World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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Assessment of the relationship between oxidative stress markers and anti-oxidative co-factors in subjects with diabetes mellitus

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World Journal of Advanced Research and Reviews, 2025, 27(02), 2131-2143

Publication history: Received on 14 July 2025; revised on 26 August 2025; accepted on 28 August 2025

Article DOI: https://doi.org/10.30574/wjarr.2025.27.2.3056

Abstract

Diabetes mellitus is characterized by impaired glucose metabolism and a resultant effect of persistent hyperglycaemia. Type 2 diabetes also known as adult-onset diabetes is majorly caused by insulin resistance. The objective of this study is to determine the relationship between oxidative stress markers and anti-oxidative co-factors in subjects with diabetes mellitus in Edo South Senatorial district of Edo state. A total of 150 participants (100 subjects with diabetes mellitus and 50 apparently healthy individuals used as control) were recruited into this study. Oxidative stress markers, glutathione peroxidase (Gpx), catalase (CAT) and malondialdehyde (MDA) were analyzed using chemiluminiscence techniques. While the trace elements; magnesium, zinc, selenium and copper were analyzed using atomic absorption spectrophotometry. The data were analyzed using statistical software for social science version 23 (IBM, Chicago IL, USA). The mean value of the oxidative stress markers shows that there was a statistically significant increase in MDA in diabetic subjects as compared to their non-diabetic counterparts (P=0.014) while Gpx and CAT experienced a significant decrease in diabetic group than the controls (P=0.000 and P=0.000 respectively). For the trace elements, only magnesium showed a significant decrease in the subject group when compared to the control group (P=0.007), with zinc, copper and selenium depicting no statistical significance. The relationship between anti-oxidative co-factors(Copper, Zinc, Magnesium and Selenium) and redox parameters (CAT, MDA and GSH) depicted no significant correlation.

Keywords: Oxidative Stress; Oxidative Markers; Anti-Oxidative; Diabetes Mellitus; Glutathione Peroxidase (Gpx); Catalase (CAT); Malondialdehyde (MDA)

1. Introduction

Diabetes mellitus is a metabolic disorder characterized by elevated blood glucose levels due to the body's inability to produce or effectively utilize insulin (American Diabetes Association, 2023). This chronic condition can lead to various complications, including dysthyroidism, inflammatory responses, and oxidative stress. Exploring the relationship between these factors and the role of anti-oxidative co-factors could be pivotal in the management of diabetes mellitus. The co-existence of these conditions can exacerbate metabolic disturbances, leading to poor glycemic control and an

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increased risk of complications (Kuo *et al.*, 2019). The underlying mechanisms involve alterations in insulin sensitivity, glucose metabolism, and thyroid hormone regulation (Kalra and Sahay, 2022).

Oxidative stress, characterized by an imbalance between the production of reactive oxygen species (ROS) and the body's anti-oxidant defense mechanisms, plays a significant role in the pathogenesis of diabetes mellitus (Yaribeygi *et al.*, 2020). Hyperglycemia, a hallmark of diabetes, promotes the formation of ROS, which can damage cellular components, including proteins, lipids, and DNA. This oxidative stress contributes to the development of various diabetic complications, such as neuropathy, nephropathy, and retinopathy. Hyperglycemia and dyslipidemia contribute to the increased production of ROS, which can damage cellular components, including lipids, proteins, and DNA (Newsholme *et al.,* 2016). Oxidative stress is also implicated in the development of diabetic complications, such as nephropathy, neuropathy, and retinopathy (Newsholme *et al.,* 2016). To combat the detrimental effects of oxidative stress, the body relies on various anti-oxidant defense mechanisms, including enzymatic and non-enzymatic anti-oxidants.

Anti-oxidative co-factors, such as vitamins C and E, carotenoids, and trace elements like zinc and selenium, play a vital role in counteracting oxidative stress and mitigating its deleterious effects (Asemi *et al.*, 2015). These co-factors act as scavengers of ROS, enhancing the body's anti-oxidant defense mechanisms and potentially reducing the risk of diabetic complications (Asemi *et al.,* 2015). However, the efficacy of anti-oxidant supplementation in managing diabetes mellitus remains a subject of ongoing research and debate (Asemi *et al.*, 2015).

These co-factors can help neutralize ROS, prevent oxidative damage, and potentially mitigate the progression of diabetic complications. Understanding these intricate relationships is crucial for developing effective strategies to manage and prevent diabetic complications. The incorporation of anti-oxidative co-factors, in conjunction with appropriate medical interventions, may hold promise in mitigating the deleterious effects of oxidative stress and improving overall outcomes for individuals with diabetes mellitus.

The objective of this study is to determine the relationship between oxidative stress markers and anti-oxidative co-factors in subjects with diabetes mellitus in Edo South Senatorial district of Edo state.

