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The Effect of Oxidative Stress And Inflammatory Status In Pre-Diabetic Subjects

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Abstract
Background: *Impaired glucose tolerance and impaired fasting glucose, referred to as pre diabetes form an intermediate stage in the natural history of diabetes mellitus. Oxidative stress is a determinant of insulin resistanceand markers of systemic oxidative stress are elevated in clinical type 2 diabetes, but there are only limited data relating the degree of oxidative stress to insulin resistance in pre-diabetic state.*
Objective: *1. To estimate the markers of oxidative stress and inflammatory status in pre-diabetic subjects.*
2. To correlate the association between oxidative stress and inflammation with pre diabetes.
Materials and Methods: *This was a hospital based cross sectional study and it included 100 subjects (50 cases and 50 controls). Age & sex matched normal healthy individuals between the age group 18 – 50 served as control. Markers like serum Malondialdehyde (MDA), Reduced glutathione (GSH), Glutathione peroxidase (Gpx), Catalase and C Reactive Protein(CRP) were estimated. Data was analyzed using student t test and chi square test using SPSS version 17.*
Results: *In pre diabetic individuals, GSH, Gpx and Catalase levels were significantly decreased (p value 0.001, 0.001, 0.001 respectively) and serum MDA levels were increased (p value 0.001) compared to the control. The levels of serum CRP were also increased in pre diabetic individuals and significant correlation was observed in oxidative stress parameters.*
Conclusion: *The present study revealed that oxidative stress and inflammatory changes only will lead the pre diabetics to frank diabetics and make all possible life threatening complications. So early detection of these parameters will be helpful to prevent their risk of progression into frank diabetes.*
Keywords: *Catalase, Glutathione peroxidase, Malondialdehyde, Oxidative stress, Pre diabetes, reduced glutathione.*

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I. Introduction

Type 2 diabetes is an emerging epidemic. The epidemicity has affected both developed and developing countries alike. The worldwide prevalence of diabetes is projected to increase dramatically by 2025[1]. The increase in type 2 diabetes is related to lifestyle changes, especially overweight, obesity, and decreased physical activity. These environmental changes, added on to a genetic background, increases the insulin resistance.
Insulin resistance will cause stress to the beta cells of pancreas, producing beta cell dysfunction. This causes rising glycemia in a non diabetic range. Further reduction in insulin secretion over time results in the development of microvascular and macrovascular complications[2]. Impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) represent intermediate states of abnormal glucose regulation that exist between normal glucose homeostasis and diabetes. IFG can be defined by an elevated fasting plasma glucose (FPG) concentration (≥100 and <126 mg/dl)[2]. IGT is defined by an elevated 2-h plasma glucose concentration (≥140 and <200 mg/dl) on the oral glucose tolerance test (OGTT) in the presence of an FPG concentration <126 mg/dl[3] . Data from the World Health organization (WHO) and American Diabetes Association (ADA) estimates that around 27% of individuals with normal fasting glucose migrate to pre-diabetes and 8% to diabetes. The development of chronic complications of diabetes, either micro or macrovascular, can begin earlier in the pre-diabetes phase[4].

Insulin resistance can influence various cardiovascular complications by several mechanisms. One such mechanism includes cellular damage produced by free radicals or oxidative stress. Oxidative stress is a pathologic condition resulting from either increased production of free radicals or decreased levels of antioxidants[5]. Oxidative stress is a determinant of insulin resistance. Markers of systemic oxidative stress are elevated in clinical type 2 diabetes, but there are only limited data relating the degree of oxidative stress to insulin resistance in pre-diabetic states[6] .

Inflammation has a vital role in the pathogenesis of diabetes. C-reactive protein (CRP), a marker of inflammation, is associated with increased risk of diabetes. However, studies examining the association between CRP and prediabetes are limited[7].A good control of diabetes can prevent complications. Undiagnosed diabetes and prediabetes, therefore needs to be detected and treated early through community based screening. Hence the present study was done to screen for the prediabetic individuals and correlate the effect of free radical damage and inflammation on prediabetes.

II. Materials And Methods

This was a hospital based cross sectional study and was conducted in and around Sri Venkateshwara Medical College Hospital and Research Centre, Puducherry. About 100 subjects (50 pre-diabetic + 50 controls) were included in the study. An informed consent was taken from the subjects. Institutional ethical committee clearance was obtained before the commencement of the study.Patients with impaired fasting glucose (100-126mg/dl), impaired glucose tolerance (≥140 and <200 mg/dl)) after a 75-g of glucose load on the oral glucose tolerance test (OGTT), random blood glucose 110 – 145mg/dl[8] and those with family history of diabetes, body mass index > 25, previous history of gestational diabetes were included in the study. Age and sex matched normal healthy individuals free of any chronic diseases were considered as normal. All individuals were of age group18 to 60 yrs.

