STUDY OF MALONDIALDEHYDE AS OXIDATIVE STRESS AND SUPEROXIDE DISMUTASE AS ENZYMATIC ANTIOXIDANT IN TYPE II DIABETES MELLITUS PATIENTS WITH AND WITHOUT PERIPHERAL NEURITIS.

Ahmed Mohamed Abohassan, Said M. Al-Barshomy, and Sultan Ahmed Al-kobaty

1. Internal medicine department, Faculty of Medicine, Al-Azhar and Zagazig University—Egypt.
2. Clinical pathology department, Faculty of Medicine, Sanaa University—Yemen.

Abstract

Background:-
Diabetic neuropathy is the most common complication of diabetes mellitus (DM). Poor glycemic control and long standing diabetes lead to diabetic complications. The cause of diabetic complications is the accumulation of diabetic stress factors and decrease of antioxidant stress factors. Oxidative stress, defined as a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses, one of the most important intracellular antioxidant substances is an enzyme, superoxide dismutase which scavenges free radicals by converting the harmful superoxide ion into stable hydrogen peroxide.

Aim of the work:- Evaluation of superoxide dismutase (SOD) as antioxidant factor and malondialdehyde (MDA) as oxidative stress factor in type II diabetes and its relation to control and duration of diabetes.

Patients and methods:- 90 patients with type II diabetes were involved in this study, 50 patients with peripheral neuritis (group I) and 40 patients without peripheral neuritis (group II) in addition to 20 normal subjects as control (group III). All individuals were subjected to full history taking, full clinical examinations including examinations of peripheral nerves, measurement of blood sugar, HbA1c, serum lipids, superoxide dismutase (SOD) and serum malondialdehyde.

Results:- the level of serum lipids, HbA1c, and serum malondialdehyde were significantly increased in patients with peripheral neuritis compared to the other groups. Superoxide dismutase was significantly decreased in peripheral neuritis patients compared to the other groups.

Conclusion: Long standing diabetes and poor glycemic control is associated with decreased serum superoxide dismutase and increased plasma malondialdehyde, which can be considered as markers for diabetic control and diabetic peripheral neuropathy complications in type II diabetes.

Introduction:-
Hyperglycemia is the primary clinical manifestation of diabetes which is essential for the development of diabetic neuropathic complications. Diabetic neuropathies are the most common, and at the same time the least recognized and understood long-term complication of diabetes, occurring both in type 1 and type II diabetic patients. Oxidative stress are compounds resulting from enhanced free radical formation and or a defect in antioxidant defenses. Reactive oxygen species is one major factor in the onset and the development of diabetic complications. Superoxide dismutase (SOD) destroys the highly reactive radical superoxide by conversion into the less reactive peroxide (H2O2). Reactive oxygen species generated in vivo during time of hyperglycemia play an important role in nerve damage. Poor glycemic control for long time leads to elevated level of reactive oxygen species and increase
oxidative stress. An increased generation of reactive oxygen species such as superoxide is the cause of oxidation and modification of structure and cellular proteins, nucleic acid and membrane lipids because unclear mechanism there is no effective drug for diabetic peripheral neuropathy.

Recent studies have focused on the intracellular oxidative stress, especially from mitochondria and find that the mitochondrial organelle is affected in many aspects after hyperglycemia. Mitochondrion has been proved the main place for producing the oxidative stress and play important role in the development of diabetic peripheral neuropathy. There is some biochemical pathways strictly associated with hyperglycemia as non-enzymatic glycosylation, glucose auto oxidation, polyol pathways. These pathways increase the production of free radicals. There is association between the degree of hyperglycemia and increase risk of micro vascular complications. Diabetic neuropathy was directly related to oxidative damage and change in antioxidant defense of nerve cell. Oxidative stress appears to be due to nerve ischemia, hyperglycemia and auto oxidation. hydrogen peroxide (H2O2) are formed in minute quantities and are very rapidly scavenged by natural cellular defense mechanisms mainly enzymes like superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase. increase production of malondialdehyde, a marker for lipid peroxidation has been found in erythrocytes membrane of diabetic patients together with depressed erythrocyte contents of anti oxidant enzymes. Expression and induction of enzymes that protect against reactive oxygen species induced damage and play an important role in the risk of neuropathy in human. One of the best characterized protective genes proven to be effective in decreasing neurovascular complications of diabetes and associated oxidative stress is superoxide dismutase. Endothelial dysfunction in diabetes mellitus is one of the important reasons for the loss of nerve function and nerve conduction deficits.

