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SHORT TERM STUDENTSHIP
STS 2019

This is to certify that Ms. Mohini Chauhan student of MBBS-III of Santosh Medical College, Ghaziabad has been awarded the Short Term Studentship (STS) for a period of two months during 2019 under the guidance of Dr. Jyoti Batra for the project entitled, "Association of depression with oxidative stress and diabetes mellitus type-2: A case-control study" (Ref. No. 2019-02483) and the Report was found to be satisfactory.

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Dr. N.C Jain
Scientist G & Head, HRD
ICMR

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Prof. (Dr.) Balram Bhargava
Secretary, Department of Health Research &
Director General, ICMR

Gmail

jyoti batra <jyotivinay89@gmail.com>

Fwd: Selection of ICMR- Short Term Studentship (STS) 2019 application- reg.

2 messages

mohini chauhan <mohinichauhan21@gmail.com>
To: jyoti batra <jyotivinay89@gmail.com>

Wed, May 1, 2019 at 4:46 PM

——— Forwarded message ———
From:**mohini chauhan** <mohinichauhan2019@gmail.com>
Date: Wed 1 May, 2019, 4:46 PM
Subject: Fwd: Selection of ICMR- Short Term Studentship (STS) 2019 application- reg.
To: <mohinichauhan21@gmail.com>

——— Forwarded message ———
From:**ICMR-STS** <sts@bmi.icmr.org.in>
Date: Wed 1 May, 2019, 4:30 PM
Subject: Selection of ICMR- Short Term Studentship (STS) 2019 application- reg.
To: <mohinichauhan2019@gmail.com>

No: 21/1/2019-HRD-STS

MUST forward this E-mail to your guide.

Dear Students,

This is in reference to your application for ICMR- Short Term Studentship-2019 submitted ONLINE to the ICMR.

I am pleased to inform you that the DG, ICMR approves selection of your application for STS to carry out the proposed research work in any two months (between April to July, 2019) to work on the proposed STS research project. **The award of Stipend Rs. 20,000/- and a Certificate is subjected to conditions and approval of your STS Report (to be submitted in October 2019) by ICMR.** Kindly complete the research at the earliest, so that the Report can be prepared and submitted in time. (Report Submission guidelines are avaible at: http://14.139.60.56:84/Instuctions.aspx)
Please note that NO Report shall be considered/accepted after the last date i.e. 31st October 2019 (till 3:00 PM) only.

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Please note that NO Report shall be considered/accepted after the last date i.e. 31st October 2019 (till 3:00 PM) only.

The research carried out by the student must be in compliance with ICMR's *ICMR Ethical Guidelines for Biomedical Research on Human Participants, 2017* and *National Ethical Guidelines for Biomedical Research Involving Children*, which may be downloaded from ICMR website (http://www.icmr.nic.in/guidelines/ICMR_Ethical_Guidelines_2017.pdf) and http://www.icmr.nic.in/guidelines/National_Ethical_Guidelines_for_BioMedical_Research_Involving_Children.pdf

On completion of the studentship, a Report of the actual work done may please be submitted ONLINE as per detailed formal and instructions specified on ICMR website.
IMPORTANT: It is advised that prior to writing/submitting the report, please go through the detailed Report Submission Guidelines/Instructions given on ICMR-STS website.

In case of any queries, please send email to stshrd2017@gmail.com

Best wishes,

(Mrs. Harjeet Bajaj)
Administrative Officer-STS
(For Director General)

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STS REFERENCE ID: 2019-02483

ASSOCIATION OF DEPRESSION WITH OXIDATIVE STRESS AND DIABETES MELLITUS TYPE 2 - A CASE-CONTROL STUDY

INTRODUCTION

India is home to an estimated 57 million people affected by depression, known to be associated with high incidences of morbidity, disability as well as mortality, along with significant socioeconomic losses. World Health Organisation, in 2017, had given the slogan "Depression–Let's talk'' thereby increasing awareness regarding mental health diseases. Depression is one of the two diagnostic categories, other being anxiety disorder, which may be a cause and consequence of several non-communicable diseases such as cancer, ischemic heart disease, diabetes, obesity, hypertension, etc. As per National Institute of Mental Health and Neurosciences, one in 20 people (5.25%) over 18 years of age have suffered (at least once in their lifetime) from depression, suggesting a multi-fold rise in near future[1,2] .

Diabetes mellitus is a chronic disease caused by inherited and or/acquired deficiency of insulin by the pancreas, diabetes mellitus type 1 (T1DM), or by the ineffectiveness of insulin produced, which accounts for around 90% of diabetics worldwide , diabetes mellitus type 2 (T2DM), damaging many of the body's systems, including the blood vessels and nerves which may undergo long term damage and dysfunction[3].This metabolic disorder which is characterized by hyperglycaemia, can also result in activation and production of reactive oxygen species which can ultimately lead to oxidative stress. According to Wild et al. the prevalence of diabetes is predicted to double globally reaching to 366 million in 2030, with a maximum increase in India by 2030[4].

Oxidative stress is an imbalance between cellular production of reactive oxygen species and the counteracting antioxidant mechanisms. The brain with its high oxygen consumption and a lipid-rich environment is considered highly susceptible to oxidative stress or redox imbalances. The oxidative stress is implicated in several mental disorders including depression, anxiety and is known to be the underline cause for the development of insulin resistance, β-cell dysfunction, impaired glucose tolerance and T2DM through the production of reactive oxygen species that may increase the risk of atherosclerosis [5, 6].

Depression being one of the most neglected symptoms especially in diabetics, lowers the quality of life for the individual. However, there is a limited data available on the association of depression with T2DM. It is still not clear whether one condition can lead to the pathogenesis and emergence of the other condition, debilitating the body and function of the brain .A physician must provide an accurate treatment for the disease in such a way that the quality of life for the patient improves with minimum amount of complication. Keeping this gap in view, the present study was designed to find an association between these two endemic diseases, which is the need for the hour.

REVIEW OF LITERATURE

Studies of the World Health Organization suggest that in the year 2020, depressive disorder will be the illness with the highest burden of disease. In the last several years, oxidative stress has received much attention with regard to psychiatric illnesses like depression and it has been proposed as a contributing factor in the pathogenesis of depression [5, 7]. Oxidative stress is defined as a persistent imbalance between oxidation and anti-oxidation, which leads to the damage of cellular macromolecules. Interestingly, the brain appears to be more

susceptible to the reactive oxygen species (ROS) and reactive nitrogen species (RNS), because of the high content of unsaturated fatty acids, high oxygen consumption per unit weight, high content of key ingredients of lipid peroxidation and scarcity of antioxidant defence systems. In depression oxidative products include products of oxidative damage of lipid peroxidation, protein and DNA. As a product of lipid peroxidation, abnormal malondialdehyde (MDA) levels in depression have been reported. On the other hand, some studies have reported that patients of depression have significant alterations in total antioxidant capacity, free radicals, and oxidative products. The enzymatic antioxidants include superoxide dismutase (SOD), glutathione peroxidase, catalase, glutathione reductase and so on [8].

Although several past studies have established a link between oxidative stress and psychiatric disorders, the causal relationship between oxidative stress and psychiatric diseases is not fully determined due to its complexity .The current thought is that the antidepressants exert their therapeutic effect by suppressing pro inflammatory cytokines and ROS/RNS production or enhancing anti-oxidant defence. It was reported that individuals who suffer from depression displayed lower serum/plasma antioxidant potentials and reduced brain glutathione levels. Thus, substantial data support the concept that depression is accompanied with heightened oxidative stress and antidepressant treatments may reduce oxidative stress, suggesting that perhaps augmentation of antioxidant defences is one of the mechanisms underlying the neuroprotective effects of antidepressants[5].

Increasing evidences have suggested that oxidative stress plays a major role in the pathogenesis of diabetes mellitus as well. Oxidative stress also appears to be the pathogenic factor in underlying diabetic

complications that may be due to any imbalance between the reactive species and antioxidants [9].

Most of the studies reveal the inference of oxidative stress in diabetes pathogenesis by the alteration in enzymatic systems, lipid peroxidation, impaired glutathione metabolism and decreased Vitamin C levels. Lipids, proteins, DNA damage, Glutathione, catalane and superoxide dismutase are various biomarkers of oxidative stress in diabetes mellitus. Oxidative stress induced complications of diabetes may include stroke, neuropathy, retinopathy, nephropathy and many more micro vascular complications as well [10].

However, the inter-relationship between the pathogenesis of depression and T2DM still remains uncertain, due to their complexity and depriving many patients of right approach to their treatment. Present study and its discussion focusses on this concept proposing that oxidative stress mechanisms mediate cognitive, emotional and physiological health.