2. Research methodology

2.1. Study Area and Population

This study was carried out at Edo Specialist Hospital, Benin City, Edo State. The study population for this research is diabetic patients attending the endocrinology clinic of Edo Specialist Hospital, Benin City, Edo State. Apparently healthy male and female subjects; with a good glucose metabolism and no history of testing positive to tuberculosis, HIV, Hepatitis B and C virus served as the control group. Therefore, a minimum of 100 test samples and 50 controls were used for this study.

2.2. Study Criteria

Adult male and female subjects with laboratory evidence of diabetes mellitus, adult male and female subjects who gave an informed consent to participate in these work and adult male and female subjects with no laboratory evidence of reactivity to tuberculosis, Human Immunodeficiency Virus(HIV), Hepatitis B and C were included in the study. While, adult male and female subjects with no evidence of impaired glucose metabolism, adult male and female subjects who refused to grant informed consent for participation and adult male and female subjects who tested positive to tuberculosis, HIV, hepatitis B and C were excluded from the study.

2.3. Sample Collection

Sputum of study participants were collected into sterile universal container for Acid Alcohol Fast Bacili(AAFB) screening. About five millilitres of blood was collected aseptically from the cubital fossa of each subject by an experienced phlebotomist using an aseptic collection procedure as described by Cheesbrough (2000), dispensed into plain sample container and allowed to clot. After clot retraction, samples were centrifuged and the supernatant was collected into another plain container. Sample was then stored at freezing temperature until analysis.

3. Laboratory analyses

3.1. Screening for tuberculosis using Gene Xpert method

3.1.1. Principle

The GeneXpert test is a molecular diagnostic test that uses real-time polymerase chain reaction (PCR) technology to detect the genetic material (DNA or RNA) of pathogens such as bacteria, viruses, and parasites. The test is performed using a small cartridge that contains all the necessary reagents, so there is no need for complex laboratory equipment or extensive sample preparation.

3.1.2. Method

- The sample was obtained from the sputum of the patient
- The sample was added to a small cartridge that contains all the necessary reagents for the test.
- The cartridge was inserted into the GeneXpert instrument, which performs the test automatically.

The instrument heats and cools the sample, which causes the genetic material to be amplified (copied) millions of times. The instrument also detects the amplified genetic material in real-time, allowing for the rapid and accurate detection of the pathogen.

The results are automatically interpreted by the instrument and can be printed out or viewed on a computer screen.

3.2. Screening for HIV, Hepatitis B and C using ELISA

3.2.1. Principle

Enzyme-linked immunosorbent assay (ELISA) is a commonly used method for screening for HIV and hepatitis infections. The principle of the ELISA method involves the use of specific antibodies that can recognize and bind to viral antigens in a patient's blood or serum.

3.2.2. Method

A microtiter plate was coated with specific viral antigens, such as HIV or hepatitis antigens, that are immobilized on the surface of the plate.

- A small amount of the serum was added to the coated microtiter plate.
- The plate was incubated to allow the patient's antibodies to bind to the viral antigens on the plate.
- The plate was washed to remove any unbound antibodies
- A secondary antibody that recognizes and binds to the patient's antibodies was added to the plate.
- The plate was incubated once more to allow the detection antibody to bind to the patient's antibodies that are bound to the viral antigens.
- An enzyme substrate was added to the plate that reacts with the enzyme conjugated to the detection antibody. This produces a color change that indicates the presence of antibodies.
- The color change was measured using a spectrophotometer, and the intensity of the color is proportional to the number of antibodies present in the patient's sample.

3.3. Determination of Catalase, Glutathione peroxidase and Malondialdehyde Using Abbott Auto-analyzer

3.3.1. Principle

Abbott Auto-analyzer is an automated clinical chemistry analyzer used for the analyses of a wide range of biochemical markers in patient’s blood and serum samples. The principle of operation of the Abbott Auto-analyzer is based on chemiluminescence. Activated ions gets excited by absorbed energy and move from their respective ground states to higher energy levels. Upon return to their initially occupied ground state, these ions emit their absorbed energy.

3.3.2. Method

The patient's blood was prepared by centrifugation to separate the cells from the serum. The serum was then loaded onto the instrument.

The Abbott Auto-analyzer uses pre-packaged reagent kits that contain all the necessary reagents for the analysis of specific biochemical markers. These reagents were loaded into the instrument, along with calibrators and quality control samples.

The instrument automatically dispenses a measured volume of the patient's sample into a reaction vessel containing the appropriate reagents. The reaction between the sample and the reagents produced a coloured compound, the intensity of which is proportional to the concentration of the analyte being measured.

The reaction vessel was placed into the instrument, which measures the absorbance of the coloured compound at a specific wavelength of light. The absorbance measurement is compared to a calibration curve generated using the calibrators, and the concentration of the analyte in the patient's sample was determined.