Gene transfer of copper, zinc and manganese SOD to diabetic aorta improved endothelial-dependant relaxation. Mitochondrion contains variety of important antioxidants as manganese SOD that able to deoxygenize O2 to H2O2. SOD work as the first line of enzymatic protection against superoxide radicals. There are several forms of SOD, these are metallo proteins each containing a metal ion in its center copper superoxide dismutase is responsible for catalytic activity of SOD, while zinc SOD has a role in the stabilization of enzymatic conformation.

Extracellular SOD is structurally similar to intracellular SOD, but it is present in the extracellular space. Mitochondrial SOD (manganese SOD) is located in the mitochondrial matrix. There are two forms of mitochondrial SOD, dimeric and tetrameric forms.

Aim of the work:-
Study and Evaluation of superoxide dismutase and serum malondialdehyde in type II diabetic patients with and without peripheral neuritis and its relations to duration of diabetes and glycemic control.

Patients and methods:-
This study was done in Al-Salam Saudi Hospital at Saadah, republic of Yemen in the period between January 2014 and December 2014. Ninety type II DM patients in addition to 20 healthy volunteer subjects included in our study. These subjects were divided into three groups:

Group I: fifty patients with type II DM suffering from peripheral neuritis.

Group II: forty patients with type II DM without evidence of peripheral neuritis.

Group III: 20 healthy volunteers (control group).

All individuals were subjected to: full history taking involving duration of diabetes and manifestation of peripheral neuritis, full clinical examination, including tests to detect PN, laboratory investigation including fasting, 2 hours postprandial blood sugar, glycosylated hemoglobin, liver function tests, renal function tests, lipid profile, and Measurement of superoxide dismutase and Serum malondialdehyde.

Superoxide dismutase activity in erythrocytes was measured according to procedure by Misra and Ridovich and expressed in adrenaline units (U/g Hob/100 ml. One unit of SOD activity is defined as the amount of enzyme inhibiting the adrenaline auto oxidation at 50 %). Superoxide dismutase level: Serum MDA measured spectrophotometrically by thiobarbituric acid method in which one molecule of MDA react with two molecules of thiobarbituric acid giving a pink crystalline pigment measured at wave length 520.
Inclusion criteria:
Patients considered diabetic if fasting blood glucose more than 5.6 mmol per liter or 2 hours value more than 7.8 mmol per liter and the diagnosis of type II diabetes was based on the American Diabetes Association definition of diabetes.  

Exclusion criteria: from our work we excluded patients with lower limb amputation, psychiatric disorders, cancer or any genetic disease, terminal illness, evidence of peripheral arterial disease, claudication symptoms, alcohol abuse, smokers, thyroid disorders, vitamin B₁₂ or folate deficiency, spondyloarthropathy, foot edema, hepatic disease, lumbosacral pathology, and toxin exposure including chemotherapeutic agents.

The diagnosis of diabetic peripheral neuritis in patient with supine position and was based on presence of combination of symptoms and sings of neuropathy including decreased distal sensation and decreased or absent ankle reflexes. Pinprick, vibration, temperature sensation, and achilles reflexes were done and modified neuropathy disability score were used to asses peripheral neuritis. Pin prick test was performed proximally to big toe nail and temperature sensation was performed on the dorsum of the foot. Vibratory perception was tested at the apex of the big toe with a 128 Hz graduated rydel – seiffer tuning fork.  

All the values expressed as mean ±standard deviation. Data were subjected to analysis using unpaired t test for comparison between two groups and one way ANOVA (F-test) for multiple group comparison.