AIMS AND OBJECTIVES

The aim of the present study is to analyse the association between depression and T2DM, with the following objectives –

1. To measure the level of insulin resistance in the patients of newly diagnosed depression with and without T2DM.
2. To study the ratio of oxidants/antioxidant in the patients of newly diagnosed depression with and without T2DM.
3. To study the correlation of depression with oxidative stress.
4. To study the correlation of depression with T2DM.
5. To study the correlation between oxidative stress and T2DM.

MATERIAL AND METHODS

Study-design: A case-control observational study.

Site: Department of Biochemistry, Tertiary care Hospital, Ghaziabad.

Duration of Study: 2 months.

Sample Size: 60 newly diagnosed patients of depression from either sex in the age group between 40-65 years, with and without T2DM.

Source of sample: After approval of the Institutional Ethics Committee (letter attached), a total of 60 newly diagnosed patients of depression attending the Psychiatry outpatient department of tertiary care hospital at Ghaziabad, were enrolled. The participants were requested to answer a predesigned and structured questionnaire which included data on medical history related to diabetes, mental health, relevant family history, treatment regimen, etc. after the Informed consent was taken. The subjects were divided into 2 groups-

Group I: 30 newly diagnosed patients of depression without T2DM

Group II: 30 newly diagnosed patients of depression with T2DM, which included newly diagnosed patients of T2DM as part of routine /clinical investigations at the psychiatry outpatient department of tertiary care hospital at Ghaziabad

Exclusion Criteria: The subjects with hepatic diseases, cardiovascular disease, renal disease, pulmonary tuberculosis, acute/chronic inflammatory and prolonged illness, any mental-health disorder except depression as well as smokers, alcoholics or any drug-addicts were excluded from the study.

Informed consent: The patients were informed regarding the observational study required to be made by the student and their blood samples were collected after their complete consent, by signing of patient information sheet / consent form by the patient or the guardian.

Anthropometric Data: Anthropometric data including weight and height were measured using a Seca scale (Seca 725; GmbH and Co.) to nearest 100g and 0.5cm respectively. Body Mass Index (BMI) were calculated as weight (kg) divided by height in square metre (m^2).

Blood Pressure: Blood pressure was measured on the left arm using mercury column sphygmomanometer in resting state.

Biochemical estimations:

Collection of blood sample: After overnight fasting, under all aseptic precautions, 7ml of blood was collected from the subjects. Thereafter, blood samples was centrifuged at 3000 rpm for 10 minutes, the serum was separated and stored at -20°C in Eppendroff tube until the analysis was carried out . Serum was dispensed into different tubes and analysed for fasting plasma insulin level, fasting plasma glucose level, oxidant marker (MDA) and antioxidant marker (SOD).

Different biochemical tests were done to analyse -

1) THE LEVEL OF INSULIN RESISTANCE

Method: HOMA-IR (Homeostatic Model Assessment of Insulin Resistance)

HOMA predicts the relationship between glucose and insulin dynamics by defining fasting steady-state glucose and insulin concentrations for a wide range of possible combinations of insulin resistance and β-cell function.

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HOMA-IR = (Glucose × Insulin) / 22.5

- Estimation of fasting insulin- Chemiluminescence enzyme Immunoassay.

  The Insulin (Human) CLIA Kit is based on a solid phase enzyme-linked immunosorbent assay. The assay system utilizes one anti-Insulin antibody for solid phase (microtiter wells) immobilization and another anti-Insulin antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The standards and test specimen (serum) are added to the Insulin antibody coated microtiter wells. Then anti-Insulin antibody labelled with horseradish peroxidase (conjugate) is added. After 1 hour incubation at room temperature, the wells are washed with water to remove unbound labelled antibodies. A solution of a chemiluminescent substrate is then added and read. The intensity of the emitting light is proportional to the amount of enzyme present and is directly related to the amount of Insulin in the sample [11].

- Estimation of fasting glucose - GOD-POD Method.

  Glucose oxidase (GOD) catalyses the oxidation of glucose to gluconate. The formed hydrogen peroxide (H_2O_2) is detected by a chromogenic oxygen acceptor, phenol, 4- Aminophenazone (4-AP) in the presence of Peroxidase (POD):

$$\beta\text{-D- Glucose} + O_2 + H_2O \xrightarrow{GOD} \text{Gluconate} + H_2O$$

$$H_2O_2 + \text{Phenol} + (4\text{-AP}) \xrightarrow{POD} \text{Red Quinone dye} + H_2O$$

The intensity of the colour formed is proportional to the glucose concentration in the serum [12].

2) TO MEASURE THE LEVEL OF ANTI-OXIDANT (SOD)

Method: Estimation of Superoxide Dismutase (SOD) by Marklund and Marklund method [13].

This method utilizes the inhibition of auto-oxidation of pyrogallol by superoxide dismutase. Pyrogallol auto-oxidises rapidly in aqueous or alkaline medium solution and this has been employed for the estimation of superoxide dismutase. SOD inhibits the auto-oxidation of pyrogallol.

Reagents-

a) Tris buffer – 50 ml of tris buffer was prepared. To this, 50 ml of HCL was added to adjust the pH at 8.5 and volume was made up to 100 ml.
b) Pyrogallol (20 mM concentration): 25 mg of pyrogallol was dissolved in 10 ml of distilled water.

Procedure: Labelled two test tubes as 'control' and 'test' and the following additions were done to start the reaction.

REAGENT	CONTROL	TEST
Tris buffer	3.0 ml	2.95 ml
Pyragallol	0.3ml	0.3 ml
Serum	-	0.05ml

Mixed and measured the absorbance continuously for 4 minutes at 420 nm.

Calculations: Absorbance reading to be taken for calculation is reading at 3.5 minutes reading at 1.5 minutes. One unit of SOD is described as the amount of enzyme required to cause 50% inhibition of pyrogallol auto-oxidation per 3.0 ml of the assay mixture. If absorbance reading of control was taken as 'A' and absorbance reading of test was taken as 'B'.

Concentration of SOD in U/mL = $\frac{A-B}{A\times 50} \times \frac{100}{0.05}$

3) TO MEASURE THE LEVEL OF OXIDANT (MDA)

Method: Estimation of Malondialdehyde (MDA) by Kei Satoh method [14].

In this method, serum and TBA are heated together to form chromogen. The chromogen then extracted with n-butyl alcohol, and the reading of organic phase was noted at a wavelength of 530nm.

REAGENTS

a) Trichloroacetic acid (20%)
b) Sulphuric acid (0.05 M)
c) Sodium sulphate solution (2M)
d) TBA reagent (Thiobarbituric acid)
e) N-Butyl alcohol
f) Standard solution

PROCEDURE

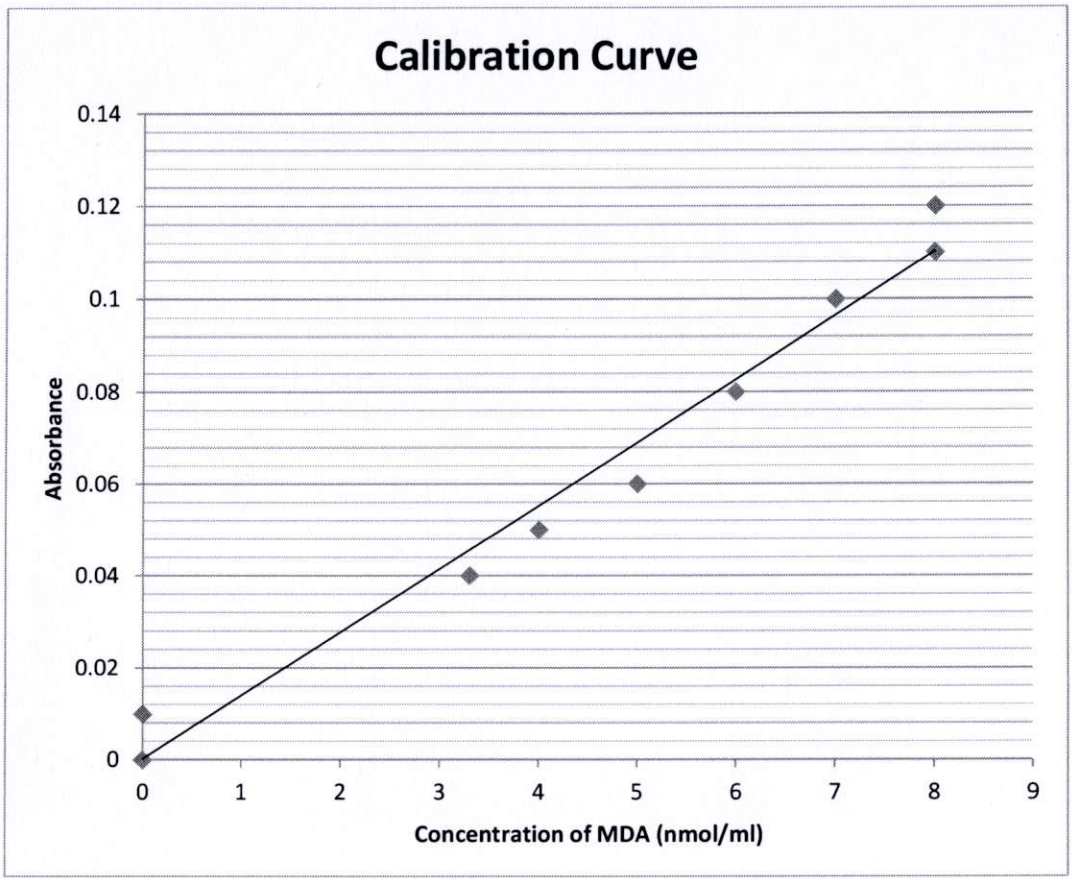
The centrifuge tubes were labelled as 'T' and 'B' for test and blank respectively. Then the following quantities were added

