidant defense system to overcome oxidation stress. Yousif *et al.,* [26], reported that there was elevated level of malondialdehye in diabetes which is associated with an increased production of plasma free radicals and a significance reduction in antioxidant mechanism. The present study observed non-significant increase in ages of type 2 diabetes. This may be due to glycemic control of which reduces hyperglycemic induced lipid peroxidation. This agrees with the work of Majeed *et al.,* [27] that reported that poorly glycemic diabetic had significant MDA level while good glycemic level can reduce its plasma level. Nemtosova *et al.,* [28] reported that increase in the level of malondialdehyde increases at the age above sixty years accompanied by poor effectiveness of antioxidant protection. The findings of this study showed that there was non-significant level of increase in the level of malondialdehyde in female type 2 diabetics when compared with male type 2 diabetics: contrary, Nakhjava *et al.,* [23] in their study suggested that uncontrolled hyperglycaemia in diabetes mellitus promotes lipid peroxidation independent of glycemic control antioxidant activity, age and sex.

The current study observed that there was lower level of glutathione peroxidase in type 2 diabetes when compared with the control subjects. This is due to increased oxidative stress which reflects an imbalance between production of reactive oxygen species such as hydrogen peroxide and neutralization by antioxidant glutathione peroxidase which is an endogenous antioxidant that scavenges free radicals by reduction of reactive hydrogen peroxides and fatty acid or lipid hydroperoxide to water and alcohol with the oxidation of glutathione respectively. Hence, continuous production of these reactive species will impair glutathione activity thereby decrease its level. Low level 0f glutathione peroxidase is due to competition for NADPH between aldose and glutathione reductase in polyol pathway where glucose is converted to sorbitol by utilizing NADPH. Increased sorbitol pathway utilizes NADPH, leading to decreased regeneration of reduced glutathione which impair antioxidant defense by glutathione peroxidase and decreases it concentration. The low activity of glutathione peroxidase may be due to nonavailability of selenium due to glycation of this selenium dependent enzyme by formation of advanced glycation end product which deprived functional antioxidant activity. This may lead generation of excessive reactive species such as hydrogen peroxide and superoxide radicals which are implicated in vascular endothelial dysfunction with deprivation of nitric oxide that leads to atherosclerosis and cardiovascular complications. The present study agrees with previous studies. This study is in agreement with the study of Gawik *et al.,* [29] that observed low activity of glutathione peroxidase in type 2 diabetes due to low content of glutathione. This is in accordance with the work of Palekar and Ray [30]; Lutchmansingh *et al.,* [31] that reported reduced erythrocyte glutathione peroxidase may be attributed to metabolism of excessive glucose by polyol pathway with decreased recycling of glutathione by utilization of NADHP. Gonzaler de Vega *et al.,* [32] observed low level of glutathione peroxidase in advanced diabetic complication due to decrease dietary selenium due to glycation of selenoprotein found in type 2 diabetes. Also, Waggillah and Alzo Hairy [33] found decreased level of GPX due to increased free radicals.

Production such as hydrogen peroxide, superoxide radicals which participate in oxidation of red cell component such as hemoglobin and decreased it oxygen capacity function which resulted in anaemia found in type 2 diabetics. Bikkad *et al.,* [34] reported decreased activity due to advanced glycated end products which is involved in development atherosclerosis and vascular complications. There was inconsistent non-significant difference in the level of glutathione peroxidase in relation to ages. This is due to compensatory defense of GPX mechanism on the may not associate with ages in type 2 diabetes. The study in accordance the work of Briggs *et al.,* [35] who reported no significance difference in different ages, Also, Hisalkar *et al.,* [36] reported no significant correlation in level glutathione in various age groups. The finding of this work observed no significance difference in male and female type 2 diabetes. This is also in with the work of Hisalkar *et al.,* [36] that found no significant difference in both sex.

The present study observed lower level of superoxide dismutase in type 2 diabetes when compared with control subjects. Superoxide dismutase is an endogenous antioxidant first line of defense against induced oxidative stress. Decrease in superoxidase dismutase found in type 2 diabetics results from mitochondrial overproduction of superoxide in endothelial cells blood vessels and mycardium through polyol pathway flux, over reacting of hexokinase pathway, formation of advanced glycated end products and its receptors. Superoxide directly inactivate anthersclerotic enzymes; nitric oxide synthase and prostacyclin synthase causes increase in intracellular ROS causing angiogenesis in response to ischaemic pathway, pro inflammatory pathway that is associated with cardiovascular events. Also superoxide can inhibit gleyceraddehyde 3-phosphase which is an important enzyme in glycolytic pathway which leads to

accumulation of glucose with alternative due to increased production of advance glycated end products. The findings of this work are consistent with previous studies. This is in agreement with the study by conducted by Zhao *et al.,* [37] that reported decreased level of superoxide dismutase in diabetic associated with cardiovascular disease due to reduced SOD activity and generation oxygen intermediate reactive species such as superoxide .Also, the study is in agreement with the study of Singh *et al.,* [38] that concluded significantly lower level in SOD in type 2 diabetes due to superoxide and other reactive radicals that induced lipid peroxidation.

