Evaluation of Catalase and Manganese in Type 2 Diabetic Patients in University of Port Harcourt Teaching Hospital

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Authors’ contributions

This work was carried out in collaboration among all authors. Authors AN, EIO, CCNV and EFC designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors CJO, AMI, CAN, UC and BII managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This study was carried out to evaluate catalase and manganese in type 2 diabetes mellitus patients. A total of 40 subjects were included in this study. This comprised of equal number of male and female subjects respectively. Enzyme linked immunosorbent assay (ELISA) method was used for this study. The result showed that there is a significant increase ($P < 0.05$) in the fasting blood sugar level of male diabetics (11.3±3.1) when compared with male non diabetics (4.0±0.4), and female diabetics (9.4±0.8) when compared with female non-diabetics (3.8±0.3). There is no significant increase ($P<0.05$) in the mean catalase value of male diabetics (77.3±137.7) when compared with male non-diabetics (256.3±207.6) and female non-diabetics (225.1±233.9). But there is significant increase in female diabetics (27.8±14.7) when compared with controls (77.3±137.7) and female non-diabetics (225.1±233.9). There is also significant decrease ($P<0.05$) in the mean Manganese value of male diabetics (0.5±0.6) when compared with male non-diabetics (0.9±0.3) and female diabetic(0.2±0.1) when compared with female non-diabetics (0.3±0.1). The result shows that serum catalase level and manganese is decreased in diabetic patients. It is therefore suggested that type 2 diabetes patients should consume meals and supplement rich in catalase and manganese.

Keywords: Catalase; manganese; type 2 diabetic patients.

1. INTRODUCTION

Diabetes mellitus is characterized by absolute or relative deficiencies in insulin secretion and/or insulin action associated with chronic hyperglycemia and disturbances of carbohydrate, lipid and protein metabolism. Long-term vascular complications represent a major cause of morbidity and mortality in patients with diabetes mellitus. Metal ions are known to play essential role in living systems, both in growth and in metabolism. Impaired metabolism of trace elements is observed in diabetic patients [1].

Catalase is an enzyme commonly found in all living organism that require oxygen for existence (Bacteria, plant and animals). It is a very important enzyme in protecting the cell from oxidative damage by Reactive Oxygen Species (ROS). It is involved in any process that results in a change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme production, gene expression etc) as a result of a reactive oxygen species stimulus. ROS include singlet oxygen superoxide and oxygen free radicals. The enzyme catalase, catalyzes the conversion of hydrogen peroxide to water and oxygen, using either an iron or manganese co-factor [2].

Trace elements facilitate numerous biochemical reactions, including those related to insulin and glucose metabolism [3]. In type 2 diabetes, it has been observed that alteration of most of these trace elements makes individuals with this disease more susceptible to various life threaten disease including coronary heart disease, hypertension, retinopathy, thrombosis thereby increasing the risks of diabetic complications, hence, the need of this study.

Diabetes mellitus with long term vascular complications represent a major cause of morbidity and mortality in patients having this disease. Type 2 is the most common form, of DM accounting for 85%–95% of diabetes in developed countries. Some patients cannot be clearly classified as type 1 or type 2 diabetes [4]. Trace elements in diabetes mellitus also act as markers in the progression of diabetic complications [5].

The study was to evaluate the catalase and manganese in type 2 diabetes.

2. MATERIALS AND METHODS

2.1 Study Area

The research was carried out in the University of Port Harcourt teaching Hospital (UPTH), located at east west road, Port Harcourt Rivers State.

2.2 Study Population

The study populations were both in and out patients suffering from diabetics. It consist of 30 patients with type 2 diabetics mellitus and 10 apparently healthy individuals. Type 2 diabetes patients were divided in 15 males and 15 female subjects and 10 apparently healthy individuals 5 males and 5 females.
2.3 Inclusion Criteria

Subjects that were considered eligible in the study were within the age group of 20 to 74 years, subjects who have given consent for the study, those who had type 2 diabetes, and those who had blood glucose level above (8.0 - 18.5 mmol/L) and within (3.8 - 6.5 mmol/L) reference ranges.

2.4 Exclusion Criteria

Non diabetics were excluded from the study. Subjects suffering from type 1 diabetes mellitus, gestational diabetes, and without significant increase in glucose level were excluded. Those with illness like Hypertension, liver damage were excluded.