Results:  
There was high significant difference between diabetic patients with and without peripheral neuritis regarding palpitation easily fatigability and exercise intolerance but only significant difference regarding constipation and erectile dysfunction (Table: 1).  
There was very high significant difference between studied groups regarding HbA1c, fasting blood sugar and postprandial blood sugar but only significant difference regarding serum cholesterol and serum triglycerides (Table: 2).
Superoxide dismutase level was significantly higher in diabetic patients without peripheral neuritis ( group II) than those with peripheral neuritis( group I) (p≤0.0001). (Table: 3).  
Malondialdehyde level was significantly higher in group I (DM with PN) than group II (DM without PN) and control group (Table: 3).  
There was direct positive correlation between MDA level and both blood sugar level and duration of diabetes with high significant difference between groups (Table: 7).  
On the other hand there was negative correlation between SOD level and both blood sugar level and duration of diabetes with high significant difference between groups. (Tables 5 and 6).

| Parameter/group | Group I Diabetes with PN (n=50) | Group II Diabetes without PN (n=40) | P value |
|-----------------|--------------------------------|----------------------------------|--------|
| Palpitation     | 45(90%)                        | 5(12.5%)                         | ≤0.0001|
| Easy fatigability | 47(94%)                       | 6(15%)                           | ≤0.0001|
| Exercise intolerance | 49(98%)                    | 2(5%)                            | ≤0.0001|
| Constipation    | 17(34%)                        | 3(7.5%)                          | ≤0.05  |
| Erectile dysfunction | 18(36%)                  | 4(10%)                           | ≤0.05  |

| Parameter| Group I DM with PN | Group II DM without PN | P value |
|----------|--------------------|------------------------|--------|
| HbA1c (%) | 8.32±1.86          | 5.9±1.1                | ≤0.0001|
| Fasting blood glucose(mg\dl) | 272±66.85          | 198±43.65              | ≤ 0.001 |
| Postprandial blood glucose (mg\dl) | 312±67.84          | 226.65±27.35          | ≤0.0001 |
| Serum cholesterol (mg\dl) | 250.5±44.77        | 176.2±55.36           | ≤0.05  |
| Serum triglycerides (mg \dl) | 222.72±107.6       | 173.74±82.95          | ≤0.02  |
Table 3: Superoxide dismutase and serum malondialdehyde levels in both groups.

| Parameter | Group I DM with PN | Group II DM without PN | Group III Control | P value |
|-----------|--------------------|------------------------|-------------------|---------|
| Superoxide dismutase (U | ml)     | 3.19±0.29             | 4.84±0.29         | 5.51±0.41 | ≤0.0001 |
| Malondialdehyde (nmol/ml) | 6.45±0.46           | 4.37±0.26             | 3.01±21           | ≤0.0001 |

There was very high significant difference between patients with and without peripheral neuritis regarding superoxide dismutase level.

Table 4: Malondialdehyde and superoxide dismutase in diabetic patients depending on glycemic control:

| Patients group | MDA (nmol/ml) | SOD (U | ml) |
|----------------|---------------|-------|
| Blood sugar ≤ 200mg/dl | 4.18±13       | 4.72±0.16 |
| Blood sugar from 200 - 300mg/dl | 5.32±0.38 | 4.09±0.15 |
| Blood sugar > 300mg/dl | 6.22±0.49     | 3.34±0.43 |
| P – value        | ≤0.0001       | ≤0.0001 |

MDA : malondialdehyde  SOD: superoxide dismutase

Table 5: SOD level and duration of diabetes.

| Test          | Mean (SOD) | ± SD |
|---------------|------------|------|
| 0 to 5 years  | 6.3        | 2.4  |
| 6 to 10 years | 3.7        | 1.9  |
| More than 10 years | 2.9      | 0.7  |
| T. test       |            | ≤0.001 |

Table 6: Correlation between SOD and blood sugar parameters and duration of diabetes.

| Test              | r         | P     |
|-------------------|-----------|-------|
| H.A1c             | -0.389    | 0.001 |
| FBS               | -0.352    | 0.02  |
| 2h blood sugar    | -0.329    | 0.02  |
| Duration of diabetes | -0.339 | 0.01  |