The findings agrees with the study of Younus *et al.,* [39] that reported decreased activity of SOD and over expression of superoxide which cause shift to alternative glucose pathway with the production of AGES. Contrary to the findings of this study, Ganjifrockwala *et al.,* [40] found increased expression of SOD due to compensatory activities in increased oxidative stress and reduced binding to heparin. Similarly, Aouacheri *et al.,* [22] found increased SOD activity with suggesting an adaptive or compensating mechanism in response to increased oxidative stress. The present study found decrease in the level of superoxide dismutase in various ages which was not significant. Contrast to the findings, Hisikar *et al.,* [36] observed that the level of SOD lowers from the age above 50 years which indicated that oxidative stress is an important factor in aging process. Similarly, Briggs *et al.,* [35] reported that SOD level decreases at the age 61 years and above which showed excessive production of ROS and reduced antioxidant defense contribute to aging. There was no significant difference in the level of SOD in both male and female type 2 diabetes. This indicates that age difference is not associated with the level of SOD in type 2 and decreases in all due to increase oxidative stress. This study is in agreement with the study conducted by Hisikar *et al.,* [41]. In contrary to the present Jabeen *et al.,* [42] who observed that female has increased level of superoxide dismutase when compared with male and indicated that male are more at risk for development of microvascular and macro vascular complications.

The present study observed decrease level of vitamin C in type 2 diabetics when compared with the control. The vitamin C is water soluble dietary antioxidant which is obtained exogenously in natural and synthetic form. It acts as a reducing agent in free radical-mediated oxidation process. Deficiency found in diabetes may be due to insufficient intake and also competition of vitamin C with glucose due to structural similarity for uptake into cells. Hyperglycaemia delays vitamin C absorption and metabolism which may increases cellular uptake and turnover for biological functions which results in deficiency. Deficiency of vitamin C in diabetes involved defective formation of collagen and connective tissue in the skin, cartilage dentine, bone, blood vessels and interfere with vasodialation and endothelial function of blood vessels. Also, Low level found may be due to increased oxidative stress which depletes this antioxidant in metabolic process that increases glucose dependent inhibition of enhanced erythrocyte fragility that potentially contributes to complications of type 2 diabetes. This study is consistent with the study of Wilson *et al.,* [43] that reported decrease level of vitamin C in pre-diabetes and type 2 diabetes mellitus. It is in accordance with the findings of Santosh and David [44] reported low level of vitamin C in type 2 diabetes due to presence of hyperglycemia which decreases it's up like and appears impaired. Also, Christie -David *et al.,* [45] suggested that decrease in vitamin C level in type 2 diabetes may contribute to insulin resistance and decrease blood vessel vasodilation in the muscle that may lead to endothelial dysfunction. In contrary, Fagbohun *et al.,* [46] reported higher significant level of vitamin C in type 2 diabetes when compared with the control subjects. The present study observed no significant difference in the level of Vitamin C across all age groups which signifies that deficiency can occur in diabetes irrespective of ages. In contrary to this study, Zhou *et al.,* [47] reported of vitamin C in older ages with increased oxidative stress. There was also no gender difference in the level of vitamin C in type 2 diabetes. This because vitamin C is obtained exogenously and its' absorption and metabolism depends on the amount each person intake. This study is not in agreement with Wali *et al.,* [48] higher level of vitamin C in female when compared with the male type 2 diabetics due to differences in their fat content This is consistent with the study by Aliyu *et al.,* [49] who indicated that 72% and 67% of female and male diabetics were vitamin C deficient. This may be due to disorder of lipid and glucose metabolism and insufficient daily supplement.

The lower level of vitamin observed in type 2 diabetes when compared with the control group may be due to inadequate compensatory antioxidant defense in oxidative stress intracellular pathway vitamin E is the major lipid-soluble, chain breaking lipid peroxyl scavenging antioxidant which protects polysaturated fatty acid and component of cell membrane, low density hypo protein from oxidation. Deficiency of vitamin E in type 2 diabetes is because of glucose and lipid metabolism dysfunction that affect absorption and metabolism which impair the antioxidant activity. Also, insufficient intake affect immune function, cell signaling regulation of gene expression, inhibitory effect of smooth muscle proliferation, platelet adhesion which may result in micro vascular and macro vascular complication in type 2 diabetes. Decrease level of vitamin E involved in glycation of protein, haemoglobin, lipid peroxidation decrease, acceleration of diacylglycerol kinase activity which is an activator of protein kinase C involved that prevents phospholylation, transportation of glucose to adequate

cells and tissues and accierates hyperglycaemia. Also, deficiency can affect immodulatory properties which may result in neuromuscular problems such as loss of vibration sensation and permanent nerve damage in type 2 diabetes. This study is in accordance with previous studies. This is in line with the work conducted by Olid *et al.*, [50] suggested that low level of vitamin E may be involved in vascular diabetic complications. Alamdiri [51] reported decrease level of vitamin E due to increased oxidative stress caused by fatty acid induced free radicals such as lipid peroxides. This is in accordance with the line study conducted by Ayeleso *et al.*, [52] that reported decreased level that may results in that activator of transcription factor and endothelial procoagulant tissue involved in diabetes complication. There was increase in level of vitamin E in ages between 60-69 years women was significant but no significant difference as the ages progresses above 50 years. It may be due to increase in biological function of vitamin E among type 2 diabetes. The present study observed significant increase in female when compared with the male type 2 diabetics. This is due to more visceral adiposity found in woman which triggers hyperglycaemia oxidative stress and activate proinflammatory cytokines that interfere with vitamin E biological functions. This is consistent with the study by Aliyu *et al.*, [49] that reported vitamin E which was higher in female (72%) when compared with the male (67%) type2 diabetes

CONCLUSION

The findings of this study has shown that type 2 diabetics mellitus is associated with an increase acute phase response by inflammatory protein over expression of lipid perioxide and decreased activity of endogenous and endogenous antioxidant which mediate oxidative stress state. These undesirable state which play roles in the pathogenesis of type 2 diabetes may result in unexpected complications increased morbidity and premature death in type 2 diabetics.

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