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Dr. Meena Verma


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    sugar levels in diabetic patients. 

21. Serum Proteins and Sialic Acid changes in malignancy. 

22. Estimation of serum hepatoglobin and its correlation with serum 
    cholesterol and plasma fibrinogen in diabetic patients. 

23. Serum ADA estimations in TB patients. 

24. Transminase and phosphatases estimations in malignancy. 

25. Correlation between serumcholesterol, plasma fibrinogen and 
    hepatoglobin in diabetic patients. 
26. Changes observed in serum Amulase levels in lung cancer. 

27. Malignant lung cancer and serum Amylase. 

28. A comparative study of serum cholesterol, plasma fibrinogen and 
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29. Biochemical changes observed in the patients with organophosphorus 
    poisoning. 

30. Study in Biochemical changes observed in relation to Age. 

31. Hypoglycemic and hypolipidemic effects of raw garlic on diabetic 
    patients. 

32. A study on the effect of raw Fenugreek Seed powder (\textit{Methidae}) on 
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33. Hypercholesterolemia in hypothyroid patients. 

34. A study of the changes observed in lipid metabolism in hypothyroid 
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    2000.


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EFFECT OF INCREASING DURATION OF DIABETES MELLITUS TYPE 2 ON GLYCADED HEMOGLOBIN AND INSULIN SENSITIVITY

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Department of Biochemistry* and Medicine**, M.G.M. Medical College, Indore (M.P.)

ABSTRACT

Non-insulin dependent diabetes mellitus (NIDDM) is the most rapidly growing chronic metabolic disorder in the world. With advancement in the age and duration of diabetes there is a gradual tendency for the level of blood sugar to rise along with a subsequent increase in the HbA1c as well as in the fasting insulin level. Whether this is an aging process or increased frequency of diabetes is still controversial. The correlation between glucose and insulin sensitivity is consistent with the idea that the degree of chronic hyperglycemia is a cause of excessive insulin resistance in type 2 diabetes, i.e. the insulin resistance which characterizes type 2 diabetes but not non-diabetic subjects matched for age, gender, family history and duration of diabetes. The study comprised a total of 76 subjects out of which 30 were normal, non-diabetic persons and the rest 46 were diabetics with different duration of age in years, after being diagnosed diabetic. Data was analyzed after dividing the subjects into four groups - Group 1 comprised of one year old diabetics, Group 2 was made up of those, who had diabetics, for the past 2-5 years, Group 3 included patients who were diabetic since more than 5 years and Group 4 included non-diabetics as the normal control group. The results obtained indicated that the HbA1c levels showed a significant increase with the duration of diabetes as well as the insulin level showed a significant correlation after adjustment for age, sex and duration of diabetes.

KEY WORDS

Glycation, HbA1c, Insulin Resistance, Fasting Blood Glucose, Post Prandial Blood Glucose

INTRODUCTION

Diabetes mellitus is a life-long disease, which makes many people worry about the quality and longevity of their life after being diagnosed with it. The complications of diabetes are influenced not only by the duration of diabetes but also by the average level of chronic glycemia (1-2), which is measured most reliably with glycated hemoglobin assay. In normoglycemic subjects a small proportion of hemoglobin A is attached to a carbohydrate moiety thus creating what is called glycated hemoglobin (3). In conditions of sustained hyperglycemia, such as in diabetes mellitus, the proportion of hemoglobin that is glycated is increased substantially (4-5). Studies conducted by Arnetz et al. (6) and Kilpatrick et al. (7) in diabetic patients have shown a significant positive correlation between HbA1c and age as well as duration of diabetes. In contradiction to this Kabadi (8) found no significant relationship between age, duration of diabetes and fasting blood glucose (FBG), glycated hemoglobin, glycated protein or glycated albumin. According to the results of many longitudinal and cross-sectional studies it has been demonstrated that the earliest detectable abnormality in NIDDM is impairment in the body’s ability to respond to insulin (9). Studies have shown that insulin sensitivity correlated inversely with fasting insulin and the insulin level increased with the duration of diabetes (10).

Though such detailed investigations have been carried out in different parts of the world to prove a correlation between the different parameters, the results were contradictory, blurring the diagnostic significance of these parameters. It is thought worthwhile to investigate the significance of such correlations in the Indian diaspora, where such a biochemical equation on the effect of these parameters for the progression of diabetic sequel has not yet been postulated. The aim of this study is to evaluate the correlation between the above detailed parameters so that they can be

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used as diagnostic or prognostic markers for the assessment of the degree of control of this lifestyle disease, to delay or prevent the multi-faceted complications before they can eventually manifest.

MATERIAL AND METHODS

The study was conducted in the Department of Biochemistry and Clinical Biochemistry of M.G.M. Medical College and OPD of M.Y. Hospital, Indore.

Clinical Material: Subjects

The clinical material for the present study comprised of a total of 76 subjects. Three groups were formed on the basis of difference in duration of diabetes. The fourth group comprised of subjects who were normal and non-diabetic, as a control group. Data on therapy of diabetes, HbA1c values, FBG and PPBG was obtained by structured questionnaires and by clinical and laboratory assessments. Insulin-sensitive subjects were defined as having an insulin-sensitivity estimate the median in non-diabetic subjects participating in the study. Using this definition 88% of all type 2 diabetics was insulin resistant. The study subjects were established diabetics. At the time of this study the patients were not under any kind of treatment but were controlling their blood sugar level by diet and exercise.

1. One-year old diabetics (Group 1)

These subjects had been diagnosed with diabetes only one year before.

2. Two-Five year old diabetics (Group 2)

This group of patients was diabetic since the last 2-5 years.

3. More than five-year old diabetics (Group 3)

This group of patients was diagnosed with diabetes for more than 5 years. The maximum duration in this group was found to be of a patient with a diabetic history of 22 years.

4. Normal subjects (Group 4)

These individuals were screened for the presence of diabetes based on the diagnostic criteria of the American Diabetes Association (ADA) (11). They were found to be normal, healthy individuals without any prior family history of diabetes. The exclusion criteria also included hypertension, use of alcohol or cigarettes and other factors affecting blood sugar level.

Collection of material: Blood

In all the above groups 5 ml whole blood was collected in the fasting state. 0.5 ml whole blood is mixed with EDTA regent (anticoagulant) and kept for HbA1c estimation. The remaining blood is kept at room temperature for 1 hour after which the supernatant clear fluid is pipetted out into another tube. This tube is then centrifuged for 10 min. the clear serum is pipetted into a clean dry test tube and used for estimation of blood sugar and insulin. Similarly, 0.5 ml blood is collected from the subjects, 2 hours after having food for the estimation of post-prandial blood sugar, along with the urine sample.

Clinical method: estimation of HbA1c, FBG, PPBG and insulin

1. HbA1c estimation

For the estimation of HbA1c, 10 microliter of the whole blood + EDTA reagent, is mixed with 1ml HbA1c reagent and direct reading is taken on the auto-analyzer (Selectra E). The value recorded is in percent.

2. FBG and PPBG estimation

10 microliter of the clear serum is mixed with 1ml glucose reagent and incubated for 10 minutes at 37°C, then direct reading is taken on the auto-analyzer (Selectra E). The value recorded is in milligram percent. The same process is repeated for the estimation of PPBG with the post-prandial blood sample.

3. Insulin level estimation

The insulin level is estimated from the clear serum separated from the fasting whole blood sample by fully Automated Radio Immuno Assay System. The value recorded is in mu IU / mL.

Statistical analysis

The statistical analysis was done by student 't' test. The values were expressed as Mean ± S.D.

