COMPARATIVE ASSESSMENT OF METHODS OF ESTIMATION OF SUPEROXIDE DISMUTASE (SOD) IN TERMS OF SENSITIVITY AND SPECIFICITY IN TYPE-II DIABETES MELLITUS.

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Abstract

Now a days it is a known fact that the oxidative stress is in the foreground of type-II diabetes mellitus. It is the main cause of macro and microvascular complications in type II diabetes mellitus. Oxidative stress can be served as a good marker of degree of diabetes in addition is HbA_{1c}. Superoxide (O₂⁻) is one of the free radical produced during oxidative stress. Superoxide dismutase (SOD) is an enzyme which quenches superoxide radicals produced due to oxidative stress. Hence it is very obvious that the estimation of SOD should be very sensitive and specific. To attain this aim we compared three different methods.

Key words: SOD, Diabetes, Oxidative stress, Superoxide Dismutase.

Introduction

Diabetes is a metabolic disorder characterised by hyperglycemia resulting from defects in the insulin secretion or insulin action or both. It is generally accompanied by increased level of free radicals, oxidative stress and decreased concentration of antioxidants suggest increased oxidative stress in diabetes mellitus may due to formation of Glycated Haemoglobin (HbA_{1c}).⁽¹⁾ Oxidative stress is a condition in which metabolites of oxidants exert their toxic effect because of an increased production or an altered cellular mechanism of protection.⁽²⁾ Oxidative stress thought to be increased in a system where the rate of free radical production is increased and/or the antioxidant mechanisms are impaired.⁽³⁾ Increased oxidative stress is a widely accepted participants in the development and progression of diabetes and its complications.⁽⁴⁾ Normally there is a balance between tissue oxidant and antioxidant activity. The latter is achieved by the antioxidant scavenger system, which includes enzymes like Superoxide Dismutase (SOD), Catalase, Glutathione Peroxidase (GPx) and antioxidants vitamin (vitamin C, A, E and other carotenoids).⁽⁵⁾

Superoxide Dismutase is an important enzyme found in both cell cytosol and mitochondria. The cytosol form is dependent upon zinc and copper co-factors while mitochondrial form requires manganese.⁽⁶⁾ Superoxide Dismutase (SOD) is the major intracellular antioxidants (substances that neutralize the potentially ill effect of free radicals are generally grouped in the so-called 'Antioxidants-defence system') in the

human body it catalyzes the dismutation of superoxide to hydrogen peroxide and molecular oxygen.

H₂O₂ is then removed by catalase or glutathione peroxidase.^(7, 8)

It is observed that Superoxide Dismutase (SOD) is estimated by different methods with different sensitivity and specificity in different laboratories. So it is necessary to find out a method with high sensitivity and specificity. Hence the present work was undertaken to compare the present available

methods of SOD estimation in order to find out better specific and sensitive method.

To achieve this aim three methods i) Kajari-Das Method, ii) Marklund G. and Marklund S. Method and iii) Winterbourn *et al* Method were selected. From this we concluded that Kajari-Das Method was most sensitive and specific.

Aims and Objective

The aim of the present study was to estimate,

1) a) Activity of RBC Superoxide Dismutase (RBC-SOD) by

- i) Kajari-Das Method,
- ii) Marklund G. and Marklund S. Method and
- iii) Winterbourn *et al* Method

b) Fasting and Post Prandial blood / Plasma glucose level along with Glycosylated haemoglobin, in 110 type II D.M. patients (without any complications) and 110 healthy volunteers (age and sex matched) who served as control-group for this study.

2) To assess statistical significant difference (if any) in the levels of above biochemical parameters between the healthy controls and the patients having type II D.M. (without any complications).

3) To evaluate the correlation (if any) in the level of above parameters in the patients having type II D.M. (without any complications)

4) To assess the sensitivity, specificity, predictive values of positive and negative test of methods of estimation of SOD.

Materials and methods

Selection and Distribution of subjects:-

In this study total 220 subjects were included, they were grouped as follows:

Group No. of Patients

Healthy Controls 110

Diabetes Mellitus (type-II) 110

Sensitivity and Specificity was determined by using following method:

Screening Test Results	Diagnosis		Total
	Diseased	Not Diseased	
Positive	TP(a)	TN(b)	a + b
Negative	FN(c)	FP(d)	C + d
Total	a + c	b + d	a + b + c + d

TP = True positive result – Number of diseased patients correctly classified by the test.