2.5 Experimental Design

The subjects were group into 4 groups, Group 1 (male diabetics), Group 2 (female diabetics), Group 3 (male control), Group 4 (female control).

2.6 Sample Collection

All samples were obtained after overnight fast; 10mls of blood was obtained from the patient's ante-cubital vein by using the standard phlebotomy procedure. Immediately after collection, 5mls of the blood was dispensed into a plain bottle for the evaluation of catalase and Manganese. The remaining blood was dispensed into a Flouride oxalate container for fasting blood sugar analysis. Each bottle was properly corked and labelled with patient's details. Each of the blood samples dispensed into the plain bottle were processed for separation of serum after about thirty minutes of sample collection. The blood sample was allowed to clot, dislodge using an Applicator stick, centrifuged at 3000rpm for 5 minutes. The serum was then separated and preserved in the Refrigerator at temperature 4 degree Celsius. The separated serum was then transported to Madonna University Teaching Hospital, Elele using the standard dispatched method for sample analysis.

2.7 Quantitative In Vitro Determination of Fasting Plasma Glucose

Method

The samples were analyzed using Glucose oxidase method as modified by Randox laboratories Ltd, (2017). Cat no- GL364.

Procedure

1 ml of the Glucose working reagent was added to the tubes labelled test, standard and blank. To the tube labeled standard and test, 0.01 ml of the cholesterol standard and samples were added respectively. The mixtures were then mixed properly and incubated for 10 minutes at temperature 37°C in the water bath. The absorbance of the samples and the standard were measured spectrophotometrically against reagent blank at 500 nm.

Calculation

\[
\text{Glucose conc. (mmol/l)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{conc. of standard}
\]

Reference Range: 3.8 – 6.5 mmol/l

2.8 Quantitative Determination of Human Catalase

Method

The samples were analyzed using Enzyme linked immunosorbent assay (ELISA) Cat number- E3053Hu Body mass index is determined

Procedure

All reagents, standard solution and samples were prepared, bringing all to room temperature before use.

The number of strip required for the assay was determined, the strips for use was inserted in the frames and the unused strips were stored at 2-8 degrees Celsius 50ul of standard was added to standard well. Note the antibody was not added to the standard solution contains biotinylated antibody. 40ul of sample was added to well and 10ul of anti-CAT antibody was also added to sample wells. 50ul of streptavidin-HRP was added to sample wells and standard wells (not blank control well), it was mixed well. The plate was covered with a seal and incubated for 60mins at 37 degrees Celsius. The seal was removed and the plate was washed 5times with wash buffer (it was soaked well with at least o.35ml wash buffer for 30secs for each wash). The plate was blotted onto a paper.

50ul of the substrate solution A was added into each well and then the substrate solution B to
each well. The plate was covered with a new seal and incubated for 10 minutes at 37 degrees Celsius in the dark. 50 ul of the stop solution was added to each well, the blue color changed to yellow immediately. The optical density (OD value) of each well was determined immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

Calculation

Construct a standard curve by plotting the average OD for each standard on the vertical curve (y) axis against the concentration on the horizontal (x) axis and draw a best fit curve through the points on the graph. These calculations can be best performed with computer–based curve–fitting software and the best fit line can be determined by regression analysis.

2.9 Quantitative Determination of Manganese

Method

The samples were analysed using Agilent FS240AA atomic absorption spectrometer (AAS).

Procedure

1 ml of the sample was measured and 4mls of distilled water was added. It was mixed properly and allowed to boil for 20 mins at 37°C. The mixture was then measured using FS240AA Agilent atomic absorption spectroscopy at 279.5 nm wavelength with 0.2nm slit and air acetylene flame.

Stock standard solution: 1000 mls of stock metal solution was dissolved in a minimum volume of (1+1) HNO3. It was then diluted to 1 litre with 1% (V/V) HCL, appropriate dilution were carried out to produce 2, 4 and 6 ppm working solution.

2.10 Statistical Analysis

Data obtained from this study were analyzed using Statistical Package for Social Sciences (SPSS) version 16.0 for windows 7. The results were expressed as mean ± Standard deviation. Independent sample t-test which was used to compare means and values were considered significant at p<0.05.