REAGENT	TEST	BLANK
Serum	0.5	-
20% TCA	2.5	-
Distilled water	-	3.0

The tube 'T' was allowed to stand for 10 minutes at room temperature and then centrifuged. After centrifugation, the supernatant was decanted and the precipitate was washed once with 0.05 M sulphuric acid. Then the following quantities were added.

REAGENT	TEST	BLANK
Sulphuric acid	2.5 ml	2.5 ml
TBA	3.0 ml	3.0 ml

The coupling of lipid peroxide with TBA was carried out by heating in boiling water bath for 30minutes. After cooling in cold water, the chromogen was extracted with 4.0 ml of n-butyl alcohol by vigorous shaking. Separation of the organic phase was facilitated by centrifugation at 3000 rev/min, and its absorbance was determined at the wavelength of 530nm. Obtained values were plotted on the graph. From the linear graph concentrations of sample were determined.



Statistical analysis: Data was analysed by SPSS statistical software and the values were expressed as Mean ± SD. The statistical differences between cases and controls was determined by an independent student's 't-test'. Pearson's correlation coefficient was calculated to determine the correlation between the studied parameters. As Pearson correlation, the increase in one variable with increase in the other variable is called positive correlation while a decrease in one variable increases the other variable is known as negative or inverse correlation. The p-value less than 0.05 was considered as statistically significant.

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OBSERVATIONS AND RESULTS

Total number of study participants were 60 individuals who fulfilled the inclusion criteria.

The participants were placed into two groups according to their manifestation of depression with or without diabetes mellitus.

GROUP-1 PATIENTS OF DEPRESSION WITHOUT DIABETES MELLITUS	***GROUP-2*** PATIENTS OF DEPRESSION WITH DIABETES MELLITUS
N=30	N=30

(N=number of patients)

The baseline characteristics of both groups are shown below in Table-1

Variables	**Group-1 (N = 30)**	**Group – 2 (N = 30)**	**T- value**	**p-value**	**Conclusion**
	Mean ±S.D	**Mean ±S.D**			
Age	50.77 ± 7.86	55.77± 6.38	2.70	0.009	Significant
BMI	22.41 ± 0.63	21.75 ± 0.45	4.40	0.001	Significant

Table-1: **Anthropometric parameters in patients of depression with and without T2DM. All values are expressed in mean ± SD. p <0.05 considered as statistically significant. The total number of subjects in each group are 30. Student t-test was applied between depressive patients with or without T2DM and it was found that all the variables**

had significant difference between the two groups with p-value of <0.05 at 95% Confidence Interval.

This table shows the mean value of age and BMI in both the groups. There was a significant age gap in the patients of depression with and without T2DM.The patients with depression who were not suffering with T2DM were in the lower age group. (Figure- 1a)

The mean BMI of depressive patients without T2DM was 22.41 ± 0.63. Whereas, the mean BMI of depressive patients with T2DM was 21.75 ± 0.45. There was a significant decrease in the values of BMI in the patients of depression with T2DM. Maximum BMI was recorded between 19-24.5 kg/m^2 as shown below for both the groups of depressive patients with and without T2DM. (Table- 1b and Figure- 1b)

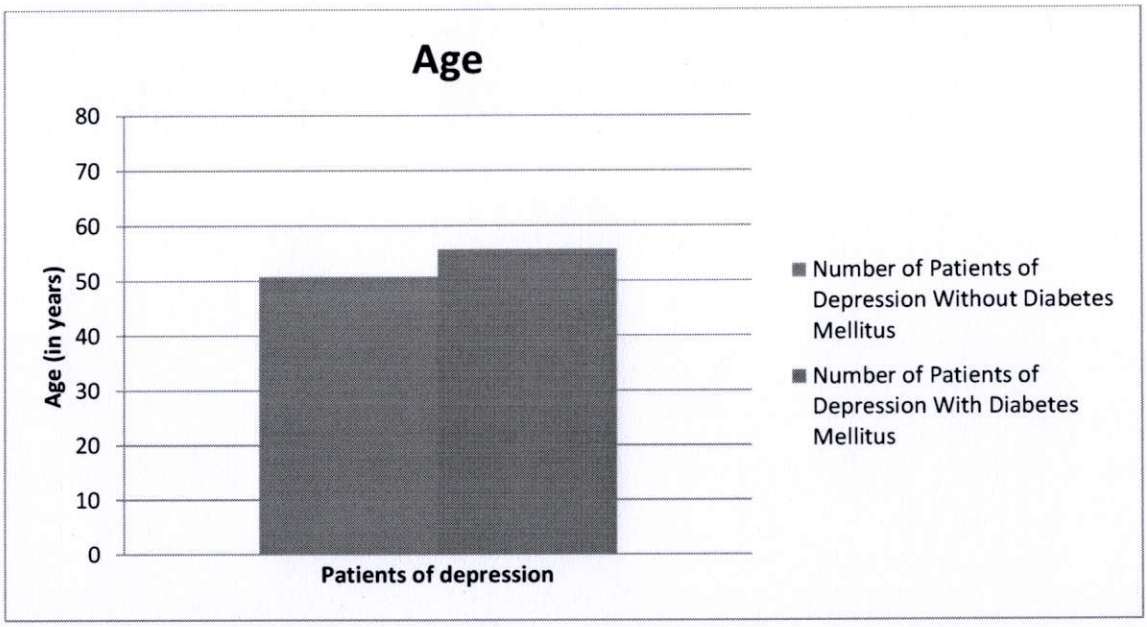

Figure-1a

Shows the mean value of age of the depressive patients without T2DM (Mean ± S.D=50.77 ± 7.86) and with T2DM (Mean ± S.D- 55.77 ± 6.38). All values are expressed in mean ± SD. p <0.05 considered as statistically significant. The total number of subjects in each group are

30. Student t-test was applied between depressive patients with or without T2DM and it was found that all the variables had significant difference between the two groups with p-value of <0.05 at 95% Confidence Interval.

BMI (kg/m$^{2)}$	Patients of Depression Without Diabetes Mellitus		Patients of Depression With Diabetes Mellitus	
	Frequency	Percentage	Frequency	Percentage
< 18.5	0	0	0	0
19-24.5	20	66.7	21	70
24.5-29.5	10	33.3	9	3
> 29.5	0	0	0	0
Total	30	100.0	30	100

Table-1b: **The BMI of patients suffering from depression ranged from 19.8 to 29 kg/ m^2. The mean BMI of depressive patients without T2DM was 22.41 ± 0.63. Whereas, the mean BMI of depressive patients suffering from T2DM was found to be 21.75 ± 0.45. All values are expressed in mean ± SD. p <0.05 considered as statistically significant. The total number of subjects in each group are 30. Student t-test was applied between depressive patients with or without T2DM and it was found that all the variables had significant difference between the two groups with p-value of <0.05 at 95% Confidence Interval.**