3.3.3. Quality control

The Abbott Auto-analyzer also includes built-in quality control features to ensure the accuracy and precision of the analysis. These features include the use of quality control samples, automated calibration, and instrument maintenance checks.

3.4. Determination of Copper, Zinc, Selenium and Magnesium using Atomic Absorption Spectrophotometry

3.4.1. Principle

Atomic absorption spectrophotometry (AAS) is a commonly used analytical technique for the quantitative analysis of trace elements in a wide range of samples, including environmental, clinical, and biological specimens. The principle of the AAS method involves the measurement of the absorbance of light by free atoms in a gaseous state.

3.4.2. Method

The sample was prepared by acid digestion that convert the sample into a solution that can be aspirated into the AAS instrument. The solution containing the analyte was aspirated into a flame, usually a flame produced by burning acetylene and air, where the sample is vaporized and the atoms of the analyte was excited to higher energy levels. A beam of light, usually from a hollow cathode lamp that emits light at the specific wavelength corresponding to the element being analyzed, was passed through the flame. The atoms of the analyte in the flame absorb some of the light, resulting in a decrease in the intensity of the transmitted light. The amount of light absorbed by the analyte was measured by a detector, and the absorbance was converted to concentration using a calibration curve generated using standard solutions of known concentration.

3.4.3. Quality control

AAS instruments include built-in quality control features to ensure the accuracy and precision of the analysis. These features include the use of blank solutions, reference standards, and control samples.

4. Result

Table 1 Showing the results of the demographic characteristics of study participants

Table 1 Demographic Characteristics of Study Participants

Parameters	Diabetics	Control
No. of Sample	100	50
Age of Subject	6.25 ± 1.70	6.25 ± 1.52
Cu (Mg/L)	0.81 ± 0.03	0.86 ± 0.02
Zn (Mg/L)	1.08 ± 0.05	1.26 ± 0.06
Mg (Mg/L)	19.07 ± 0.47 *	20.96 ± 0.49 *
Se (Mg/L)	0.09 ± 0.01	0.11 ± 0.01
Catalase (u/mg)	7.57 ± 0.57 *	16.89 ± 0.99 *

GPx (u/mg)	4.72 ± 0.52 *	11.49 ± 0.93 *
MDA (µM/g)	0.54 ± 0.03 *	0.44 ± 0.01 *

The table below portraying the anti-oxidants analysis shows that there is significant increase (P<0.05: P=0.014) for the MDA values for the diabetic group when compared with the non-diabetic group while a significant decrease was recorded for the catalase and GPx levels when the diabetic group was compared with non-diabetic group. This depicts that accompanied by hyperglycemia in diabetes mellitus is the induction of oxidative stress as shown by the result algorithm.

Table 2 Comparison of oxidative stress markers among study participants

Variables	Diabetics (Test) n=100	Non-Diabetics (Control) n=50	t-value	P-value
Catalase	7.5736±4.0878	16.8934±7.01801	8.114	0.000
MDA	0.5499±0.2806	0.4422±0.1108	-2.524	0.014
GPx	4.7212±3.7003	11.4898±6.6404	6.296	0.000

Results for the anti-oxidative co-factors, only magnesium showed a significant (P<0.05: P=0.007) decrease for the diabetic group when compared to the non-diabetic group while Cu, Zn and Selenium were not significant. This invariably implies that accompanied by impaired glucose metabolism as seen in diabetes mellitus is hypomagnesemia.

Table 3 Comparison of anti-oxidative co-factors among study participants

Variables	Diabetics (Test) n=100	Non-Diabetics (Control) n=50	t-value	P-value
Copper	0.8126±0.2219	0.8626±0.1950	1.197	0.234
Magnesium	19.0702±3.3523	20.9584±3.5065	2.752	0.007
Zinc	1.2318±0.4492	1.1140±0.4055	-1.377	0.172
Selenium	0.0986±0.0357	0.1118±0.0377	1.796	0.076

Table 4 shows the relationship between oxidative stress markers and anti-oxidative co-factors, with a non-significant correlation being established amongst these respective parameters.

Table 4 Correlation between Oxidative Stress Markers and Anti-Oxidative Co-Factors

MDA and GPx	Anti-oxidative Co-factors	r-value	P-value	Remark
MDA GPx	Copper	-0.044	0.761	NWNSC
	Magnesium	-0.004	0.978	NWNSC
	Selenium	-0.201	0.162	NWNSC
	Zinc	0.030	0.838	PWNSC
GSH	Copper	0.139	0.337	PSNSC
	Magnesium	-0.047	0.748	NWNSC
	Selenium	0.162	0.260	PSNSC
	-Zinc	0.213	0.138	PWNSC

Key: NWNSC= Negative Weak Non-Significant Correlation**;** PWNSC= Positive Weak Non-Significant Correlation