2.1 Exclusion Criteria:

1. Diabetic patients
2. Pregnant women
3. Critically ill patient

The person's age, sex, height, weight and waist circumference were recorded. A fasting and post prandial blood sugar was measured. Body mass index was calculated (weight in Kg/ height in m^2) and was used as criteria for diagnosis of overweight and obesity. Those with BMI between 18.5 – 22.9 kg/m2 were considered normal, 23 – 24.9 kg/m2 as overweight and > 25 kg/m2 as obese.

2.2Biochemical Analysis:

a. Estimation of fasting and post prandial Blood sugar (enzymatic method)
b. Estimation of markers of oxidative stress like plasma MDA, reduced glutathione, glutathione peroxidase, catalase using semi autoanalyzer
c. CRP – serum latex agglutination test

The results were reported as mean ± SD. The blood sugar level were correlated with markers of oxidative stress using students 't' test and chi square test using SPSS version 17. A 'p' value < 0.05 was considered significant.

III. Result

Table 1: Descriptive statistics of the studied population.

S. No	Parameters	Study group (n = 50)				Control group (n = 50)			
		Min	Max	Mean	SD	Min	Max	Mean	SD
1.	Age	23	59	43.02	8.77	21	54	38.14	8.32
2.	BMI	21.1	30	25.56	1.85	18.2	24.9	22.7	1.73
3.	FBS	75	126	110.74	10.05	59	113	84.32	13.5
4.	PPBS	122	198	149.62	19.65	73	132	106.68	15.63
5.	Hb	6.7	15.2	12.17	1.76	8.9	15.5	12.37	1.82
6.	GSH	1.9	5.78	3.09	0.74	2.1	7.2	4.56	1.18
7.	GPx	38.04	70.03	50	6.96	42.91	101.05	72.36	17.5
8.	Catalase	58.23	98.07	75.7	10.8	83.51	130.1	111.67	10.5
9.	MDA	3.18	5.87	4.62	0.66	2.08	4.21	3.18	0.58

The mean age in the study group was 43.02 ± 8.77 yrs and in the control group was 38.14 ± 8.32 yrs. Regarding the blood sugar, the mean fasting and postprandial blood glucose was 109.08 ± 11.53 mg/dl, 155.28 ± 19.33 mg/dl, 84.32 ± 13.5 mg/dl and 106.68 ± 15.63 mg/dl in the study and control group respectively. There was no change in the mean hemoglobin between study and control group.

Table 2: Distribution of IFG and IGT patients in the study group

	Study group (n= 50)
IGT	28 (56%)
IFG	22 (44%)

Table 2 indicates the number of impaired fasting glucose and impaired glucose tolerance patients in the study group. They were categorized based on the fasting blood sugar and OGTT. Of the total 50 individuals in the study group, 44% of them were having impaired fasting glucose and 56% of them, impaired glucose tolerance.

Table 3: Mean erythrocyte GSH mg/gm Hb level in the study and control group

GSH	**Mean**	**SD**	**Statistical inference**
Control (n=50)	4.56	1.18	T = 7.43
Study (n = 50)	3.09	0.74	p = 0.001, highly significant

Tables 3 shows the mean erythrocyte glutathione levels in the study and control group. The mean glutathione level was 4.56 ± 1.18 mg/gm Hb in the control group and 3.09 ± 0.74 mg/gm Hb in the study group. There was a high significant association between the study and control group.

Table 4: Mean Gpx U/gm Hb level in the study and control group

GPx	**Mean**	**SD**	**Statistical inference**
Control (n = 50)	72.36	17.56	T = 8.369
Study (n = 50)	50	6.96	p = 0.001, highly significant

The GPx level was 72.36 ± 17.56 U/gm Hb and 50 ± 6.96 U/gm Hb in the control and study group respectively. There was a high significant association between the study and control group, as evident by the p value of 0.001.

Table 5: Mean catalase kU/ml level in the study and control group

Catalase	**Mean**	**SD**	**Statistical inference**
Control (n = 50)	111.68	10.5	T = 16.786
Study (n = 50)	75.7	10.8	p = 0.001, highly significant

The mean catalase level was 111.68 ± 10.8 kU/ml and 75.7 ± 10.8 kU/ml in the control and study group. On comparing the two groups, a significant association was found. The catalase level seems to be low in the pre diabetic people.