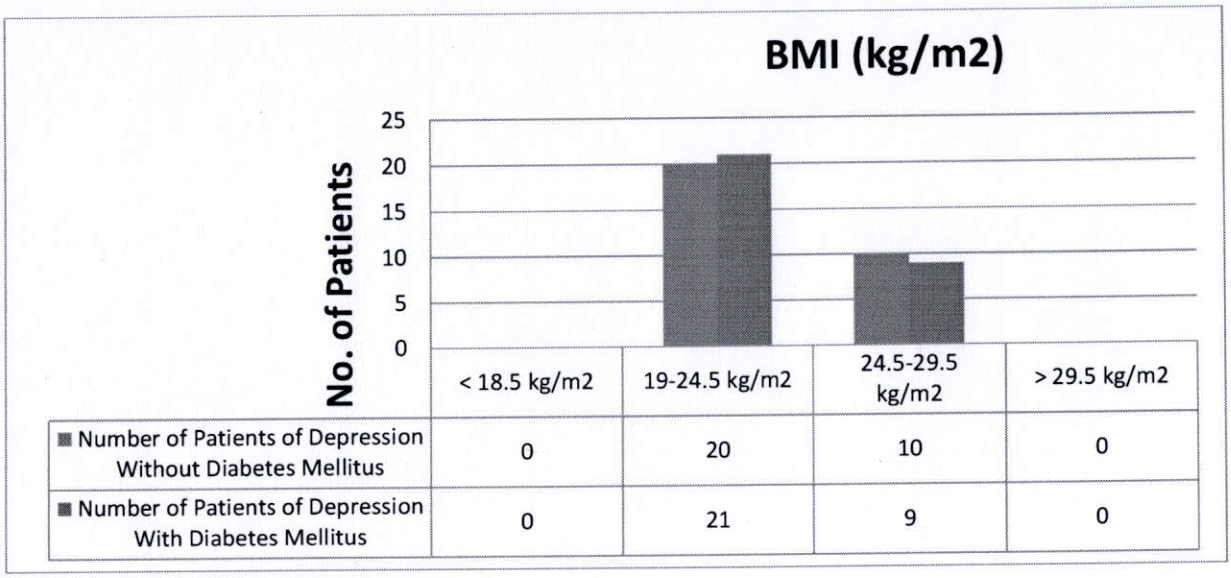

Figure -1b: **Distribution of depression patients with and without diabetes mellitus according to Body Mass Index.**

Variables	Group-1 (N = 30)	Group – 2 (N = 30)	T- value	p-value	Conclusion
	Mean ± SD	Mean ± SD			
Insulin Resistance	0.97 ± 0.34	2.40 ± 0.66	10.45	0.001	Significant
MDA	4.52 ± 0.67	6.23 ± 1.05	7.45	0.001	Significant
SOD	8.50 ± 0.92	6.48 ± 1.23	7.19	0.001	Significant

Table-2: **Biochemical parameters in patients of depression with and without T2DM. All values are expressed in mean ± SD. p <0.05**

considered as statistically significant. The total number of subjects in each group are 30. Student t-test was applied between depressive patients with or without T2DM and it was found that all the variables had significant difference between the two groups with p-value of <0.05 at 95% Confidence Interval.

We also found a significant increase in the levels of oxidants as shown by MDA and a significant decrease in the values of anti-oxidants as shown by SOD. However, there was a significant increase in insulin resistance in the group-2, which represents the subjects of depression with T2DM.

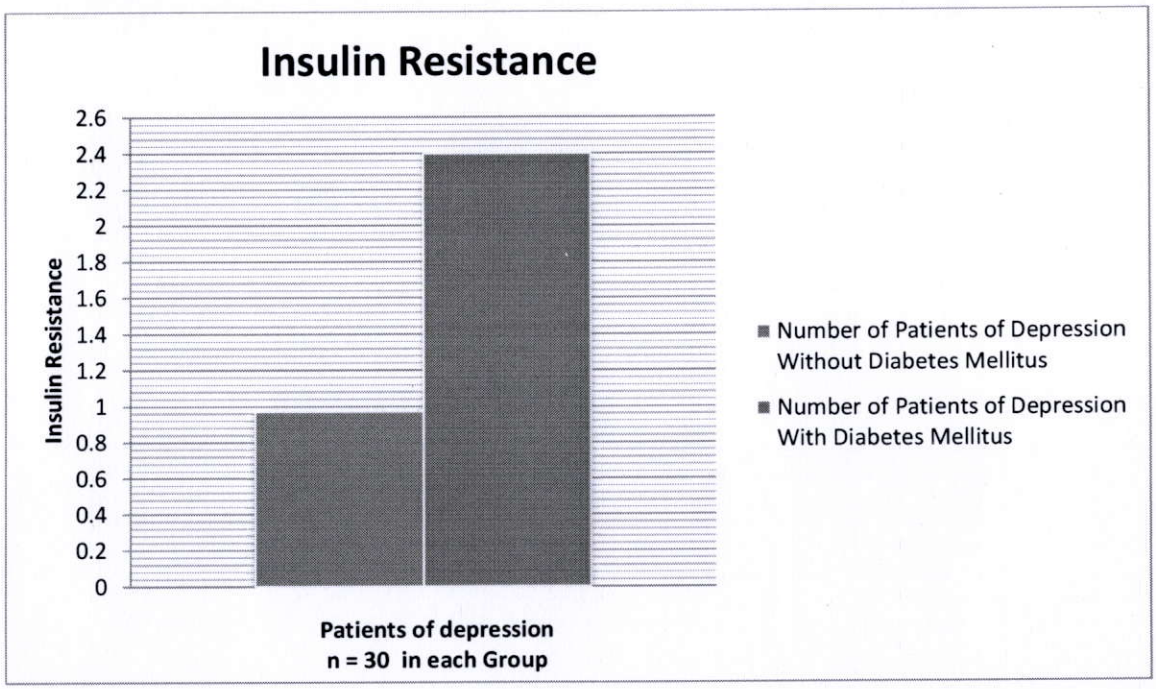

Figure -2a

Shows the mean value of Insulin Resistance in the depressive patients without T2DM (Mean ± S.D=0.97±0.34) and with T2DM (Mean ± S.D=2.40 ± 0.66). All values are expressed in mean ± SD. p <0.05 considered as statistically significant. The total number of subjects in each group are 30. Student t-test was applied between depressive

patients with and without T2DM and it was found that all the variables had significant difference between the two groups with p-value of <0.05 at 95% Confidence Interval.

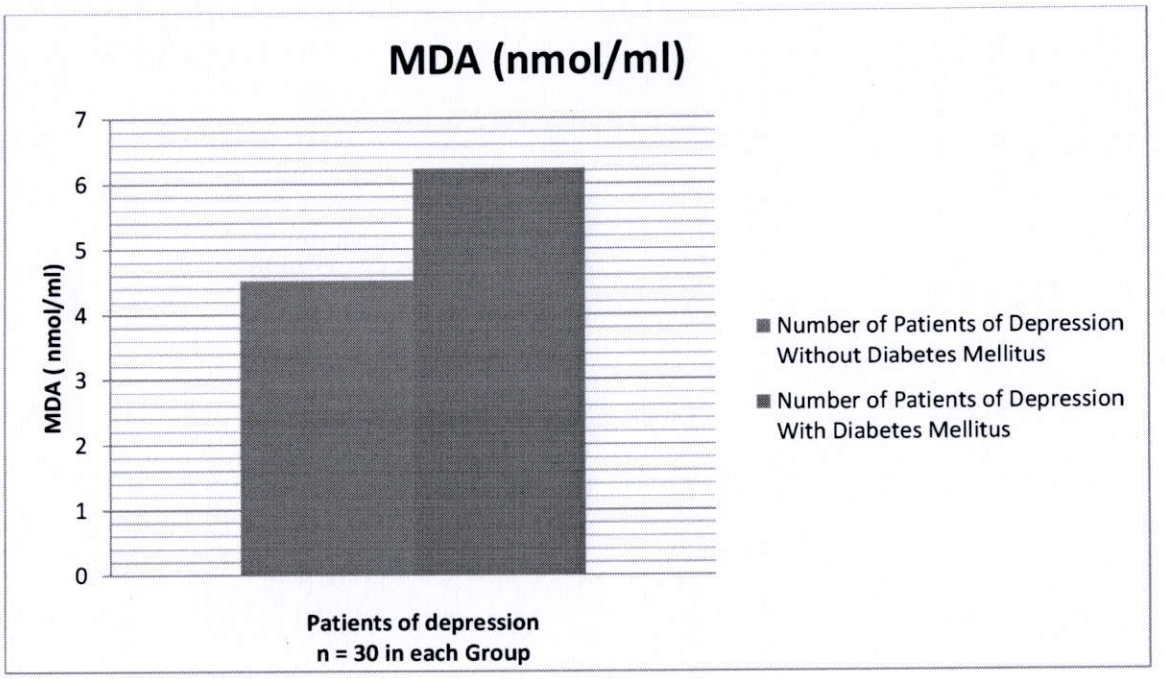

Figure -2b

Shows the mean value of MDA in the depressive patients without T2DM (Mean ± S.D=4.52±0.67) and with T2DM (Mean ± S.D=6.23 ± 1.05). All values are expressed in mean ± SD. p <0.05 considered as statistically significant. The total number of subjects in each group are 30. Student t-test was applied between depressive patients with and without T2DM and it was found that all the variables had significant difference between the two groups with p-value of <0.05 at 95% Confidence Interval.