FP = False positive result – Number of non-diseased patients misclassified by the test.

FN = False negative result- Number of diseased patients misclassified by the test.

TN = True negative result – Number of non-diseased patients correctly classified by the test.

Predictive Value of laboratory diagnosis –

Sensitivity, Specificity and Predictive value define diagnostic accuracy of laboratory test.

Sensitivity indicates the frequency of the positive test results in patients with a particular disease; it is positive in disease.

Sensitivity = $[TP / (TP + FN)] \times 100$

Specificity indicates the frequency of the negative test results in the patients without that disease; it is negative in healthy or absence of a particular disease.

Specificity = $[TN / (FP + TN)] \times 100$

The predictive values of a positive test result indicates the frequency of diseased patients in all patients with positive test results.

Predictive value of a positive test = $[TP / (TP + FP)] \times 100$

The predictive value of a negative test result indicates the frequency of non-diseased patients in all patients with negative test results.

Predictive value of a negative test = $[TN / (TN + FN)] \times 100$

Inclusion Criteria:

Patients diagnosed as type-II Diabetes Mellitus without any complications.

Exclusion Criteria –

Patients having type-I Diabetes Mellitus, Gestational Diabetes Mellitus,

type-II diabetes mellitus patients with complications.

Collection and preservation of sample –

After obtaining prior consent, after 12 hrs. fasting, about 10 ml venous blood was collected from the subjects. Under aseptic condition by venipuncture using sterile disposable syringe and needle and around 2 ml blood sample was collected 2 hrs. after intake of food from the control and patient population. The blood sample were collected in

EDTA, Heparinised and Fluoride bulb and transported to the laboratory for further process.

About 1 ml. whole blood was mixed with EDTA and was used for the determination of HbA1c, 2ml blood collected in Fluoride bulb was used for determination of Fasting and post prandial glucose. Remaining blood was used to measure the activity of Superoxide Dismutase by different methods. The blood samples collected were analyzed for biochemical parameters mentioned below –

Parameter	Methods of estimation
1. Plasma Glucose (Fasting and Post Prandial)	GOD-POD ⁽⁹⁾
2. Glycated Haemoglobin (HbA1c)	Ion Exchange Resin ^(10, 11)
3. RBC Superoxide Dismutase	Kajari Das ⁽¹²⁾
	Winterbourn <i>et al</i> ⁽¹³⁾
	Marklund G. And Marklund S. ⁽¹⁴⁾

Statistical Analysis

All the biochemical parameters measured in study group subjects were statistically compared with those estimated in controls. Results were presented as mean $\pm$ SD. Student unpaired 't' test used for statistical analysis between controls and cases for numerical variables and 'Receiver Operating Characteristics' (ROC) curve was applied to find out the more sensitive method.

The results obtained in the study were evaluated using MYSTATISTICAL PACKAGE at 95% Confidence interval and at a significance level of $P < 0.05$.

Results and Observations

n= number of cases

p > 0.05 not significant

p < 0.05 Significant value

p < 0.001 Highly significant.

Table 1: Statistical Analysis of the Parameters of Glycemic Index in Healthy Controls and Type 2 D.M. (without any complications)

Parameters	Healthy Controls n = 110 Mean $\pm$ SD	Type-II D.M. n = 110 Mean $\pm$ SD	't' value	'p' value
Fasting Glucose	88.99 $\pm$ 9.14	162.63 $\pm$ 44.33	16.99	<0.001
Post Prandial Glucose	117.52 $\pm$ 8.45	288.05 $\pm$ 71.23	24.79	<0.001
Glycosylated Hb.	4.96 $\pm$ 0.60	9.03 $\pm$ 0.57	4.99	<0.001

Table 2: Statistical Analysis of the activity of RBC-SOD in the Healthy controls and Type 2 D.M. (without any complications).

Activity of RBC-SOD BY	Healthy Controls n = 110 Mean $\pm$ SD	Type-II D.M. n = 110 Mean $\pm$ SD	't' value	'p' value
Kajari Das Method	5.25 $\pm$ 0.49	3.00 $\pm$ 0.44	25.83	<0.001
Winter Bourn et al method	3.11 $\pm$ 0.19	2.92 $\pm$ 0.22	11.58	<0.001
Marklun G and Marklund S. Method	7.47 $\pm$ 0.68	4.93 $\pm$ 0.41	10.7	<0.001

Table 3: Table showing Comparative Assessment of SOD estimation.