Table 7: Correlation between MDA and blood sugar parameters and duration of diabetes

| Test              | r         | P     |
|-------------------|-----------|-------|
| H.A1c             | 0.761     | ≤0.0001 |
| FBS               | 0.722     | ≤0.0001 |
| 2hours blood sugar | 0.364 | ≤0.01  |
| Duration of diabetes | 0.337 | ≤0.01  |

Discussion:
Recent researches have confirmed that oxidative stress is the main mechanism for diabetic peripheral neuritis (DPN). Mitochondria is the most important part to start and produce oxidative stress. Hyperglycemia leads to elevation of mitochondrial membrane action potential and change of mitochondrial respiratory chain and structure protein. Also hyperglycemia inhibits activity of mitochondrial antioxidant which damage mitochondrial DNA that increase oxidative stress. Increased free radicals leads to lipid peroxidation and have a role in diabetes mellitus complications as DPN. Chronic hyperglycemia leads to glycosylation of proteins like hemoglobin. Poorly controlled diabetes mellitus and glucose oxidation lead to excessive formation of NADPH which promote lipid peroxidation in the presence of cytochrome P-450. Increased antioxidative glycosylation of hemoglobin lead to imbalanced generation of free radicals like superoxide causing depletion of SOD. Diminished activity of SOD leads to exhausted antioxidant reserve which further exacerbates the oxidative stress. Excessive peroxidation is associated with reduced SOD activity in diabetic patients. The level of MDA was higher in diabetic patients with longer disease duration if compared with newly diagnosed patients. Serum MDA was positively correlated with disease duration and also depends on the level of glycemic control. The mean MDA level is elevated in diabetic patients with complications when compared with diabetic patients without complication as DPN. SOD deficiency is observed after two years of diagnosis of type II diabetes and become prominent with the development of diabetic complications.
In our study there was high significant difference between studied groups regarding palpitation, easily fatigability, exercise intolerance, erectile dysfunction and constipation due to affection of nervous system specially autonomic nervous system. This result was in agreement with Vinik et al., 2003 who stated that diabetic autonomic neuropathy is a common complication of diabetes. Also there was high significant difference between studied groups regarding the level of fasting, postprandial blood sugar and HbA1c and this was in agreement with Seckin et al., 2006 who said that the development of micro vascular complications of diabetes mellitus is related to the duration of disease and the degree of glycemic control.

In our study the level of serum cholesterol and triglycerides is much higher in patients with peripheral neuropathy group than the other patient group and this was in agreement with Lachin et al., 2008 who mentioned that the presence of diabetic neuropathy was related to the duration of diabetes, HbA1c and serum lipids. also there was marked decrease in the superoxide dismutase level in patients with diabetic peripheral neuritis if compared to the other patient group and this in agreement with Ziegler 2009 who said that the erythrocyte activity of SOD was significantly decreased in type II diabetes mellitus patients with distal symmetric polyneuropathy and also in agreement with Vivan and Smilee 2010 who stated that there is deficiency of SOD level with decreased activity in type II diabetes mellitus due to increased oxidative glycosylation of hemoglobin and excessive peroxidation leading to peripheral neuritis and diabetic complications.

In the current study there was marked increase in the serum malondialdehyde level in patients with diabetic peripheral neuritis if compared to the other studied groups and this in agreement with Gupta and Chari 2005 who reported that plasma malondialdehyde increase with the increase in severity and duration of diabetes and also in agreement with Shen et al., 2010 and Geeta et al., 2011 who stated that increase in plasma malondialdehyde level as markers of lipid peroxidation in type II diabetes mellitus with poor glycemic control may contribute to the development of diabetic complications.

Also there was direct positive correlation between MDA level and both blood sugar level and duration of diabetes with high significant difference between groups. On the other hand there was negative correlation between SOD level and both blood sugar level and duration of diabetes in agreement with Edwards et al., 2008.

Conclusion:

Long standing diabetes and poor glycemic control is associated with decreased serum superoxide dismutase and increased plasma malondialdehyde level, which can be considered as markers for diabetic control and diabetic peripheral neuropathy complications in type II diabetes. Poor glycemic control is associated with diabetic complications especially diabetic peripheral neuritis.

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