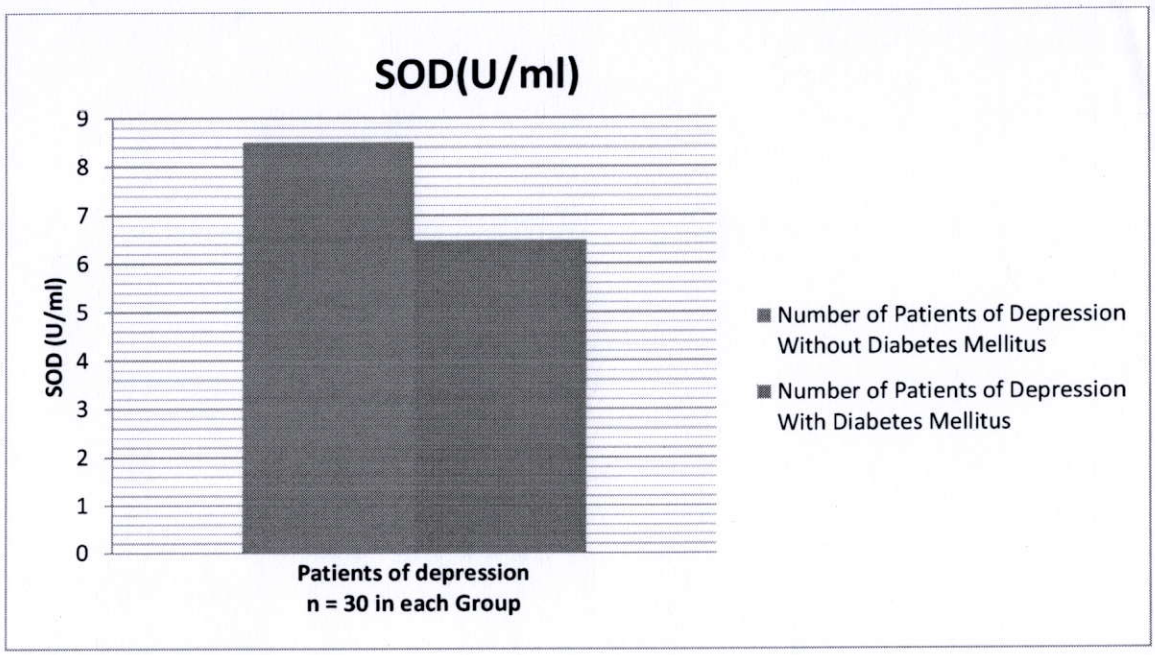

Figure-2c

Shows the mean value of SOD in the depressive patients without T2DM (Mean ± S.D=8.50±0.92) and with T2DM (Mean ± S.D=6.48 ± 1.23). All values are expressed in mean ± SD. p <0.05 considered as statistically significant. The total number of subjects in each group are 30. Student t-test was applied between depressive patients with and without T2DM and it was found that all the variables had significant difference between the two groups with p-value of <0.05 at 95% Confidence Interval.

Group-1 (N=30)	Variables	Correlation (r value)	p -Value	Conclusion
Patients of Depression Without Diabetes Mellitus	INSULIN RESISTNCE	0.476	0.008	Significant
	MDA			
	INSULIN RESISTNCE	-0.096	0.040	Significant
	SOD			
	MDA	-0.512	0.004	Significant
	SOD			

Table- 3

Correlation between MDA and insulin resistance, SOD and insulin resistance, SOD and MDA in patients of depression without diabetes mellitus. r- Value represents the Pearson coefficient, p<0.05 considered as significant. The total number of subjects in each group are 30.

Among the depressive patients who did not have T2DM, insulin resistance was positively correlated with MDA with a significant p value <0.05. Whereas, insulin resistance was negatively correlated with SOD with a significant p value <0.05. MDA and SOD were negatively correlated with each other as well, with a significant p value of <0.05.

Group-2 (N=30)	Variables	Correlation (r value)	p Value	Conclusion
Patients Of Depression With Diabetes Mellitus	INSULIN RESISTANCE	0.634	0.001	Significant
	MDA			
	INSULIN RESISTANCE	-0.180	0.009	Significant
	SOD			
	MDA	-0.434	0.017	Significant
	SOD			

Table-4

Correlation between MDA and insulin resistance, SOD and insulin resistance, SOD and MDA in patients of depression with diabetes mellitus. r- Value represents the Pearson coefficient, p<0.05 considered as significant. The total number of subjects in each group are 30.

Among the depressive patients who were suffering from T2DM as well, insulin resistance was positively correlated with MDA with a significant p value <0.05. Whereas, insulin resistance was negatively correlated with SOD with a significant p value <0.05. MDA and SOD were negatively correlated with each other as well, with a significant p value of <0.05.

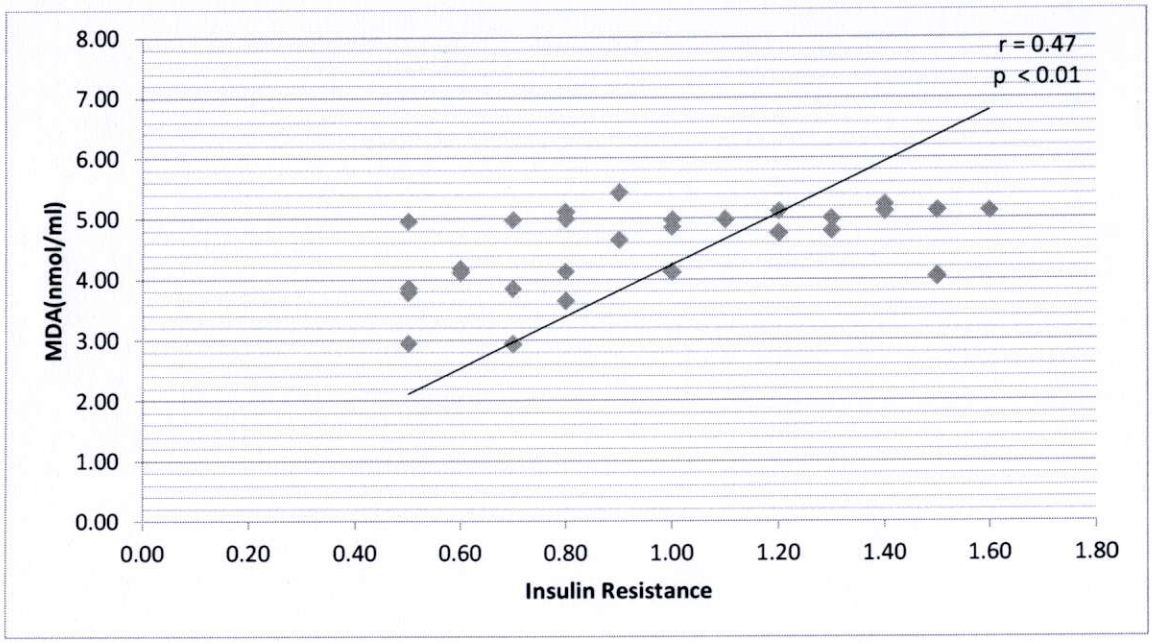

Figure 4 a: **Correlation between MDA and Insulin Resistance in patients suffering from depression without T2DM.**

('r' value represents the Pearson coefficient , p<0.05 considered as significant)