Name of Method	Sensitivity	Specificity	Predictive value of Positive test.	Predictive value of Negative test.	Area Under Curve.
Kajari Das	96.36%	97.27%	97.24%	96.39%	0.995
Winter Bourn et al method	88.18%	89.09%	90.65%	88.28%	0.813
Marklun G and Marklund S. Method	90.90%	94%	91.66%	92.85%	0.957

Discussion

SOD is one of the enzyme of oxidative stress which can be a good marker for diagnosis as well as prognosis of the disease. Superoxide Dismutase (SOD) is thought to play a very important role in protecting living cell against toxic oxygen derivatives. The enzyme catalyzes the dismutation of two superoxide radicals (O_2^-) and H_2O_2 .⁽¹⁵⁾ SOD is considered a primary enzyme since, it is involved in direct elimination of ROS.^(16, 17)

Superoxide Dismutase (SOD) is one of the defence against oxidative stress. Among various antioxidant mechanisms in the body, SOD is thought to be one of the major enzyme that protect cells from ROS. It exists in three major cellular forms viz. Copper-Zinc SOD (SOD 1), Manganese SOD (SOD 2) and extracellular SOD (SOD 3).

In the literature we found that SOD is estimated by different methods with different sensitivity and specificity, It is necessary to compare Kajari Das, Marklund and Marklund and Winterbourn *et al* methods in order to get proper method. So the present study is an attempt to find out the proper method for estimation of SOD which will be more sensitive and specific as compared to others. In the present study Erythrocytic SOD activity was estimated by the methods Kajari Das, Marklund and Marklund and Winterbourn *et al* were significantly decreased in type 2 diabetic patients(without any complications) as compared to healthy controls($P<0.001$). Many studies have been done on SOD activities and most of them showed similar results.^(18, 19, 20)

In order to assess the reliability of the method for the estimation of RBC- Superoxide Dismutase activity by Kajari Das, Marklund and Marklund, Winterbourn *et al* method we compared the sensitivity, specificity, area under curve and predictive values of above mentioned methods.

This study is an attempt to find out proper method to estimate SOD with high sensitivity and specificity. Now a day oxidative stress is foreground in most of the disease, to achieve this aim we have selected type II diabetic patients in which oxidative stress is found to be increased.

In the present study, our results represent that the activity of SOD (estimated by Kajari Das, Marklund and Marklund and Winterbourn *et al* method) was significantly decreased in all the methods in type 2

D.M. (without complications). But the method of Kajari Das for the estimation of RBC-SOD activity had highest sensitivity and specificity in type 2 D.M. (without complications) among three methods of estimation.

Thus, the results reveal that the estimation of activity of RBC-SOD in type 2 D.M. (without any complications) can be of clinical importance by using Kajari Das method which has more sensitivity and specificity in the present study. Further studies from different areas involving large scale sample size are needed to confirm and to elucidate the findings of the present study.

SUMMARY AND CONCLUSION

The present study was aimed to assess the comparative methods of estimation of RBC-Superoxide Dismutase (RBC-SOD) in terms of sensitivity and specificity along with glycated haemoglobin in type 2 diabetes mellitus. Present study demonstrated significantly decreased activity of RBC-SOD in type 2 diabetes as compared to healthy controls which were estimated by Kajari Das, Marklund and Marklund and Winterbourn *et al* methods. In diabetic patients, the autoxidation of glucose results in the formation of hydrogen peroxide which inactivates SOD and this accumulated hydrogen peroxide as well as utilization of SOD to scavenge excessive superoxide anions might be one of the explanations for lowered activity of SOD in type 2 diabetic patients.

From the result we cannot come to a conclusion that which method is better so all these results were subjected to ROC analysis. From the ROC analysis we found that area under curve is higher in Kajari Das method. So we concluded that Kajari Das method is better than Marklund and Marklund and Winterbourn *et al* method.

Our results suggest that activity of RBC-SOD estimated by Kajari Das method had highest sensitivity and specificity than method of Marklund and Marklund and Winterbourn *et al* method. From the present study, it can be concluded that activity of RBC-SOD in type 2 diabetes mellitus (without any complications) may be estimated by Kajari Das method which have highest sensitivity and specificity.

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