5. Discussion

There is significant increase (P<0.05: P=0.014) for the MDA values for the diabetic group when compared with the non-diabetic group while a significant decrease was recorded for the catalase and GPx levels when the diabetic group was compared with non-diabetic group. This depicts that accompanied by hyperglycemia in diabetes mellitus is the induction of oxidative stress as shown by the result algorithm. The findings of this study are consistent with those of Trivedi *et al.,* (2024) in their study on "Evaluation of anti-oxidant enzyme activity and malondialdehyde levels in patients with diabetes mellitus." They also reported a significant increase in malondialdehyde (MDA) levels among diabetic subjects compared to non-diabetic patients. Earlier studies suggest that glycemic balance appears to impact plasma MDA levels, leading to increased production of free radicals. This phenomenon likely occurred due to either enhanced glycosylation and platelet aggregation or impairment of cellular anti-oxidant protective systems. The heightened production of free radicals may contribute to the development of metabolic vasculopathy. Their study on "The investigation of the oxidative stress-related parameters in type 2 diabetes mellitus" also reported a significant reduction in catalase and glutathione peroxidase (GPx) levels. The changes in anti-oxidant status may stem from heightened oxidative stress induced by factors such as hyperglycemia.

Barbagallo and Dominguez (2015) noted a decline in serum magnesium levels correlated with increases in HbA1c levels and the duration of Type 2 Diabetes Mellitus, which corresponds with the findings of this study. Likewise, the decrease in magnesium levels could be a consequence of heightened urinary excretion triggered by hyperglycemia.

Studies have showed that in diabetic mellitus patients, trace elements like selenium, zinc, and copper play crucial roles in modulating redox parameters such as anti-oxidant enzyme activities and oxidative stress levels. Alterations in trace element status can disrupt the balance between oxidant and anti-oxidant defenses, contributing to increased oxidative stress and tissue damage observed in diabetes. Conversely, supplementation or optimization of trace element levels may help restore redox balance and mitigate complications associated with diabetes.

Based on the results of this study, diabetic subjects exhibited a significant decrease in magnesium, catalase, and glutathione peroxidase compared to control subjects. Conversely, a significant increase in malondialdehyde levels was observed in diabetic subjects compared to control subjects. The results of this study regarding magnesium levels are consistent with those of Arpaci *et al*., 2017, whose research on "Associations of serum Magnesium levels with diabetes mellitus and diabetic complications" identified a significant magnesium depletion. They suggest that hyperinsulinemia-related urinary magnesium excretion, insufficient nutrition, and possibly a particular renal defect might contribute to this occurrence. Consistent with the outcomes of this study, Mishra and Mishra (2017) reported a notable rise in MDA levels and a significant decrease in glutathione peroxidase levels among diabetic subjects compared to non-diabetic individuals.

The relationship between age, trace elements and oxidative stress markers in diabetic mellitus patients is complex. Aging exacerbates oxidative stress. These factors collectively contribute to the pathogenesis and progression of diabetes mellitus, impacting its management and complications.

In investigating the influence of age on various factors including trace elements and oxidative stress markers in patients with Diabetes Mellitus, as observed in the results revealed that age led to a significant increase in Fasting Serum Glucose and Malondialdehyde. Conversely, age was associated with a significant decrease in Magnesium, Catalase and glutathione peroxidase. The correlation observed in this study regarding Fasting Serum Glucose aligns with the results of Animaw and Seyoum (2017), whose Multiple logistic regression tests similarly indicated a significant difference among age categories. Individuals aged 37 to 50 years old were approximately six times more likely to have diabetes, with an adjusted odds ratio (AOR) of 5.5 (95% CI: 1.5–19.6), compared to those aged 18–23 years old. The likelihood of having diabetes increases to over six-fold as participants age beyond 50 years. The results regarding magnesium in this study are consistent with those of Liamis *et al*., 2014, who observed hypomagnesemia in patients with diabetes mellitus. They suggested that glomerular hyperfiltration might be responsible for this occurrence. Regarding anti-oxidant status, the results of this study are entirely consistent with those of Rani and Mythili, (2014), whose research on "Study on total anti-oxidant status in relation to oxidative stress in type 2 diabetes mellitus" noted a notable increase in malondialdehyde levels and a significant decrease in glutathione peroxidase and catalase levels among diabetic subjects compared to control subjects. The elevation in malondialdehyde levels could be attributed to heightened peroxidative damage to lipids resulting from oxidative stress experienced during diabetes. Furthermore, the reduction in glutathione peroxidase and catalase levels among diabetic subjects may be linked to increased oxidative stress, as evidenced by lipid peroxidation. The decrease in anti-oxidants reflects the battle they wage against oxidative stress to minimize the resulting damage.

6. Conclusion

Diabetic subjects display increased oxidative stress, indicated by elevated malondialdehyde levels and decreased anti-oxidant enzyme levels such as catalase and glutathione peroxidase. It has also been established that there is hypomagnesemia in subjects with diabetes mellitus, with no significant effect on copper, zinc and selenium. Understanding these relationships may inform targeted therapeutic interventions to mitigate the complications associated with diabetes mellitus.