OBSERVATIONS

Table 1 shows the distribution of the normal control group and the diabetic groups of people with different duration of the disease. Out of the total subjects investigated 39 (51.3%) were males and 37 (48.68%) were females. The control group included 13 (33.33%) males and 17 (45.94%) females while the number of males and females in the study group were 26 (66.66%) and 20 (54.05%) respectively. Of the total 76 cases studied, the control cases numbered to 30 (39.47%) and the total number of study subjects numbered to 46 (60.5%).

Table 2 shows the status of mean ± standard deviation of fasting insulin level, HbA1c, fasting and post-prandial blood glucose in the males and females of the study and control group.

Table 3 shows the correlation between the duration of diabetes with the various parameters, like HbA1c,
Table 1. Analysis of the 76 cases under study

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Males (n = 39)</th>
<th>Females (n = 37)</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Study</td>
</tr>
<tr>
<td>Age</td>
<td>55 ± 12</td>
<td>57.8 ± 13</td>
</tr>
<tr>
<td>Duration of diabetes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1 Yr.</td>
<td>-</td>
<td>9</td>
</tr>
<tr>
<td>2-5 Yrs.</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>More than 5 years</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>26</td>
</tr>
</tbody>
</table>

n = 76 (number of subjects / sample size)

Table 2. Insulin Level, HbA1c, FBG, PPBG values in control and study group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n = 30)</th>
<th>Study (n = 46)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male (n=13)</td>
<td>Female (n=17)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>55±12</td>
<td>59±8</td>
</tr>
<tr>
<td>Fasting insulin level</td>
<td>8.2±2.1</td>
<td>7.4±2.0</td>
</tr>
<tr>
<td>(mu IU/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>2.75±0.82</td>
<td>2.2±0.72</td>
</tr>
<tr>
<td>FBG (mgm%)</td>
<td>81.5±18.1</td>
<td>89±11.2</td>
</tr>
<tr>
<td>PPBG (mgm%)</td>
<td>120±12.2</td>
<td>110±23.4</td>
</tr>
</tbody>
</table>

**p < 0.001

Table 3. Correlation between Duration of disease, HbA1c, fasting insulin level, FBG and PPBG in control and study groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-1 yrs.</td>
<td>2-5 yrs.</td>
</tr>
<tr>
<td>Fasting insulin level</td>
<td>7.8 ± 3.6</td>
<td>10.0 ± 2.9</td>
</tr>
<tr>
<td>(mu IU/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>2.45 ± 1.2</td>
<td>5.9 ± 2.2</td>
</tr>
<tr>
<td>FBG (mgm%)</td>
<td>85 ± 12.8</td>
<td>110 ± 10.2</td>
</tr>
<tr>
<td>PPBG (mgm%)</td>
<td>115 ± 20.1</td>
<td>138 ± 12.5</td>
</tr>
</tbody>
</table>

*p < 0.005; **p < 0.001

fasting insulin level, FBG and PPBG. The status of these parameters are shown in the mean ± standard deviation form.

RESULTS

The biochemical findings of this study can be expressed in the form of the following results.

1. With the increase in the duration of diabetes, the HbA1c values showed a significant increase. Males had a higher mean value of HbA1c as compared to the females.
2. Insulin level increases with the duration of diabetes though the increase is found to be within the normal limit. This means insulin resistance increases with the duration of diabetes.
3. NIDDM or the maturity onset diabetes occurs more frequently in the females at a slightly higher age as compared to the males as was shown in Table 1.

4. The fasting and post prandial blood glucose also showed a very significant increase with the duration of diabetes.

**DISCUSSION**

NIDDM is a chronic degenerative disease of epidemic proportions and is one of the major challenges to public health (12). India has the dubious distinction of being home to the largest number of people suffering from diabetes in any country. In theory, treating diabetes should be simple, just prevent hyperglycemia from causing damage to organs and not allow hypoglycemia to cause coma as energy supply to brain fails. In practice, it does not work that way. Glucose fluctuations occur all the time and one effective way is to monitor the HbA1c, which gives the average blood glucose level of the preceding 2-3 months. In a study of 178 Libyan men it was found that the patients having poorly controlled diabetes showed a significant correlation between HbA1c and duration of diabetes (13). HbA1c will be a valuable adjunct to blood glucose determinations in epidemiological studies. In another study of 500 diabetic patients it was found that in the group of patients with HbA1c greater than 8%, there was a significant relation to the duration of diabetes (14). Various studies prove that the amount of carbohydrate attached to the HbA1c increases with increasing duration of the disease (15).

Normal levels of insulin are healthy and necessary. But too much of a good thing, in this case, insulin, can be deadly. To be healthy our body needs to produce the right amount of insulin and respond to the insulin appropriately. A confounding factor is that hyperglycemia and hyperinsulinemia in themselves can impair insulin secretion and insulin sensitivity (16, 17, 18). The body becomes more resistant to insulin with increasing duration of diabetes, so that insulin level is high or normal in the body but the available insulin is insufficient (19). As recently pointed out in a study, because of the feedback between glucose concentration (the major stimulus for insulin release) and beta-cell insulin secretion, it is virtually impossible to develop diabetes due to severity of insulin resistance found in most type 2 diabetic patients unless the capacity to secrete additional amounts of insulin to compensate for the insulin resistance is impaired (20).

The data of Table 1 shows that females suffer from diabetes at an older age as compared to males. Various other studies also prove that the disease shows a little gender preference, although diabetes becomes slightly more frequent in women with advancing age (21). Females have estrogen hormone, which is protective for developing diabetes (22), estrogen makes the body cells more receptive or sensitive to insulin. Estrogen seems to contribute to glucose homeostasis in women (23).

Poor glycemic control and age-related pathology with duration of diabetes are thought to accelerate degenerative changes in a cooperative manner (21, 24, 25, 26). The correlation analysis carried out in another study suggests that the variables like sex, age at onset of disease, duration of diabetes and age of patients influence glycemia directly and HbA1c indirectly (27).

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ROLE OF COMBINATION OF ANTIOXIDANT AND ORAL HYPOGLYCEMIC AGENTS IN UNCOMPLICATED DIABETES

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JHANSI (U.P.) INDIA

ABSTRACT

The use of a combination of antioxidants with Oral Hypoglycemic Agent (OHA) in helping to bring down glucose levels in diabetics and also in reducing morbidity was studied. It was found that the antioxidants act better in combination with OHA and produce significantly beneficial effects even in low doses as compared to those patients who were given OHA and placebo instead of antioxidants. An improvement of 15% and more was documented in the trial group of patients.

Tables : 04  Figure : 00  References : 14

KEY WORDS: Uncomplicated Diabetes, Antioxidants, Oral Hypoglycemic agent.

Introduction

Diabetes is the biggest cause which has befallen mankind. Long have the scientists struggled to master its intricacies but till date, this dreadful disease has continued to defy them with little or no in roads being made as to the cause; the focus of attraction has shifted to controlling diabetes and its associated ill effects.

The antioxidants are at the forefront of research these days, their role in several disease processes and their control is being studied extensively at all levels with highly encouraging results. In this context the beneficial effects of antioxidants on diabetic neuropathy and as protective agents against diabetic by mopping up destructive oxygen radicals and altering the immune response have been successfully documented. It is now also known that antioxidants add in glucose utilization and also helps in reduction in now also known that antioxidants add in glucose utilization and also helps in reduction in insulin doses. Keeping all these studies in mind a project on Blood Glucose level was planned to study the role of combination of antioxidant with oral hypoglycemic agent in comparison to combination of OHA + Placebo in human subjects of uncomplicated diabetes and associated morbidity. The aim was to find out the effects (beneficial otherwise) of combination of antioxidants with oral hypoglycemic agent acting in tandem.