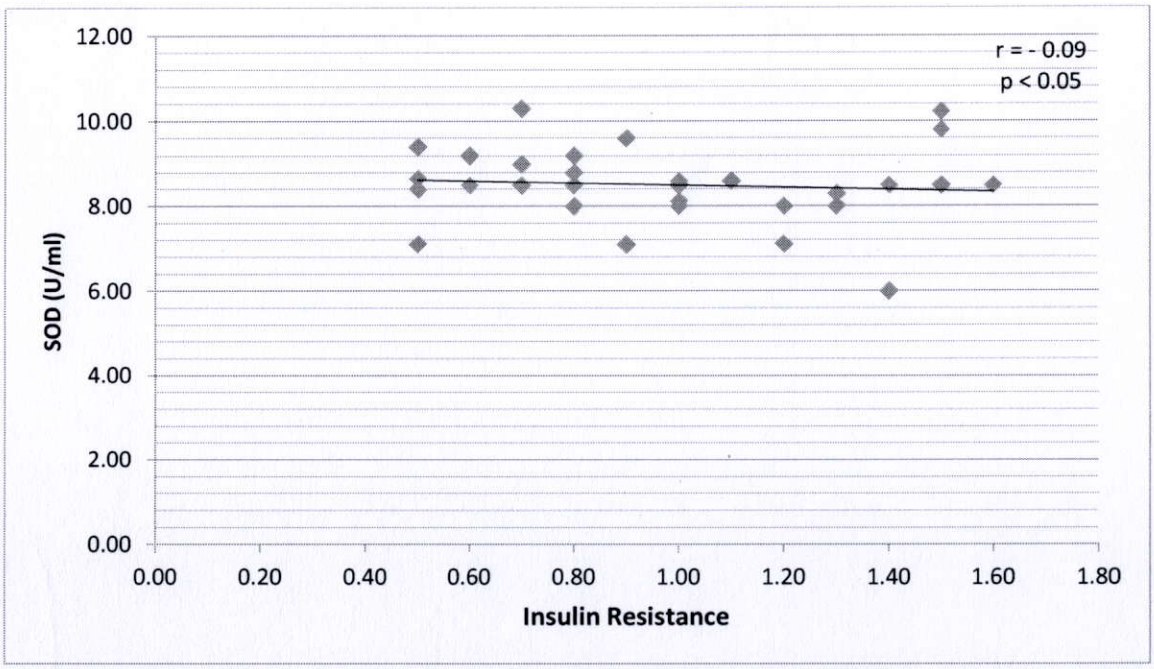

Figure 4 b: **Correlation between SOD and Insulin Resistance in patients**

suffering from depression without T2DM.('r' value represents the Pearson coefficient , p<0.05 considered as significant)

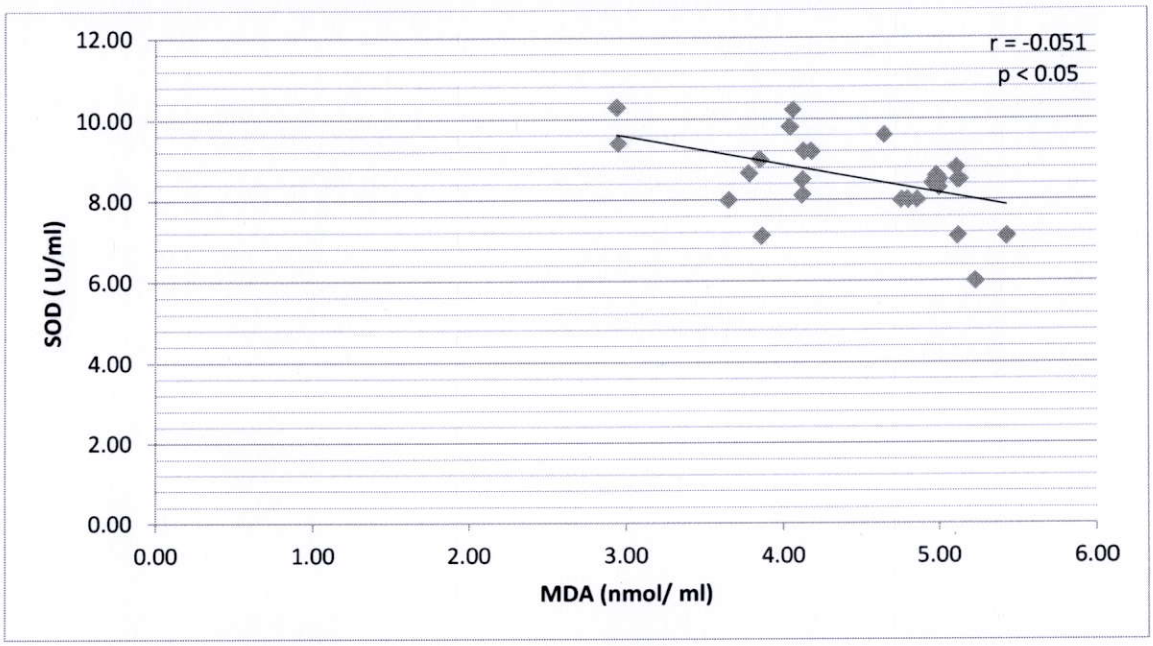

Figure 4 c: **Correlation between SOD and MDA in patients suffering from depression without T2DM.**

('r' value represents the Pearson coefficient , p<0.05 considered as significant).

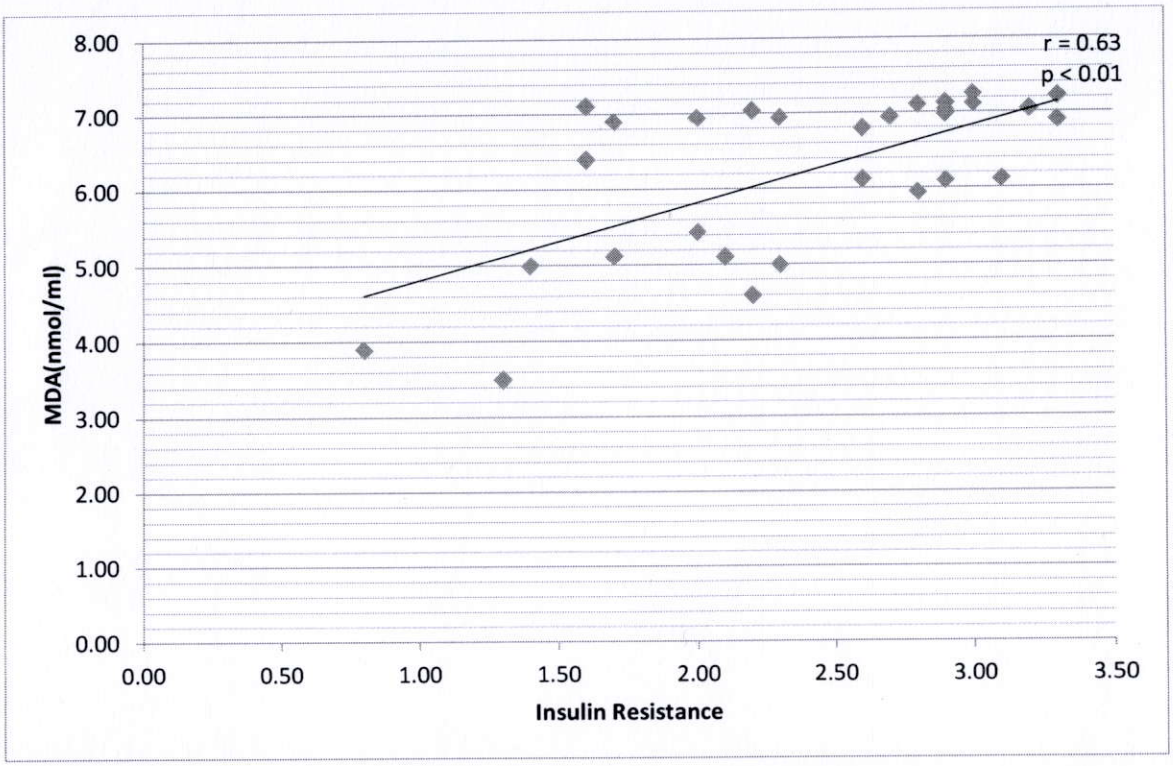

Figure 4 d: **Correlation between MDA and Insulin Resistance in patients suffering from depression with T2DM.**

('r' value represents the Pearson coefficient , p<0.05 considered as significant).

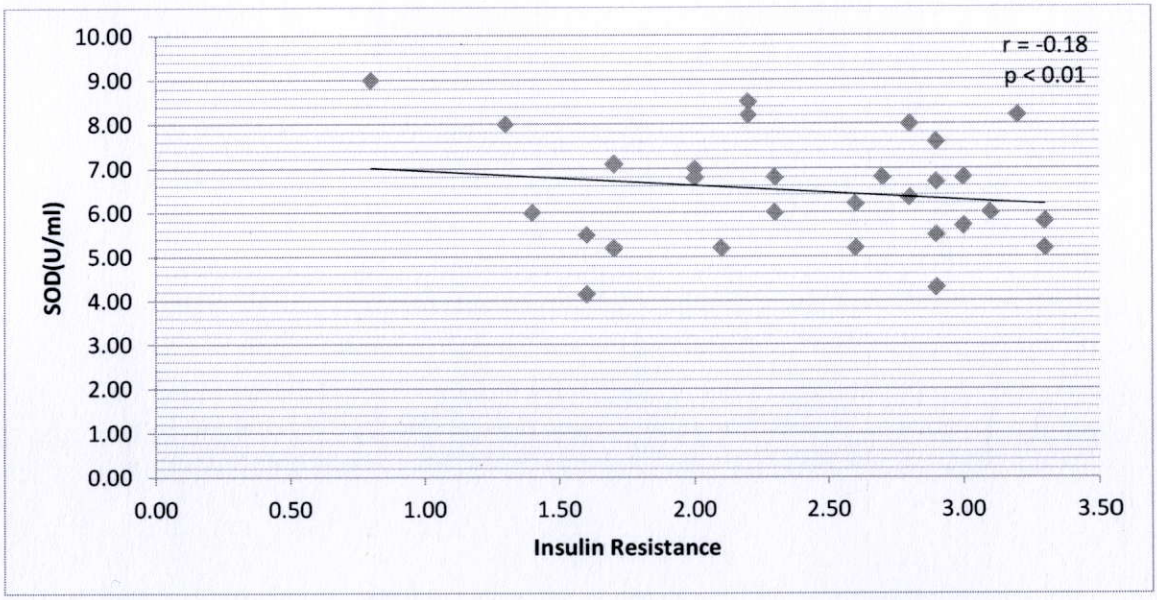

Figure 4 e: **Correlation between SOD and Insulin Resistance in patients suffering from depression with T2DM.**