Based on the findings of this study, Magnesium levels of diabetic patients should be routinely analyzed and derangement should be commensurately managed using necessary diets and/or supplementation. Also, levels of Oxidative stress markers such as catalase, glutathione peroxidase and malondialdehyde in diabetics should be frequently determined. Lifestyle modifications, therapeutic interventions and dietary adjustments should be recommended by healthcare providers to cushion the effect of oxidative stress on the pathogenesis of diabetes mellitus.

Compliance with ethical standards

Acknowledgments

- I want to specially acknowledge the scholarly guidance given me by my indefatigable supervisor, Prof. Mathew Folaranmi Olaniyan, during the course of this research work. Words are not enough to express the depth of my gratitude.
- My beloved wife and Children; Shiphrah and Shimrath Ugege, many thanks for your patience and unwavering supports in all sense of the word.
- I do also appreciate my Head of Department, Dr. G.I. Iyare, who continually provided needed support and encouragement.
- I want to also acknowledge the timely support of my former Dean, Prof. M.A. Muhibi and current Dean, Dr. Pius Omosigho. Your guidance was a stimulant.
- Sincere gratitude to Mr. Ilenagbe Teddy, Igure Babatunde Augustine and Agbonghai Chinedu for their massive supports during the sojourn.
- The greatest thanks goes to Jehovah; the provider and sustainer of life.

Disclosure of conflict of interest

No conflict-of-interest to be disclosed.

Statement of ethical approval

The approval for this study was given by the ethical committee of Health Research. Ethics Committee of Edo State Ministry of Health, Benin City, Edo State, Nigeria.

Statement of informed consent

Informed consent was obtained from each participant prior to specimen collection.

Authors' Contributions

- Conception and design the work/idea: Christian Onosetale Ugege and Mathew Folaranmi Olaniyan
- Collect data/obtaining results: Christian Onosetale Ugege and Aigbokhan Akhere Caleb
- Manuscript writing: Christian Onosetale Ugege
- Analysis and interpretation of data: David Olufemi Adebo and Odekunle Bola Odegbami
- Critical revision of the manuscript: Ekomobong Effiong Idem and Edward Eghonghon Imadojemu
- Final research review: Mathew Folaranmi Olaniyan

Funding

This research did not receive any grant from funding agencies in the public, commercial, or not-for-profit sectors.

Availability of Data and Materials

The authors declare consent for all available data present in this study.