Material and Method

The clinical material for the present study comprised of a total of 78 patients. They were the diabetic patients without associated complications attending the diabetic out door of M.Y. Hospital and M.G.M. Medical college Indore.

1. 34 diabetic patients who were having combination of placebo with oral hypoglycemic agent (Group I).
2. 44 diabetic patients who were taking combination of oral hypoglycemic agent + antioxidants (Group II).

Care was taken that none of the subjects of the study selected was suffering from hypertension or on drug therapy accept the therapy given according to study. All the subjects were non smokers and non obese with normal BMI. All the subjects having diabetes type II without any other associated complications.

The estimation of post meal blood glucose was done before combination therapy and after
### TABLE -4: Comparative Study of Reduction in Glucose Level in Both Groups (I and II)

<table>
<thead>
<tr>
<th>Days</th>
<th>% Reduction in Glucose in Group I</th>
<th>% Reduction in Glucose level in Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>After 15 Days</td>
<td>0.99</td>
<td>1.66</td>
</tr>
<tr>
<td>After 30 Days</td>
<td>-3.3</td>
<td>9.5</td>
</tr>
<tr>
<td>After 45 Days</td>
<td>4.9</td>
<td>16.2</td>
</tr>
<tr>
<td>After 60 Days</td>
<td>7.6</td>
<td>25.5</td>
</tr>
<tr>
<td>After 75 Days</td>
<td>11.2</td>
<td>35.6</td>
</tr>
<tr>
<td>After 90 Days</td>
<td>14</td>
<td>44.6</td>
</tr>
</tbody>
</table>

### Abbreviations
- OHA = Oral Hypoglycemic Agent
- AO = Antioxidant
- GOD, POD = Glucose Oxidase, Peroxidase

### Results & Discussion

The results show the very significant reduction in glucose level in patients who were taking (OHA+AO) in comparison to the patients who were taking (OHA+Placebo).

From the results obtained in this primary study it is evident that a statistically significant improvement is observed in the diabetic status\(^{1,11}\) in those patients who received the antioxidant group in addition to their usual OHA as compared to those who were taking placebos in place of antioxidant group. The associated symptoms like polydypsia, polyuria, polyphagia also affected significantly in the first group of patients i.e. those receiving combination of antioxidants and OHA.

These results correlate very well with other studies conducted along similar lines through using different and single antioxidant\(^{10,11}\). The use of combination of antioxidants + OHA seems to act in synergy and produces a compounding of beneficial effects\(^{2,4,14}\). This study has opened up an altogether new vista in the scene of control of diabetes and its associated complications.

### References

TABLE -1: Analysis of 78 Cases Under Study

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Group 1st</th>
<th>Group II nd</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Males</td>
</tr>
<tr>
<td>1.</td>
<td>18</td>
<td>24</td>
</tr>
<tr>
<td>2.</td>
<td>Females</td>
<td>Females</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td>34</td>
<td>44</td>
</tr>
</tbody>
</table>

Table No.1 shows distribution of studied cases.
Group 1st: Comprised patients who were taking OHA + Placebo.
Group II nd : Comprised patients who were taking OHA + Antioxidant.

TABLE -2: Estimation of Glucose level Before and After every 15 days of Placebo + OHA Therapy (Group 1st patients)

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameters</th>
<th>Glucose Level before combination Therapy of Placebo+OHA</th>
<th>After 15 Days</th>
<th>After 30 Days</th>
<th>After 45 Days</th>
<th>After 60 Days</th>
<th>After 75 Days</th>
<th>After 90 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Glucose Level (mg/dl)</td>
<td>302</td>
<td>299</td>
<td>292</td>
<td>287</td>
<td>279</td>
<td>268</td>
<td>260</td>
</tr>
<tr>
<td>2.</td>
<td>S.D. +</td>
<td>±18</td>
<td>±10.2</td>
<td>±9.8</td>
<td>±15</td>
<td>±10</td>
<td>±12</td>
<td>±14.8</td>
</tr>
<tr>
<td>3.</td>
<td>% Reduction</td>
<td>-</td>
<td>0.99%</td>
<td>3.31%</td>
<td>5%</td>
<td>17.6%</td>
<td>11.2%</td>
<td>12%</td>
</tr>
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TABLE -3: Estimation of Glucose level Before and After every 15 days of OHA + Antioxidant Therapy (Group II nd patients)

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameters</th>
<th>Glucose Level before OHA+ AO therapy</th>
<th>After 15 Days</th>
<th>After 30 Days</th>
<th>After 45 Days</th>
<th>After 60 Days</th>
<th>After 75 Days</th>
<th>After 90 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Glucose Level (mg/dl)</td>
<td>376</td>
<td>370</td>
<td>340</td>
<td>315</td>
<td>280</td>
<td>242</td>
<td>218</td>
</tr>
<tr>
<td>2.</td>
<td>S.D. +</td>
<td>±11.8</td>
<td>±13</td>
<td>±8.2</td>
<td>±13.4</td>
<td>±12.6</td>
<td>±9.9</td>
<td>±11.2</td>
</tr>
<tr>
<td>3.</td>
<td>% Reduction</td>
<td>-</td>
<td>1.6%</td>
<td>9.57%</td>
<td>16.22%</td>
<td>25.5%</td>
<td>35.6%</td>
<td>44.68%</td>
</tr>
</tbody>
</table>
STATUS OF ANTIOXIDANT ENZYMES AND LIPID PEROXIDATION IN ERYTHROCYTES OF OVER WEIGHT AND OBESE PATIENTS

MEENA VERMA*, SANGITA PANERI** AND S.P. SINGH***

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**Department of Biochemistry, M.G.M. Medical College, INDORE (M.P.)
***Department of Biochemistry, M.G.M. Medical College, JHANSI (U.P.)

ABSTRACT

To investigate the status of Antioxidant enzyme activity and lipid peroxidation levels in over weight and obese, we measured the levels of lipid peroxidation by measurement of thiobarbituric Acid Reactive Substances (TBARS), activity of Antioxidant enzymes like Catalase, Glutathione peroxidase. Superoxide dismutase and the level of reduced glutathione in 45 obese (23 over weight and 22 obese) and 25 non obese control subjects. It was observed in over weight and obese subjects that there was significantly increased activity of antioxidant enzymes and increased level of TBARS and reduced glutathione than non obese control subjects. In brief, these, findings showed the direct relationship between body fat and level of lipid peroxidation and antioxidant enzyme activity. The result suggests that obesity (adiposity) affect the activity of antioxidant enzymes and lipid peroxidation, likewise it is an important factors for enhanced oxidative stress.

Tables: 02  Figure: 00  References: 14

KEY WORDS: Antioxidants, Obesity, Lipid peroxidation, Oxidative Stress, Free radicals, Adiposity.

Introduction

The irradiation with UV or X-ray or by interaction of catalytic metals, metal complexes like Hb generates free radicals such as OH, Singlet oxygen (O_2), hydroxyl radical (OH), Super oxide anion (O_2^-), and peroxy radical (HO_2^-). These free radicals are very harmful to the body. They can cause lipid peroxidation of lipid membrane. The several enzymes like superoxide dismutase, glutathione oxidase, catalase present in the cytosol and mitochondria scavenge the free radicals, therefore they are called antioxidant enzymes. Normally there is an appropriate balance between free radical: Antioxidant enzyme in the body but when this balance is shifted towards the free radical, state is called oxidative stress. Oxidative stress can result in serious cell damage by lipid peroxidation. As the level of free radical increases the activity of antioxidant enzyme also increases. The Cytokines released from adipose tissues and LDL enhance the formation of free radicals. In obese, the increased amount of adipose tissue may lead to increased formation of free radicals and alteration of antioxidant enzymes activities and lipid peroxidation. The present study is therefore aimed at further understanding of antioxidant enzyme activity and lipid peroxidation with increased adiposity in overweight and obese persons.