('r' value represents the Pearson coefficient , p<0.05 considered as significant)

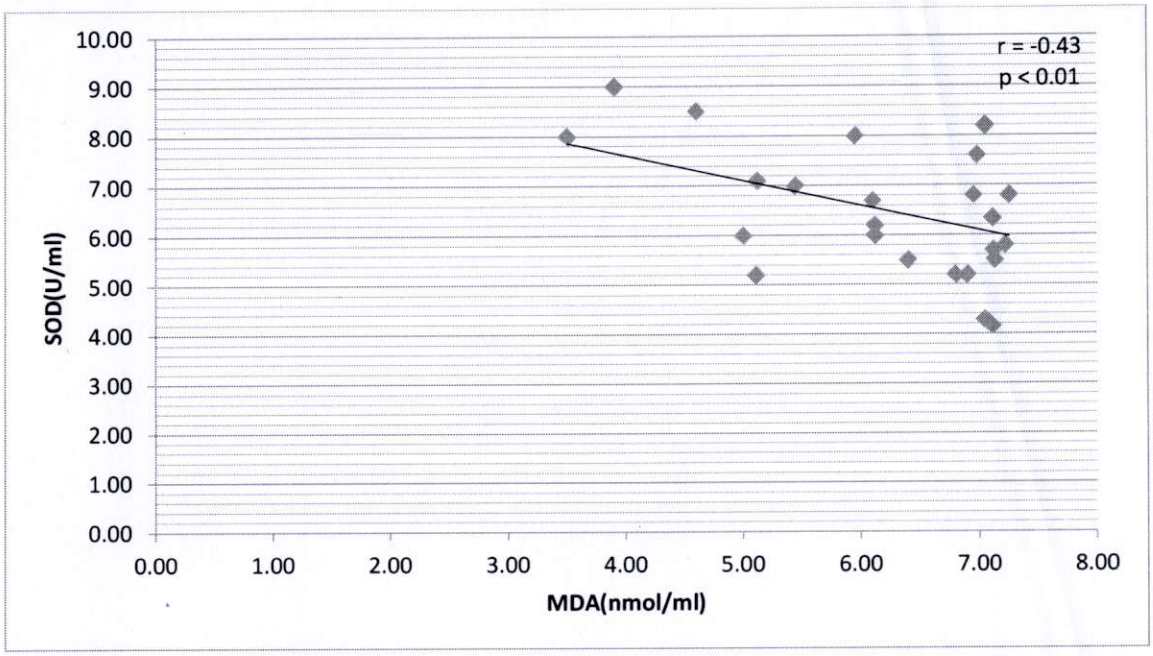

Figure 4 f: **Correlation between SOD and MDA in patients suffering from depression with T2DM.**

('r' value represents the Pearson coefficient , p<0.05 considered as significant)

★ The subjects with hepatic diseases, cardiovascular disease, renal disease, pulmonary tuberculosis, acute/chronic inflammatory and prolonged illness, any mental-health disorder except depression as well as smokers, alcoholics or any drug-addicts were excluded from the study.

★ Patients were asked to fill a questionnaire (enclosed at the end) for obtaining their personal history and for knowledge of their past history as well.

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GHAZIABAD, NCR DELHI

★ Hamilton rating scale for depression was also used by the clinician for diagnosing depression (Enclosed at the end).

DISCUSSION

Reactive oxygen species (ROS) produced by products during different metabolic reactions has the potential to cause a number of detrimental effects in the body which reduces the defence mechanism of the body as well. It was originally thought that only phagocytic cells were responsible for ROS production as they play a part in host cell defence mechanisms but ROS has also been known to be increased in depression and diabetes mellitus and is confirmed in the present study by showing an increased levels of oxidized species in depression with and without T2DM.

Number of studies, have been conducted in the past to show relationship between depression and oxidative stress markers. The ratio of oxidants and anti-oxidants have also been found to be disturbed in the patients of diabetes mellitus type 2. However, there are a very limited studies available on the relation with oxidative stress in the patients of depression suffering from T2DM.

In the present study, the levels of oxidative stress markers were measured and comparison was done between the patients of depression (group-1) and depressive patients suffering with T2DM (group-2). Both the groups consisted of 30 patients each. Majority of the patients in the group -1 were in the age of Mean ± SD= 50.77 ± 7.86. Whereas, group-2 were in the age group of Mean ± SD= 55.77 ± 6.38, implying that with increasing age the susceptibility of T2DM increases in the patients of depression.

MDA levels were raised above normal values in the patients of depression. However, there was a significant increase in the MDA levels in the patients of depression suffering from T2DM (p-value >0.001). The

SOD levels were found to be significantly lower in the patients of depression with T2DM as compared to the patients suffering from depression only (Table-2 and Figure 2c). However, the levels were below normal in the group-1. This implies that the ratio of oxidant to anti-oxidant was found to be higher in group 2 in comparison with group 1, where more oxidative stress was observed.

It was interesting to note that insulin resistance was significantly raised in the depressive patients suffering from T2DM. However, there was no change in the insulin resistance in patients suffering from depression only. As the patients were newly diagnosed in both the groups, depression may be a risk factor for development of T2DM .As earlier studies have pointed out the relationship between T2DM and insulin resistance with depression [15].

An intervention study maybe planned to see the effect of anti-depressants on the levels of oxidative stress and in turn its relation with T2DM as shown by the correlation studies between different parameters [16]. It is very clear from the Figure 4 b and 4 e , where a significant negative correlation has been found between insulin resistance and SOD in group 1 and 2, implying the relationship between T2DM and oxidative stress and Pearson coefficient was further raised in the patients of depression who were suffering from T2DM.

There is significant positive correlation between MDA levels and insulin resistance in both the groups. However, there was no significant increase in the values of insulin resistance in group- 1. Thus, correlation studies show that in patients of depression may show manifestation of T2DM in future, as a positive significant correlation was found between the levels of MDA in the patients of depression who were suffering from T2DM. This is suggestive of the fact inflammatory status and oxidative stress are proven concepts behind pathogenesis of depression and can cause

detrimental effects in the body of patient, especially if the patient is suffering from diabetes mellitus as well .

Very few studies, are available on the association of oxidative stress when depression is present along with T2DM in the same patient, oxidative stress imbalance may be the key factor in development of co-morbidities like T2DM associated with depression and vice versa. However, many factors such as insulin resistance, hyperglycaemia may also be involved as all of these conditions which result in increased production of free radicals[17].An increased MDA level inactivates the antioxidant enzyme SOD in untreated cases causing damage of various proteins that may also be one of the causes for reduced enzymatic activity of SOD. The decreased SOD activity may be further explained on the basis of the glycosylation of superoxide dismutase which has been show to lead to enzyme inactivation. Compromised antioxidant functions result in the well-known cascade of hypoxic ischemic injury, inflammation, apoptosis and finally cell death [18].

LIMITATIONS OF STUDY

The strength of the present study are its prospective nature, and application of strict selection criteria. Major limitations is the small set of patients as well as time limitation.

As the study was conducted as a pilot research for STS-2018 which could not include a wider range of patient's population, I suggest that there is a need for further research to establish a precise association between depression and T2DM and identification of other factors like nutritional deficiencies and environmental factors. Some intervention studies may be planned to see the effect of nutritional supplements like anti-oxidants.

Conclusion

In the present study, we can strongly confirm that oxidative stress plays a major role in pathophysiology of depression as well as T2DM. The presence of one disease can have detrimental effects on the other disease confirmed by the increased amount of oxidative stress in depressive patients who were suffering from T2DM as well. This can increase the morbidity and decrease the quality of life for them.

The growing prevalence of depression and T2DM in all parts of the world and the complexity involved in the pathogenesis of these two diseases calls us to bring a much more effective treatment than the ongoing one.

The use of antioxidants, with better medications and lifestyle changes can be incorporated in the treatment of depressive patients who are suffering from T2DM.