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7. Appendix

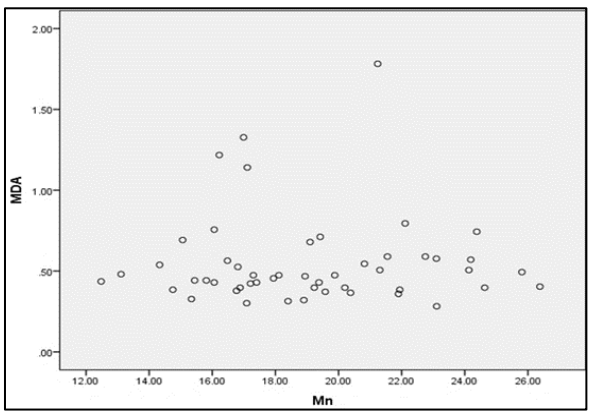

Figure 1 The Relationship Between Malondialdehyde And Magnesium

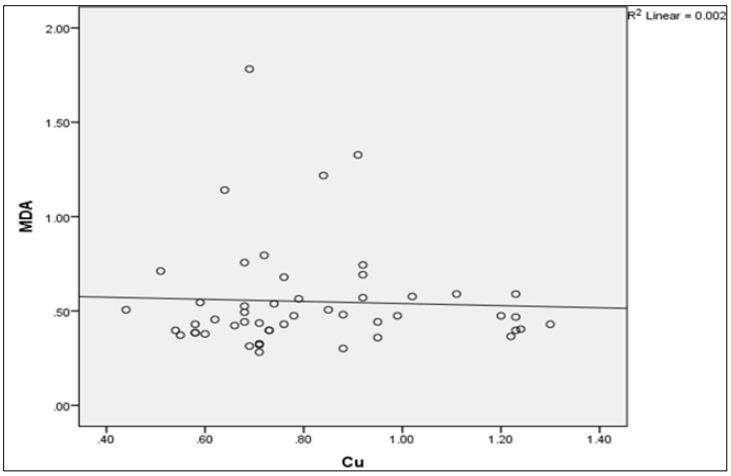

Figure 2 The Relationship Between Malondialdehyde And Copper

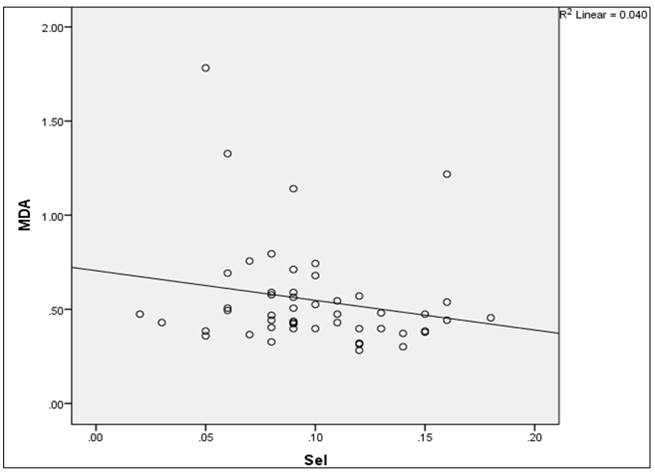

Figure 3 The Relationship Between Malondialdehyde And Selenium

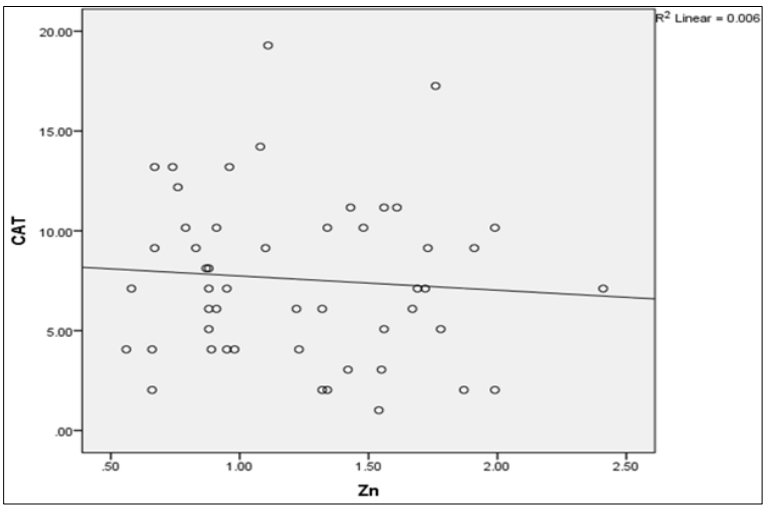

Figure 4 The Relationship Between Catalase And Zinc

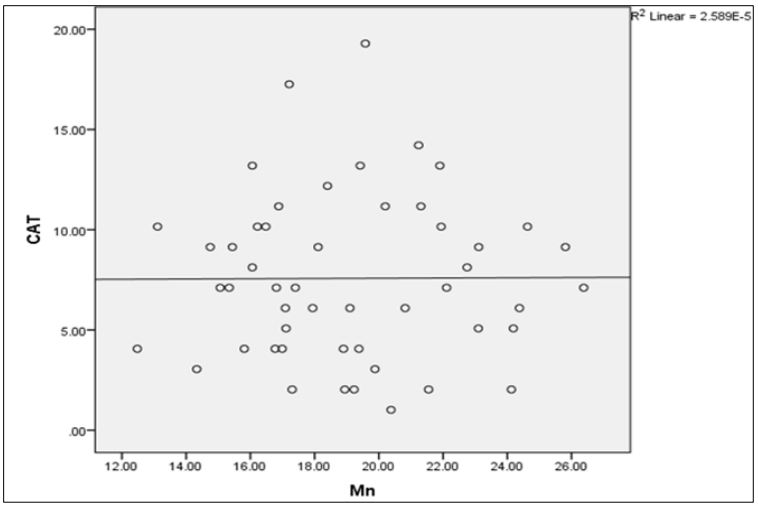