Materials and Methods

This Study Comprised 45 obese (BMI between 25 kg/m2 to 29.9 kg/m2) divided into two groups (1) over weight BMI = 25 kg/m2 to 29.9 kg/m2 (2) Obese BMI = 30 kg/m2 to 39.9 kg/m2 and 25 non obese (BMI = 18 kg/m2 to 24.9 kg/m2) healthy controls. BMI was calculated as the body weight (kg) divided by the square of the height (meters). Neither of these subjects were chronic smoker, alcoholic and hypertensive, nor they were suffering from CHD, Diabetes and Renal failure. None of the subjects were

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Parameters</th>
<th>Controls</th>
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<tbody>
<tr>
<td>1</td>
<td>Number of Subjects</td>
<td>25</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>BMI (Kg/m2)</td>
<td>20.5±12</td>
<td>28.4±17</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>TBARS (n. mol/gm of Hb)</td>
<td>4.6±0.52</td>
<td>5.8±0.28</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4</td>
<td>GSH (m. mol/gm of Hb)</td>
<td>14.17±0.72</td>
<td>15.21±0.64</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>5</td>
<td>SOD (U/mg of Hb)</td>
<td>2.98±0.40</td>
<td>3.42±0.32</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>6</td>
<td>GPX (U/mg of Hb)</td>
<td>22.19±0.84</td>
<td>26.21±0.71</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>7</td>
<td>Catalase (μ mol H2O2 decomposed/min/gm of Hb)</td>
<td>2532.2±31.2</td>
<td>5321.2±18.1</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

**TABLE-2:** Levels of TBARS, GSH and Activity of Cat, GPX, SOD in erythrocytes of Obese Individuals.

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Parameters</th>
<th>Controls</th>
<th>Obese</th>
<th>P. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Number of Subjects</td>
<td>25</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>BMI (Kg/m2)</td>
<td>20.9±12</td>
<td>36.8±22</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>TBARS (n. mol/gm of Hb)</td>
<td>4.5±0.52</td>
<td>7.0±48</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4</td>
<td>GSH (m. mol/gm of Hb)</td>
<td>14.17±0.72</td>
<td>16.1±80</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>5</td>
<td>SOD (U/mg of Hb)</td>
<td>2.98±0.40</td>
<td>3.98±0.29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>6</td>
<td>GPX (U/mg of Hb)</td>
<td>22.19±0.84</td>
<td>28.9±0.91</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>7</td>
<td>Catalase (μ mol H2O2 decomposed/min/gm of Hb)</td>
<td>2532.2±31.2</td>
<td>6802.81.2± 16.32</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>
receiving any medication, exercise or diet therapy before
the beginning of this study. Venous blood was collected
after over night fast and bed rest. The following estimates
were carried out from the haemolysate by following
methods.
1. TBARS by Ohkawa et al.
2. Reduced glutathione by Better and Kelly
3. Catalase by Sinha et al.
4. Superoxide dismutase by Kakkar et al.
5. Glutathione peroxidase by Rotruck et al.
6. Hb by cytoamin hemoglobin

Statistical Method
Data were expressed as the mean ±SE. Comparison
between obese, overweight and non obese subjects was
done by student 't' test. All statistical analyses were
performed with the computer software programs for this
study. P < 0.05 was taken as statistically significant and P <
0.001 taken as statistically very significant.

Results
The results of present study were presented in table
I and II showed that,
- The level of TBARS were very significantly
  increased in both overweight and obese subject
  (P< 0.001).
- There was significant increase in level of GSH
  (Reduced glutathione) in over weight (P< 0.05)
  and very Significant increase in obese (P< 0.001).
- The activity of enzyme SOD was significant
  increase in overweight (P< 0.05) and very
  significant increase in obese (P< 0.001).
- The activity of enzyme GPX (glutathione
  peroxidase) and catalase were very significantly
  enhanced in over weight and obese subjects (P<
  0.001).

MEENA VERMA*, SANGITA PANERI** AND S.P. SINGH****
- There was significant positive correlation between
  the body fat (adiposity) and antioxidant enzyme
  activity.
- There was significant positive correlation between
  body fat and lipid per oxidation.

Discussion
The present study showed that the lipid peroxidation
and antioxidant enzyme activity were significantly related
to body fat (adiposity).
The ratio of oxidative damage to lipid is increased in
obese subjects. A significant decrease in oxidative stress
after dietary restriction and weight loss was also reported
in obese subjects. Harper reported that the balance
between free radicals and antioxidant enzyme activity
shifted towards the free radical in obese.
Cytokines released from adipose tissue and LDL
enhances the formation of free radical which ultimately
increases the oxidative stress. However it is a fact that
reactive oxygen species (ROS) generation from leucocytes
is increased with increased adiposity.

The oxidative stress has been considered the Major
Mechanism responsible for endothelial dysfunction in human
obesity which causes atherosclerosis in future. Several
studies have shown correlation between adiposity and
free radical production in human and increased free radicals
leads to increase lipid peroxidation and increase activity of
antioxidant enzyme.

The results of present study showed significant
positive correlation of body fat and lipid peroxidation,
antioxidant enzyme activity. This suggests that increase
body weight (fat) stimulate the free radical formation and
may play a role in the pathogenesis of oxidative stress.
Which can leads to the obesity associated complication
future.

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metab Disorder. 26 : 754-764.
STATUS OF MDA AND ANTIOXIDANT ENZYMES IN HYPERGLYCEMIC POSTMENOPAUSAL WOMEN

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ABSTRACT

Diabetes mellitus is a chronic disease associated with serious complications and enhanced oxidation is the underlying abnormality responsible for some of these complications. In postmenopause, the ovaries do not produce estrogen, a hormone that has got antioxidant properties and so the antioxidant enzyme system seems to be affected in the women. Therefore, the already imbalanced antioxidant system is unable to effectively counteract the augmented oxidative stress due to hyperglycemia in diabetes mellitus type 2 and this manifests in the patients. The study comprised a total of 64 postmenopausal women who had attained menopause for average 5 years. Data regarding malondialdehyde (MDA), antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), glycated hemoglobin (HbA1c) and random blood glucose (RBG) values were analyzed in all the subjects, who were divided into two groups. Group 1 comprised of 32 normal, non-diabetic postmenopausal women while Group 2 comprised of 32 postmenopausal women with diabetes mellitus type 2. The results obtained showed increase in plasma MDA indicating increased oxidative stress (OS) in the diabetic postmenopausal women with a corresponding increase in the antioxidant enzymes, SOD and CAT showing oxidative stress in the cells.

<table>
<thead>
<tr>
<th>Tables  : 02</th>
<th>Figure : 00</th>
<th>References : 30</th>
</tr>
</thead>
</table>

KEY WORDS: Diabetes mellitus type 2, postmenopausal women, antioxidant enzymes, malondialdehyde.

Introduction

Diabetes mellitus type 2 with an increasing incidence worldwide is characterized by an increased risk for the development of neuropathic, micro and macro-vascular complications. Menopause in women with diabetes mellitus type 2 compounds the situation by increasing a wide variety of physical and psychological problems. Several experimental, epidemiological and clinical studies support the notion that oxidative stress plays a significant role in type 2 diabetes mellitus and in the development of coronary vascular diseases. Diabetes is associated with more lipid peroxidation via free radical formation and many disease states associated with free radicals are the same as those found with insulin resistance.