It was also found that the age factor of the patient also plays a major part. With increase in age the depressive patient becomes more susceptible to develop T2DM, due to increase in insulin resistance developed over the years. The bi-directionality in pathogenesis of these two major diseases with each other, can help millions of people to get accurate and better understanding of their treatment which still remains very difficult to be treated by the physicians as the medication for depression itself could be a risk factor for the development of T2DM.

SUMMARY

- The present study was designed with the objective of finding an association of oxidative stress with depression and diabetes mellitus type-2. Although there is a plenty of literature available that shows a positive association between depression and

oxidative stress and T2DM with oxidative stress, very few are available which prove association between of oxidative stress in patients of depression with T2DM in this side of globe.

- Keeping this in mind, a study was planned with the aim and objectives of finding a correlation between patients of depression of with T2DM and its association with oxidative stress.
- The study was performed in newly diagnosed patients of depression attending the Psychiatry outpatient department of tertiary care hospital at Ghaziabad. The patients were selected as per the inclusion and exclusion criteria (stated in the article). Their anthropometric data was collected and blood samples were withdrawn for analysis of oxidants-antioxidants levels in the patients of depression with and without T2DM.
- Through our results, we observed that the patients with depression (group-1) had more than normal levels of MDA (Mean ± S.D=4.52 ± 0.67) and significant increase was observed in the depressive patients suffering with T2DM (group-2)(Mean ± S.D= 6.23± 1.05). The values of insulin resistance was normal in the patients of depression without T2DM. However, significant increase was found in the value of insulin resistance in the patients of depression with T2DM. The mean value of SOD of patients suffering from depression was found to be 8.50 with a S.D of 0.92. Whereas, the mean value of patients suffering from depression as well T2DM was found to be 6.48 with a S.D of 1.23.
- In the present study, we had also tried to find a correlation between onset of T2DM in the patients of depression only. A very significant positive correlation was found between the patients of T2DM with depression and insulin resistance in comparison to patients of depression only. However, patients in group-1 were not found to be diabetic as per blood sugar testing. This signifies that insulin resistance check may be mandatory in the patients of

depression who are not suffering from T2DM as per blood sugar results but the levels of insulin resistance were found to be significantly raised showing a higher susceptibility to T2DM. This might be related to significant change in the oxidative stress levels which were found to be increased in both the groups. However, the percentage was more in group 2.

- So through the results, we could gather that co-morbidities are preventable if it is diagnosed at an earlier stage and the role of pharmacological and nutritional intervention maybe studied in further research groups having large population size.

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STUDY PROFORMA

Department of Biochemistry

Tertiary Medical College,

Ghaziabad

ASSOCIATION OF DEPRESSION WITH OXIDATIVE STRESS AND DIABETES MELLITUS TYPE 2 - A CASE-CONTROL STUDY

PERSONAL DETAILS:

Name:
Age/Sex:
Height:
Weight:
Address:

Registration no:
Education:
Occupation:
Contact No.

CLINICAL EXAMINATION:

H/O Present illness:
H/O Tuberculosis:
H/O Renal Disease:
H/O Gestational Diabetes:
H/O COPD
H/O Gout/ Arthritis:
Any other Endocrine disorders:
activity: H/O Any major illness in the past:

H/O Diabetes mellitus:
H/O PCOS:
H/O CVD:
H/O Hypertension:
H/O Cardiac Disease:
Pregnancy (Only for Ladies):
Duration of daily physical

PERSONAL HISTORY:

Any Addiction:

Any Nutritional Supplement:

FAMILY HISTORY:

Any history of depression in family *(if yes, details please):*

Any history of diabetes in family *(if yes, details please):*

Patient Name :_____

Date: _____

Hamilton Rating Scale for Depression (17-items)

1. Depressed Mood

(sadness, hopeless, helpless, worthless)

0 Absent
1 These feeling states indicated only on questioning
2 These feeling states spontaneously reported verbally
3 Communicates feeling states nonverbally, i.e., through facial expression, posture, voice and tendency to weep
4 Patient reports VIRTUALLY ONLY these feeling states in his spontaneous verbal and nonverbal communication

2. Feelings of Guilt

0 Absent
1 Self-reproach, feels he has let people down
2 Ideas of guilt or rumination over past errors or sinful deeds
3 Present illness is a punishment. Delusions of guilt
4 Hears accusatory or denunciatory voices and/or experiences threatening visual hallucinations

3. Suicide

0 Absent
1 Feels life is not worth living
2 Wishes he were dead or any thoughts of possible death to self
3 Suicide ideas or gesture
4 Attempts at suicide (any serious attempt rates 4)

4. Insomnia - Early

0 No difficulty falling asleep
1 Complains of occasional difficulty falling asleep i.e., more than ½ hour
2 Complains of nightly difficulty falling asleep

5. Insomnia - Middle

0 No difficulty
1 Patient complains of being restless and disturbed during the night
2 Waking during the night – any getting out of bed rates 2 (except for purposes of voiding)

6. Insomnia - Late

0 No difficulty
1 Waking in early hours of the morning but goes back to sleep
2 Unable to fall asleep again if gets out of bed

7. Work and Activities

0 No difficulty
1 Thoughts and feelings of incapacity, fatigue or weakness related to activities; work or hobbies
2 Loss of interest in activity; hobbies or work – either directly reported by patient, or indirect in listlessness, indecision and

vacillation (feels he has to push self to work or activities)

3 Decrease in actual time spent in activities or decrease in productivity. In hospital, rate 3 if patient does not spend at least three hours a day in activities (hospital job or hobbies) exclusive of ward chores.

4 Stopped working because of present illness. In hospital, rate 4 if patient engages in no activities except ward chores, or if patient fails to perform ward chores unassisted.

8. Retardation

(slowness of thought and speech; impaired ability to concentrate; decreased motor activity)

0 Normal speech and thought

1 Slight retardation at interview

2 Obvious retardation at interview

3 Interview difficult

4 Complete stupor

9. Agitation

0 None

1 "Playing with" hand, hair, etc.

2 Hand-wringing, nail-biting, biting of lips

10. Anxiety - Psychic

0 No difficulty

1 Subjective tension and irritability

2 Worrying about minor matters

3 Apprehensive attitude apparent in face or speech

4 Fears expressed without questioning

11. Anxiety - Somatic

0 Absent- Physiological concomitants of anxiety such as:

1 Mild- Gastrointestinal - dry mouth, wind, indigestion,
2 Moderate- diarrhea, cramps, belching
3 Severe Cardiovascular – palpitations, headaches
4 Incapacitating Respiratory - hyperventilation, sighing or
Urinary frequency

12. Somatic Symptoms - Gastrointestinal

0 None
1 Loss of appetite but eating without staff encouragement. Heavy feelings in abdomen.
2 Difficulty eating without staff urging. Requests or requires laxatives or medications for bowels or medication for G.I. symptoms.

13. Somatic Symptoms - General

0 None
1 Heaviness in limbs, back or head, backaches, headache, muscle aches, loss of energy and fatigability
2 Any clear-cut symptom rates 2

14. Genital Symptoms

0 Absent- Not ascertained
1 Mild- Symptoms such as: loss of libido,
2 Severe- menstrual disturbances

15. Hypochondriasis

0 Not present
1 Self-absorption (bodily)
2 Preoccupation with health
3 Frequent complaints, requests for help, etc.
4 Hypochondriacal delusions

16. Loss of Weight

A. When Rating by History:

0 No weight loss

1 Probable weight loss associated with present illness

2 Definite (according to patient) weight loss

B. On Weekly Ratings by Ward Psychiatrist, When Actual Changes are Measured:

0 Less than 1 lb. weight loss in week

1 Greater than 1 lb. weight loss in week

2 Greater than 2 lb. weight loss in week

17. Insight

0 Acknowledges being depressed and ill

1 Acknowledges illness but attributes cause to bad food, climate, overwork, virus, need for rest, etc.

2 Denies being ill at all

Total Score:_____

(FORMAT ONLY)

PATIENT INFORMATION SHEET/CONSENT FORM

Part 1 Filled by Participant/Patient

I ..... understand the terms and conditions of the experiment. I understand the nature of the study which was explained by (name of the

investigator) in ..... language. I understand the purpose and procedure

of the experiment. I got a chance to ask questions about the study and my questions were answered to my satisfaction. I know my participation in this study is voluntary. I also know that I can withdraw my participation at any time during the research without giving any reason. I know that my name and identity will not be mentioned in the study & confidentiality will be maintained.

Part 2 Filled by Parent/Guardian/Attendant

I ..... permit my (relation of the participant) to participate in the experiment. I clearly understand the nature, risks and benefits of the study.

....

Signature of Parent/Guardian/Attendant

Date