3. RESULTS

This table shows that there is a significant increase (P < 0.05) in the FBS level of male diabetics (11.3±3.1) when compared with male nondiabetics (4.0±0.4), and female diabetics (9.4±0.8) when compared with female nondiabetics (3.8±0.3). There is no significant increase (P<0.05) in the mean Catalase value of male diabetics (77.3±137.7) when compared with male (256.3±207.6) and female non diabetics (225.1±233.9). But there is significant increase in female diabetics (27.8±14.7) when compared with male (77.3±137.7) and female non diabetics (225.1±233.9). There is significant decrease (P<0.05) in the mean Manganese value of male diabetics (0.5±0.6) when compared with male non diabetics (0.9±0.3) and female diabetics (0.2±0.1) when compared with female nondiabetics (0.3±0.1).

4. DISCUSSION

This study was carried out to compare the difference in catalase and manganese levels in type 2 diabetic patients. The results showed that there is a significant increase (P < 0.05) in the FBS level of male diabetics (11.3±3.1) when compared with male nondiabetics (4.0±0.4), and female diabetics (9.4±0.8) when compared with female nondiabetics (3.8±0.3). This correlates with Shobak et al. [6] who states that type 2 diabetes mellitus is characterized by insulin resistance, which may be combined with relatively reduced insulin secretion. This reduction in insulin leads to an increase in blood sugar when tested.

There is no significant increase (P<0.05) in the mean serum Catalase value of male diabetics (77.3±137.7) when compared with male (256.3±207.6) and female nondiabetics (225.1±233.9). But there is significant decrease in female diabetics (27.8±14.7) when compared with male (77.3±137.7) and female non diabetics (225.1±233.9). It correlates with Ceriello et al. [7] who states that Hyperglycemia is able to increase the levels of oxygen radical scavenging enzymes in cultured endothelial cells.
Table 1. The mean values for fasting blood sugar (FBS), catalase, and manganese values of various groups

| Parameter | Group 1 | Group 2 | Group 3 | Group 4 | P Value |
|-----------|---------|---------|---------|---------|---------|
| FBS       | 11.3±3.1| 9.4±0.8 | 4.0±0.4 | 3.8±0.3 | 0.000   |
| CAT       | 77.3±137.7| 27.8±14.7| 256.3±207.6225.1±233.90.004|
| Mn        | 0.5±0.6 | 0.2±0.1 | 0.9±0.3 | 0.3±0.1 | 0.005   |

Table 2. shows comparison of fasting blood sugar (FBS), catalase and manganese values of various groups

| Groups       | FBS     | Catalase | Manganese |
|--------------|---------|----------|-----------|
| GP 1 V GP 3  | 0.068   | 0.000*   | 0.127     |
| GP 2 V GP4   | 0.000*  | 0.037*   | 1.000     |
| GP 1 V GP2   | 0.1000.749| 0.213    |           |
| GP 3 V GP4   | 1.000   | 0.983    | 0.041*    |

KEYS; Group (GP) 1-male diabetics; Group (GP) 2-female diabetics; Group (GP) 3-male control; Group (GP) 4-female control; * significant increase

There is significant decrease (P<0.05) in the mean Manganese value of male diabetics (0.5±0.6) when compared with male non-diabetics (0.9±0.3) and female diabetics (0.2±0.1) when compared with female non-diabetics (0.3±0.1). It correlates with Emsley [8] who said that Manganese is concentrated in the liver and kidneys, there diabetics mellitus which affects the kidney causing kidney damage, causing loss of manganese [9-12]. Also Supplemental magnesium (200mg/day) has been shown to slightly decrease manganese bioavailability in healthy adults, either by decreasing manganese absorption or by increasing its excretion. This is in contrary to the result.

5. CONCLUSION

Serum catalase enzymes are decreased in type 2 diabetes subjects. This is because in Diabetes mellitus oxidative stress mostly seems to be due to an increase in production of free radicals leading sharp reduction in antioxidant defense. Diabetic patients may require more antioxidants in their diet or supplements compared to healthy individuals. The result of this research shows that there is a decrease in the level of manganese and catalase in diabetic patients when compared with apparently healthy individuals. Manganese is a co-factor of the antioxidant enzyme catalase and its deficiency may also lead to a decreased activity of this enzyme resulting in accumulation of free radicals.

CONSENT

Informed consent of the subjects involved was obtained. The subjects were told that their blood samples were collected for research purpose.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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