Free oxygen radicals have been proposed as important causative agents of ageing and menopause is a natural step in the process of ageing. Hence, menopausal women develop oxidative stress because of estrogen deficiency and advancing age accompanied with oxidative stress due to hyperglycemia in postmenopausal women with diabetes mellitus type 2.

The RBC has an effective mechanism to prevent and neutralize the oxidative stress induced damage. This is accomplished by a set of antioxidant enzymes like CAT, SOD etc. Recently it has been reported that the gene expression of various antioxidant enzymes i.e. SOD, CAT was substantially lower in mouse pancreatic islets than in various other tissues. This fact suggests that pancreatic islets would be more vulnerable to oxidative stress than other tissues. Another paper reported that cytokines might damage islet - cells by inducing oxygen free radical generation, lipid
peroxidation and consequently the formation of aldehydes such as MDA in the islet cells. The accelerated oxygen radical production can have serious adverse effects on cell membrane protein and lipid resulting in thiol oxidation and lipid peroxidation.

In the midst of the above detailed reviews, it is thought worthwhile to investigate the status of MDA, SOD, CAT and to assess the effect of hyperglycemia on these parameters and compared with those in age matched normal postmenopausal women treated as control.

**Material and Methods**

The study was conducted in the Department of Biochemistry and Clinical Biochemistry of MGM Medical College and OPD of M.Y. Hospital, Indore.

**Clinical Material : Subjects**

The clinical material for the present study comprised of a total of 64 subjects. They were divided into two groups.

1. **Normal subjects (Group-1)**: This group comprised of 32 normal, non-diabetic postmenopausal women without any prior family history of diabetes. These individuals were screened for the presence of diabetes based on the diagnostic criteria of the ADA.

2. **Hyperglycemic postmenopausal women (Group-2)**: This group comprised of 32 postmenopausal diabetes mellitus type 2 women. Blood glucose was controlled by balanced diet and exercise. All the patients had normal hepatic and kidney functions. None of the subjects had received hormone replacement therapy or any supportive treatment for menopausal symptoms for at least 6-8 months prior to the study. The exclusion criteria also included other factors affecting blood sugar level.

Data on therapy of diabetes, HbA1c, blood glucose levels, MDA, SOD, CAT and other parameters were obtained by structured questionnaires and by clinical and laboratory assessments.

**Collection of Material : Blood**

From all the above groups 5ml whole blood was collected along with 24 hrs. urine sample. 0.5ml whole blood was mixed with EDTA reagent (anticoagulant) and kept for the estimation of HbA1c. The remaining whole blood was kept at room temperature for 1 hour after which the supernatant clear fluid was pipetted out into another tube and the sample was used for estimation of blood sugar. The blood samples were also analyzed for plasma lipid peroxidation (MDA) and antioxidant enzymes like superoxide dismutase and catalase.

**Clinical Method: Estimation of HbA1c, Blood Sugar, MDA, SOD and CAT**

1. **HbA1c estimation**: 10 microliter of whole blood + EDTA reagent was mixed with 1ml HbA1c reagent and direct reading was taken on the auto-analyzer (selectra E) the value recorded is in percent.

2. **Blood glucose estimation**: 10 microliter of the clear serum was mixed with 1ml glucose reagent and incubated for 10 min. at 370c, then direct reading was taken on the autoanalyzer (selectra E). The value recorded was in milligram percent.

3. **MDA, SOD, CAT estimation**: The blood sample was also analyzed for MDA, SOD and CAT levels.

**Statistical Analysis**

The statistical analysis was done by student 't' test. The values were expressed as Mean ± Standard Deviation. P < 0.05 was taken as statistically significant and P < 0.001 was taken as statistically very significant.

**Observation**

**TABLE- 1: Physical Analysis of Subjects ( n = 64 )**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control ( n = 32 )</th>
<th>Study ( n = 32 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age ( years)</td>
<td>50 ± 12.5</td>
<td>56 ± 13.6</td>
</tr>
<tr>
<td>BMI ( Kg/m²)</td>
<td>26 ± 9.2</td>
<td>30 ± 8.2</td>
</tr>
<tr>
<td>Systolic B.P. ( mm Hg )</td>
<td>126 ± 20.5</td>
<td>138 ± 18.2</td>
</tr>
<tr>
<td>Diastolic B.P. ( mm Hg )</td>
<td>82 ± 8.8</td>
<td>92 ± 12.4</td>
</tr>
<tr>
<td>Pulse (per minute)</td>
<td>72 ± 4.4</td>
<td>74 ± 2.2</td>
</tr>
</tbody>
</table>
### TABLE- 2: RBG, HbA1c, MDA, SOD, CAT values in Control and Study Groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n = 32)</th>
<th>Study (n = 32)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBG ( mg % )</td>
<td>112 + 22</td>
<td>154 + 28</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>HbA1c ( % )</td>
<td>6.2 + 0.4</td>
<td>7.8 + 0.8</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>MDA (nmol/mL)</td>
<td>5.47 + 0.8</td>
<td>6.8 + 1.2</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>SOD (U/mg protein/mL)</td>
<td>6.5 + 0.632</td>
<td>8.2 + 1.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CAT (U/g protein/mL)</td>
<td>7.0 + 0.8</td>
<td>8.6 + 1.4</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

### Results

The biochemical findings of the study could be expressed in the form of the following results:

1. Diabetic postmenopausal women have high blood pressure as compared to the postmenopausal women treated as control table 1.

2. The random blood glucose values showed a significant increase in the diabetic postmenopausal women (P < 0.05) along with a corresponding significant increase in the HbA1c values (P < 0.05).

3. The level of MDA was significantly increased in the study group subjects as compared to the control (P < 0.05).

4. The level of antioxidant enzyme SOD showed a very significant increase in the diabetic post-menopausal women (P < 0.001).

5. The level of CAT showed a significant increase in the study group subjects (P < 0.05).

### Discussion

The multi-faceted toxic effects that high sugar level inflicts through out the body has been profusely studied, with many studies showing that sugar damages cells via multiple mechanism and is a causative factor in common diseases of ageing\textsuperscript{11,17-20,22}. Menopausal phase, an important physiological phenomenon in a woman's life, also is a natural step in the process of ageing. The deficiency of estrogen in postmenopausal women develops oxidative stress, due to release of free radical or reactive oxygen species and becomes the cause of various pathologies like development of hypertension, as is evident from table 1. Estrogen is a powerful antioxidant, which prevents lipid peroxidation and change in lipid profile\textsuperscript{24}.

Higher blood glucose also causes increased oxidative stress. The blood sugar levels were increased in the study group subjects (table 2). Accelerated generation of reactive oxygen species has been shown to occur in diabetes mellitus in association with hyperglycemia\textsuperscript{10,23}. Lipid peroxidation products impair insulin secretion induced by glucose probably through affecting both the glycolytic pathway and citric acid cycle\textsuperscript{12}. As is evident from table 2, the HbA1c levels were also increased in the study group subjects.

Due to hyperglycemia increase in non-enzymatic glycation occurs accompanied with glucose oxidation and these reactions are catalyzed by Cu\textsuperscript{2+} and Fe\textsuperscript{2+}, resulting in formation of oxide and hydroxide radicals which further accelerates the risk of cardiac diseases\textsuperscript{4}.