Figure 5 The Relationship Between Catalase And Magnesium

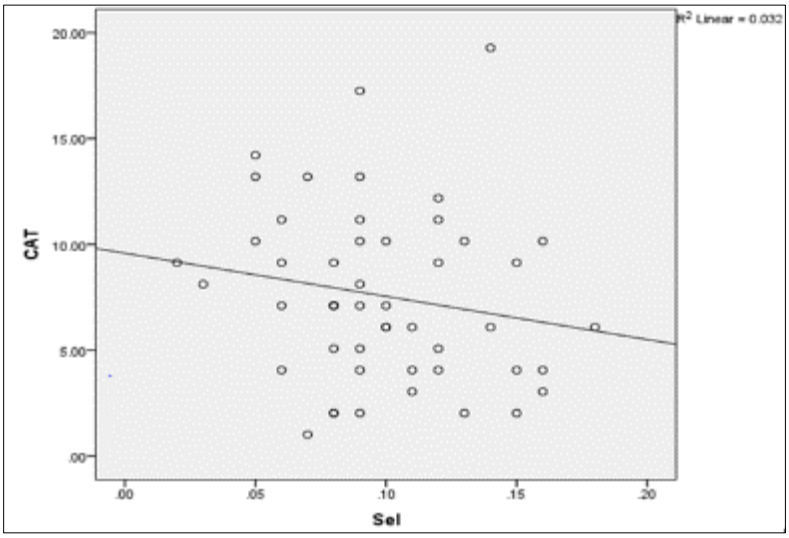

Figure 6 The Relationship Between Catalase And Selenium

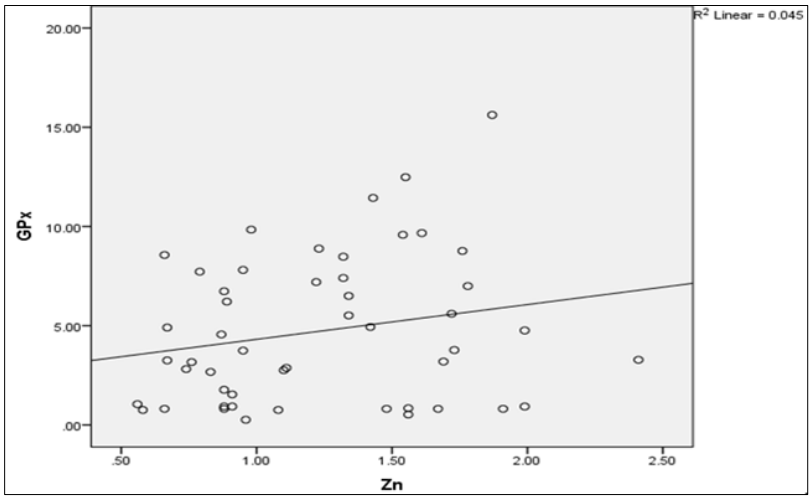

Figure 7 The Relationship Between Glutathione Peroxidase And Zinc

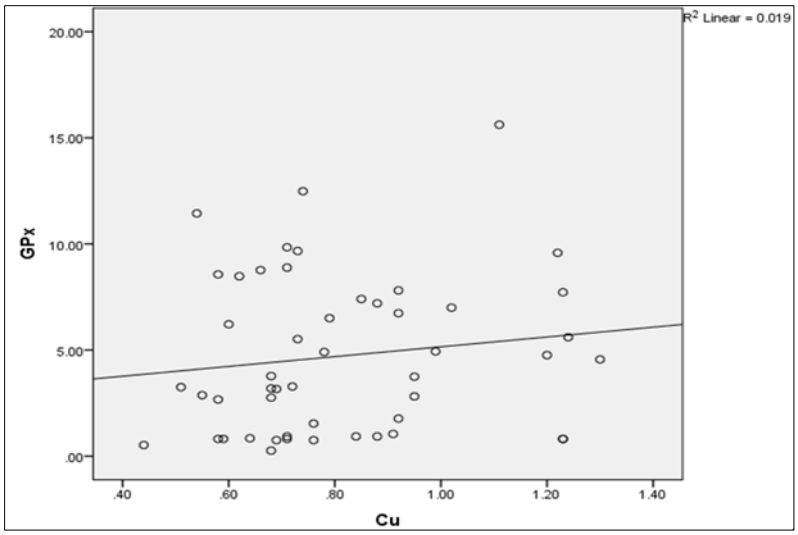

Figure 8 The Relationship Between Glutathione Peroxidase And Copper

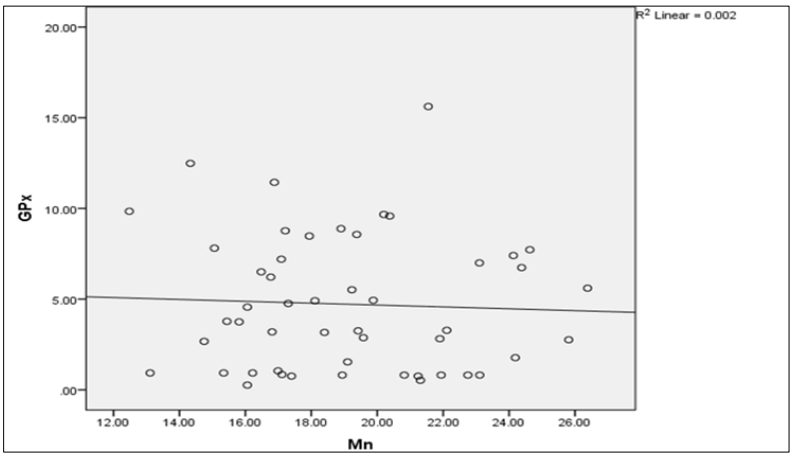

Figure 9 The Relationship Between Glutathione Peroxidase And Magnesium