Table 6: Mean plasma MDA µmol/L level in the study and control group

MDA	**Mean**	**SD**	**Statistical inference**
Control (n =50)	3.19	0.58	T = 11.408
Study (n = 50)	4.62	0.67	p = 0.001, highly significant

The plasma MDA level was 3.19 ± 0.58 µmol/L and 4.62 ± 0.67 µmol/L in the control and study group. Plasma MDA level, which is the marker of oxidative stress is elevated in pre diabetic subjects.

Table 7: CRP status in the study and control group

CRP	**Control (n = 50)**	**Study (n = 50)**	**Statistical inference**
Positive	3 (6%)	27 (54%)	Chi sq value – 27.43
Negative	47 (94%)	23 (46%)	pvalue = 0.001, highly significant

Among the study group, 54% of the individuals had a positive CRP and 46% with a negative CRP status. In the control group, 94% of the individuals had a negative CRP and 6% with a positive CRP. A chi square test was performed which indicated that there was a significant association between the two groups (p value – 0.001).

III. Discussion

People with pre diabetes are at an increased risk of developing diabetes[9]. According to a study, fasting endogenous glucose production is inappropriately increased and glucose disappearance is inappropriately decreased in patients with IFG; whereas endogenous glucose production was promptly suppressed in IGT individuals after meal ingestion[10].Though the transition from early metabolic abnormalities that happens in

prediabetes to diabetes takes many years, majority of the individuals eventually develop diabetes[11]. The risk of developing long term complications of diabetes is related to its duration and the level of glycemia. Hence, screening for prediabetes becomes mandatory.The prevalence of IFG and IGT varies widely. They differ among various ethnic groups. There is a significant difference with age and sex; however, both the metabolic disorders increase with increase in age. IGT is more frequent in women than in men[12].

The natural history of IFG and IGT are variable. According to a study, 25% of the prediabetes individuals progress to diabetes, 50% remains in their abnormal glycemia and 25% reverting back to normal glucose tolerance, over an observational period of 3 – 5 years[13]. There are a wide variety of interventions to alter the natural history of prediabetes to diabetes. Most of the clinical trials have measured the changes in glycemic status as the primary outcome[14].

Many studies have shown that hyperglycemia leads to overproduction of free radicals through several pathways. The increased production of free radicals is contributed by glucose autoxidation and protein glycation[15]. The oxidative degradation of oxidants leads to lipid peroxidation products. Markers of systemic oxidative stress are elevated in clinical type 2 diabetes[6] but there are only limited data relating the degree of oxidative stress in prediabetes[7].With respect to oxidative stress markers, the common results reported in diabetic patients are increased oxidative stress and impaired anti oxidative defense system[16]. This was very evident in our study. Compared to the control group, the oxidative stress marker MDA is increased and antioxidant markers like GSH, GPx and catalase were decreased in the study group.

According to a study done by Abdhul Kareem et al[17], in IGT patients, plasma MDA level was increased and erythrocyte GSH level decreased. The findings are consistent with our study. A similar result was present not only in IGT patients but also in IFG patients as well. These findings reveal that oxidative stress plays an important role in the natural history of diabetes[18].Similarly, a study conducted by Patel H et el[19], revealed that increased circulating blood glucose increases hydrogen peroxide production and thereby downregulates catalase enzyme activity. This is consistent with our study in which the catalase levels were decreased in the study group.With respect to the inflammatory status, in our study the serum CRP was significantly associated with IFG/IGT. This is consistent with the findings of a study done by Thiyagarajan et al, where the marker of inflammation, CRP was significantly high in IFG group[20]. The findings point out that prediabetes (IFG/IGT) is a state of subclinical inflammationAccording to a study done in 2009, on Chinese Han clinical population, it was shown that chronic inflammation is involved in the development of hyperglycemia irrespective of body mass index. A strong correlation was observed between CRP and pre diabetic state[21]. In our study, BMI was an important risk factor in screening the patients for pre diabetes. Those individuals with IFG/IGT had a mean BMI of 25.56, which according to Indian standards is considered grade 1 obesity. Hence in our study, CRP level correlated with obesity.

IV. Conclusion

In conclusion, pre diabetes is associated with a state of low- grade inflammation and oxidative stress. Serum levels of CRP as a marker of inflammation and MDA, GSH, GPx and Catalase as markers of oxidative stress may serve for identifying people at high risk for developing type 2 diabetes. Such people will be important targets for programs that are designed to prevent diabetes.

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