Increased MDA levels in patients of study group compared with healthy controls suggests increased systemic oxidative stress\textsuperscript{2}. MDA levels are clinical indicators for an oxidative process linked to diabetes mellitus type 2, especially in women\textsuperscript{8}. The reaction of free radicals with membrane lipids leads to the formation of lipid peroxidation products like MDA\textsuperscript{12}. In postmenopausal women ageing has been associated to a more atherogenic lipid profile\textsuperscript{1}.

In the diabetic postmenopausal women the level of SOD is very significantly increased as compared to control (table 2). This finding is consistent with the earlier finding for the level of SOD\textsuperscript{30}. Its product H\textsubscript{2}O\textsubscript{2} irreversibly inactivates superoxide dismutase because exposure of intact erythrocyte to H\textsubscript{2}O\textsubscript{2} resulted in inactivation of endogenous SOD activity in the concentration dependent manner\textsuperscript{24}. Catalase activity is enhanced in the red blood cells taking care of the disposal of H\textsubscript{2}O\textsubscript{2} in the cells\textsuperscript{18}. This finding is supported by the earlier studies\textsuperscript{5,16,26}.

It is therefore concluded that the oxidative stress due to estrogen deficiency as well as the oxidative stress due to hyperglycemia further compromises the already imbalanced antioxidant
Correlative study of bone related Biochemical parameters in normal postmenopausal women and hyperglycemic postmenopausal women

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Key words: Hyperglycemia, postmenopausal women, bone-related biochemical parameters, alkaline phosphatase.

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Abstract

Described and treated since ancient times, diabetes is an incurable chronic disease that affects almost every organ and system of the body. The present study comprised a total of 78 postmenopausal women out of which 40 women had diabetes mellitus type 2 and all these women had attained menopause for average five years. The remaining 38 were normal, non-diabetic, and postmenopausal women. Data regarding bone related parameters like calcium, phosphorus, hydroxyproline, bone formation marker alkaline phosphatase, HbA1c and blood sugar levels were estimated. The results obtained showed that in postmenopausal diabetic women the serum alkaline phosphatase tends to be higher, while serum calcium and phosphorus levels are decreased, moreover an increase in level of urinary calcium and hydroxyproline is seen. Therefore, our findings suggest that hyperglycemia in postmenopausal women affect the bone related biochemical parameters.

Introduction

Women have an early postmenopausal phase of rapid bone loss that lasts for 5-10 years after menopause. In women the rapid phase is mediated mainly by loss of the direct restraining effect of estrogen on bone cell function, whereas the slow phase is mediated mainly by the loss of estrogen action on extra skeletal calcium homeostasis leading to net calcium wasting. Osteopenia has been ascribed to diabetes without residual insulin secretion and high insulin requirement. However, it is not known if this is partially due to disturbance in the Insulin - like growth factor system, which is a key regulator of bone cell function.

One study reports that women with diabetes had significantly higher bone mineral density levels than women with normal glucose tolerance. Recent cross-sectional studies revealed that the presence of hyperglycemia is associated with higher bone mass and lower fracture rates. One of these studies reports that metabolic improvement of poorly controlled hyperglycemia decreases bone turnover. Other studies indicate that poor glycemic control impairs the responses of osteoblasts and osteoclasts in normo-insulinemic type 2 diabetic patients. Still some studies find no evidence that hyperglycemia produces any change in bone metabolism or mass. It seems worthwhile to investigate the pattern of bone loss in postmenopausal women with hyperglycemia.

The aim of this study is to evaluate the effect of hyperglycemia on the rate of bone turnover as well as the status of calcium, phosphorus and hydroxyproline metabolism in postmenopausal diabetic women so that they can be used as prognostic markers to delay or prevent the multi-faceted complications before they can eventually manifest.

Material and Methods

The study was conducted in the Department of Biochemistry and Clinical Biochemistry of M.G.M. Medical College and OPD of M.Y. Hospital, Indore.

Clinical Material

Subjects: The clinical material for the present study comprised of a total of 78 post menopausal subjects. Two groups were formed, group 1 comprised of 40 mildly diabetic patients while group 2 comprised of 38 normal, non-diabetic women as a control group. Data regarding history of diabetes, HbA1c values, random blood glucose (RBG) levels, bone-related parameters like calcium phosphorus, alkaline phosphatase (ALP) and hydroxyproline was obtained by structured questionnaires and by clinical and laboratory assessments.
1. Hyperglycemic postmenopausal women (Group 1)

This group comprised of 40 postmenopausal mildly diabetic women. Blood glucose was controlled by balanced diet and exercise. None of the patients had a disease or were treated with drugs that would interfere with calcium or phosphate metabolism and/or bone structure. The exclusion criteria also included hypertension and other factors affecting blood sugar level.

2. Normal Subjects (Group 2)

This group comprised of 38 normal, non-diabetic postmenopausal women, without any prior family history of diabetes and not on any other drug therapy. These individuals were screened for the presence of diabetes based on the diagnostic criteria of the ADA [9].

Collection of material

Blood and urine: From all the above Groups 5ml whole blood was collected along with 24 h urine sample. 0.5ml whole blood was mixed with EDTA reagent (anticoagulant) and kept for the estimation of HbA1c. The remaining whole blood is kept at room temperature for 1 hour after which the supernatant clear fluid is pipetted out into another tube and the sample is used for estimation of blood sugar, calcium, phosphorus and ALP. Urine sample is used for estimation of calcium and hydroxyproline.

Clinical Method: estimation of HbA1c, Blood sugar and bone related biochemical parameters

HbA1c, Calcium, Phosphorus, Alkaline phosphatase and Random blood sugar are estimated on fully automated analyzer (Selectra E). Urine hydroxyproline estimation is done by Modified Neuman and Logan method.

Statistical analysis

The statistical analysis was done by student 't' test. The values were expressed as Mean ± S.D. (Standard Deviation).

Results

The biochemical findings of the study can be expressed in the form of the following results.

1. Significant increase (p < 0.001) in the alkaline phosphatase level is seen in the study group (Table 1).
2. Significant decrease (p < 0.001) in the serum calcium and phosphorus level is found in the study group (Table 1) with an associated increase (p < 0.001) of urinary calcium and hydroxyproline levels (Table 2).
3. Blood sugar levels and HbA1c values were significantly high (p < 0.001) in the study group subjects (Table 1).

<p>| Table 1: Status of biochemical parameters estimated in serum of control and study subjects. |</p>
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n = 38)</th>
<th>Study (n = 40)</th>
<th>'p' Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaline Phosphatase (IU/L)</td>
<td>46 ± 2.04</td>
<td>89 ± 4.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>10.2 ± 0.82</td>
<td>8.2 ± 1.02</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>3.8 ± 0.41</td>
<td>2.2 ± 0.24</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Random blood glucose (mg/dl)</td>
<td>108 ± 10.2</td>
<td>154 ± 20.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.2 ± 0.4</td>
<td>7.2 ± 0.94</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

<p>| Table 2: Status of biochemical parameters estimated in urine of control and study subjects |</p>
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n = 38)</th>
<th>Study (n = 40)</th>
<th>'p' Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mg/24 hrs.)</td>
<td>104.2 ± 12.4</td>
<td>125.2 ± 9.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Hydroxyproline (mg/24 hrs.)</td>
<td>20 ± 2.8</td>
<td>26.8 ± 2.4</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Discussion

During the course of their lifetime, women lose approximately 50% of their trabecular bone and 30% of all postmenopausal women eventually will have osteoporotic fractures. In the present study the postmenopausal women had an increased serum alkaline phosphatase (Table 1) level. This result is similar to the reports of many earlier studies which demonstrated that bonespecific alkaline phosphatase tends to be higher in diabetic subjects [10,11,12]. A decline in alkaline phosphatase expression with maturation of osteoblasts at mineralization phase [13] suggested that hyperglycemia causes a suppression of osteoblast.
maturation [11]. However, histomorphometric analyses of bones from diabetes type 2 patients do not show such changes [14,15]. In normal postmenopausal women, an increase in bone turnover accelerates the reduction in bone mass, whereas a decrease in bone turnover is associated with preservation of bone mass [16,17,18].