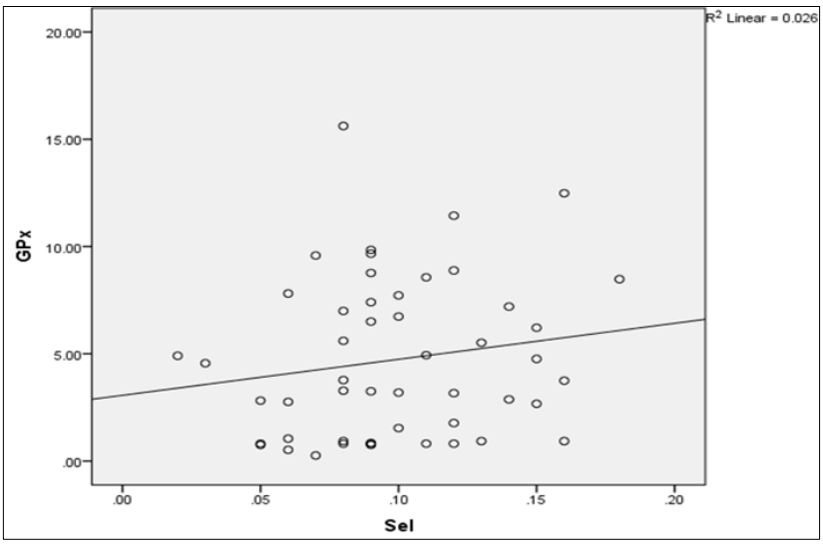

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Figure 10 The Relationship Between Glutathione Peroxidase And Selenium

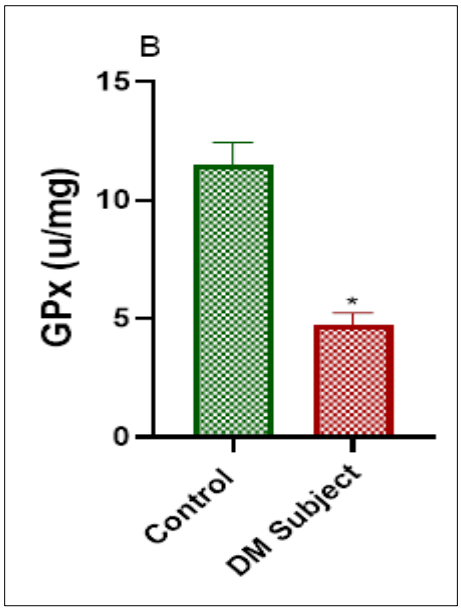

Figure 11 The Levels Of Glutathione Peroxidase Among Study Participants

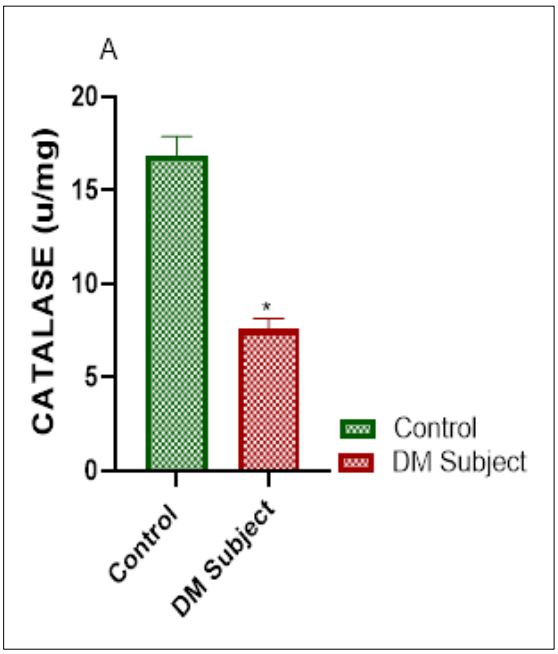

Figure 12 the Levels of Catalase among study participants

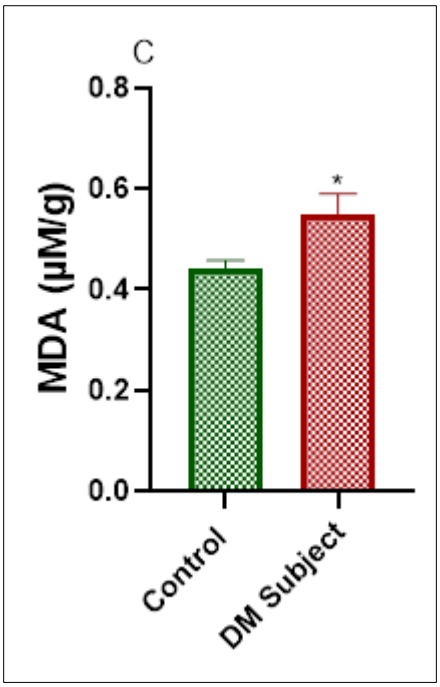

Figure 13 The Levels of Malondialdehyde Among Study participants