Although osteoporosis is reported as a potential complication of type 1 diabetes mellitus, the effects of type 2 diabetes mellitus on bone mass are conflicting in postmenopausal women. One study suggested that the bone turnover rate is remarkably lower in diabetes mellitus type 2 patients compared to healthy postmenopausal subjects [19]. In the present study the serum calcium and phosphorus levels were decreased in the study group (Table 1). Poorly controlled NIDDM patients have relative hypercalciuria probably caused by osmotic diuresis associated with glycosuria [20,21]. This could lead to negative calcium balance which might result in accelerated bone resorption and loss of bone [22]. The decrease in urinary calcium excretion after metabolic control correlated with the decrease in urinary glucose excretion as previously reported [20,21,23,24]. Another study found that there is a significant relation between the state of metabolic normalization of diabetes and the degree of biochemical aberrations concerning calcium phosphate metabolism [25]. The increased level of urinary hydroxyproline in diabetic postmenopausal women (Table 1) show increased bone loss [26].

Postmenopausal diabetic women have higher blood sugar values as compared to the control group (Table 1) because the estrogen hormone which makes the body cells more receptive or sensitive to insulin is either not secreted at all or is in limited supply. The estrogen hormone in females is protective for developing diabetes [27]. Estrogen seems to contribute to glucose homeostasis in women [28]. Bone turnover is regulated by many local cytokines, cell-cell and cell-matrix interactions as well as systemic hormones and hyperglycemia may affect any of these local micro-environments that regulate bone turnover [22].

It is therefore concluded that the biochemical indices of bone turnover estimation show significantly increased bone activity in hyperglycemic postmenopausal women as compared to normal postmenopausal women.

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Evaluation of hypoglycemic effect of Eugenia jambulana (jamun) on obese diabetic patients

The present study is conducted on 32 obese patients with recently diagnosed diabetes mellitus type 2 and 30 sex and age matched normal control subjects. Subjects were selected from Medicine OPD and staff of M.Y. Hospital & MGM Medical College, Indore. The patients with coronary heart disease, respiratory diseases and renal diseases were excluded from the study. The exclusion criteria also included use of cigarettes, alcohol and oral contraceptives.

Described and treated since ancient times, diabetes is an incurable chronic disease that affects almost every organ and system of the body. In recent years emphasis is on the development of drugs from plants for the treatment of various diseases including diabetes mellitus. The incidence of which is very high all over the world, especially in India. A number of plants have been found to be useful in diabetes mellitus. Jambulana fruit, seeds, leaf and bark have been reported to possess antimicrobial, antihistaminic, immunomodulatory, hypoglycemic and a number of other effects. The Jambulana seeds contain beta-sitosterol, essential oil, limonene, alpha and beta pinene, cineol, alpha-humulene and bornyl acetate which are effective in reducing fasting blood sugar. The aim of the present study is to evaluate the hypoglycemic effect of Jambulana seeds on obese patients with type-2 diabetes mellitus.

Material and method:

The present study is conducted on 32 obese patients with recently diagnosed diabetes mellitus type 2 and 30 sex and age matched normal control subjects. Subjects were selected from Medicint OPD and staff of M.Y. Hospital & MGM Medical College, Indore. The patients with coronary heart disease, respiratory diseases and renal diseases were excluded from the study. The exclusion criteria also included use of cigarettes, alcohol and oral contraceptives.

The glucose levels and lipid profile was estimated before administration of Jambulana seed powder to each subjects and compared with the control. Then daily does of 6 to 9 grams of Jambulana seed powder was given to each study subjects continuously for 3 months along with balanced vegetarian diet; After each month the glucose levels and lipid profile were estimated. The estimation of glucose was done by Glucose oxidase Peroxidase method, cholesteroi, triglycerides and HDL were estimated by Kinetic method.

Statistical analysis:

The statistical analysis was done by student 't' test. The values were expressed as Mean ± Standard Deviation (S.D.).

Results and discussion:

The results shown in table 1 and 2 reveal that there is a significant increase in glucose, cholesterol, HDL and

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n = 30)</th>
<th>Study (n = 32)</th>
<th>'p' Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>96.8 ± 9.8</td>
<td>188.2 ± 11.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>172.2 ± 13.2</td>
<td>282 ± 21.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>52 ± 8.2</td>
<td>38.2 ± 13.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>128.4 ± 18</td>
<td>268 ± 20.2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Department of Biochemistry, M.G.M. Medical College, Indore (M.P.)
Table 2
Status of parameters estimated after administration of Jambulana seed powder in study group and its comparison with control.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n = 30)</th>
<th>Control (n = 32) After administration of Jambulana seed powder</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 month</td>
<td>2 month</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>86 ± 8.5</td>
<td>75 ± 7.1</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>170 ± 8.6</td>
<td>168 ± 4</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>52 ± 13</td>
<td>50 ± 11</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>120.4 ± 8.2</td>
<td>129 ± 7.8</td>
</tr>
</tbody>
</table>

DECREASE IN GLUCOSE LEVEL AFTER ADMINISTRATION OF JAMBULANA SEED POWDER
triglycerides in obese patients with recently diagnosed diabetes mellitus type 2. But after treatment with Jambulana seed extract there was significant lowering of serum glucose, cholesterol and triglycerides and these values were close to normal. A slight increase in HDL level is also seen. This indicates that the Jambulana seed powder has favourable effect on the lipid and glucose metabolism of obese diabetic patients.

The increased level of fatty acids and fats in obese patients causes the insulin resistance which leads to the hyperglycemia. Hypolipidemic and hypoglycemic property of Jambulana seed powder improve the lipid and glucose level in obese patients.5 From the above results, it can be concluded that Jambulana seed powder which contains beta-sitosterol, essential oil, bornylacetate, cisocimene, alpha and beta pinene can be an useful antidiabetic agent and have the ability to cure the hyperglycemia and hyperlipidemia.

References:
Estimation of Serum Lipoproteins and Glucose in Diabetic Offsprings

Diabetes Mellitus has always been regarded primarily as a disturbance of carbohydrate metabolism. It is only in the last few decades that the disturbances in the lipid metabolism have been described in association with the disease. The clinical material for the present study comprised of a total of 60 patients. They were the offsprings of diabetic patients attending the diabetic out-door of M.Y. Hospital & M.G.M. medical College, Indore. 20 Individuals were studied as control cases of which 10 were healthy subjects and 10 were diabetic patients. 30 Cases were such whose parents were diabetic. 30 Cases were such whose one of the parent was diabetic. Estimation of Total lipids, lipoproteins, triglycerides, cholesterol and blood sugar was done in these patients. The study revealed that Serum triglycerides, lipoproteins & total lipids were raised in diabetic control cases and were within normal limits in diabetic offsprings.

DR. SANGITA PANERI, DR. MEENA VARMA

Diabetes Mellitus has been known to man since time immemorial. It has always been regarded primarily as a disturbance of carbohydrate metabolism. It is only in the last few decades that the disturbances in the lipid metabolism have been described in association with the disease.

It has now been proved that Insulin in also related to lipid metabolism disturbances of insulin will equally derange the lipid metabolism. Secondly, carbohydrate metabolism will lead to alterations in the lipid metabolism (1). The hereditary nature of diabetes mellitus is generally accepted, although the mode of inheritance is controversial. Also, the high prevalence of diabetes in families of diabetic patients is well known (2). In recent years, detail studies have been made in clinics & laboratories all over the word in an attempt to discover some abnormality which would enable one to identify those persons who are labeled prediabetic on genetic grounds & who will in reality develop diabetes later on (3).

Investigations on prediabetics were begun some years ago in Elliott P. Joslin research laboratory under the direction of several doctors. A wide variety of suitably controlled serial studies have been carried out in an attempt to detect any anatomic or biochemical abnormality in this earliest stage of diabetic state (4, 5, 6, 7).

The present study has been carried out with an aim to find out that whether there is a disturbance of lipid metabolism in diabetic offsprings. Also an attempt has been made to evaluate the diagnostic significance of these investigations.

Material & Methods

The clinical material for the present study comprised of a total of 60 patients. They are the offsprings of diabetic patients attending the diabetic out-door of M.Y. Hospital & M.G.M. medical College, Indore.

1. 20 Individual were studied as control cases; of which 10 were healthy subjects and 10 were diabetic patients.
2. 30 Cases are such whose both parents are diabetic.
3. 30 Cases are such whose one of the parents is diabetic.

Care was taken that none of the subjects of the study selected should be suffering from hypertension or taking medications such as b blockers which may alter their lipid profile. All of the subjects were non-smokers and non-obese with normal Basal metabolic Index (BMI) and Waist to Hip ratio (W/H). Estimation of total lipids, lipoproteins, triglycerides, cholesterol and blood sugar was done in these patients.

1. Normal individuals

All these persons were clinically examined and found to be normal healthy individuals not suffering from any disease. They have no family history of diabetes and their fasting blood sugar and post prandial urine & blood examination did not reveal any sign of hyperglycemia.

2. Diabetic Controls

These persons were proven diabetics receiving treatment from diabetic out-door clinic of M.Y. Hospital.

3. Diabetic Offsprings

They are the offsprings of diabetic parents and between the ages 10-40 yrs. Their Glucose tolerance tests were normal.

A detailed history of the patients was taken and through physical examination was carried out. Family history regarding diabetes was taken & following investigations were carried out:

1. Blood Sugar
2. Serum cholesterol

Department of Biochemistry, MGM Medical College, Indore (M!)
COLLECTION OF MATERIAL

In all cases blood was collected in fasting state. The blood was kept for 2 hrs at room temperature after which the supernatant clear fluid was collected with a pipette in another tube. This tube was centrifuged for 10 minutes. The clear serum was pipetted into a clean dry test tube & this was used for estimation of cholesterol, triglycerides, lipoprotein & total lipids. For blood sugar 2 ml blood was collected in an oxalated bulb.

LOCUS OF STUDY

The study was conducted in the department of Biochemistry & Pathology of M.Y. Hospital and M.G.M. Medical College, Indore.

Observations

Table No. 1

<table>
<thead>
<tr>
<th>Control Cases</th>
<th>Diabetic offspring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Cases</td>
<td>Diabetic Cases</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Males</td>
<td>5</td>
</tr>
<tr>
<td>Females</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
</tr>
</tbody>
</table>

Table No. 1 shows distribution of control cases and cases of diabetic offsprings. Out of 10 normal cases, 50% were males and 50% were females. Ten diabetic cases were selected for study so as to see any of the lipid metabolic derangements in them. In offsprings 40(66%) were males and 20(34%) were females.

Table No. 2

Relationship of blood sugar, cholesterol, triglycerides, lipoproteins & total lipids in diabetic control cases.

a) Males

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Case No.</th>
<th>R.G.</th>
<th>Cholesterol</th>
<th>Triglycerides</th>
<th>Lipoproteins</th>
<th>Total Lipids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(mg%)</td>
<td>(mg%)</td>
<td>(mg%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Beta</td>
<td>Alpha</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>200</td>
<td>240</td>
<td>180</td>
<td>72</td>
<td>28</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>140</td>
<td>200</td>
<td>160</td>
<td>76</td>
<td>24</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>280</td>
<td>200</td>
<td>1188</td>
<td>73.5</td>
<td>26.5</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>170</td>
<td>222</td>
<td>125</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>160</td>
<td>231</td>
<td>140</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>190</td>
<td>218.6</td>
<td>156.6</td>
<td>73.3</td>
<td>26.7</td>
</tr>
<tr>
<td>Std.</td>
<td></td>
<td>48.98</td>
<td>16.21</td>
<td>23.66</td>
<td>2.13</td>
<td>2.13</td>
</tr>
</tbody>
</table>

b) Females

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Case No.</th>
<th>R.G.</th>
<th>Cholesterol</th>
<th>Triglycerides</th>
<th>Lipoproteins</th>
<th>Total Lipids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(mg%)</td>
<td>(mg%)</td>
<td>(mg%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Beta</td>
<td>Alpha</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>16</td>
<td>200</td>
<td>220</td>
<td>120</td>
<td>70</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>160</td>
<td>210</td>
<td>120</td>
<td>60</td>
<td>31</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>180</td>
<td>160</td>
<td>180</td>
<td>72</td>
<td>28</td>
</tr>
<tr>
<td>4</td>
<td>19</td>
<td>185</td>
<td>182</td>
<td>185</td>
<td>76</td>
<td>24</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>190</td>
<td>185</td>
<td>115</td>
<td>71</td>
<td>29</td>
</tr>
</tbody>
</table>

Mean 183 195.4 144 71.60 28.40 681
S.D. 13.26 25.85 31.52 2.41 2.4 78.12

In diabetic control cases, beta lipoproteins were raised with hypertriglyceridemia in 4 cases. Total lipids were also high as compared to non-diabetic control levels, but serum cholesterol was within normal limits.

Table No. 3

The mean value of serum cholesterol, triglycerides, lipoproteins and total lipids in diabetic onsprings according to age group and presence of diabetes in parents are as follows:

<table>
<thead>
<tr>
<th>Cholesterol</th>
<th>Triglycerides</th>
<th>Beta</th>
<th>Alpha</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>(mg%)</td>
<td>(mg%)</td>
<td></td>
<td></td>
<td>mgm%</td>
</tr>
<tr>
<td>Both parents diabetic 10-25 yrs 171.0 140.4 67.6 34.4 658 25-40 yrs 178.5 123.4 73.6 26.4 571</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One of the parents is diabetic 10-25 yrs 182.0 120.2 73.0 27.0 707 25-40 yrs 171.6 112.6 72.0 28.0 693</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RESULTS

From the study undertaken following information is concluded.

1. a) Serum cholesterol value in non-diabetic control cases was between 120-275 mgm%, average of 199.40 mgm%.
b) In diabetic control cases it was between 160-240, average of 205 mgm between.
c) In diabetic offsprings it was found to be between 90-200 mgm%, average of 180 mgm%.

2. a) Serum triglycerides value in non-diabetic control cases was between 80-200 mgm% average of 128 mgm%.
b) In diabetic control cases it was between 115-118 mgm% average of 150.3%
c) In diabetic offsprings it was found to be 90-200 mgm% average of 130 mgm%.

3. a) Serum total lipid value in non-diabetic control cases was between 450 to 710 mgm% average of 545 mgm%.
b) In diabetic control cases it was between 420 to 890 mgm% average of 796 mgm%.
4. a) Serum lipoprotein Beta & Alpha % in non-diabetic control cases was found to be 60:40 to 70:24, mean value of 68.32.
   b) In diabetic control cases it was between 69.31 to 76.24, mean value of 69.32.
5. Serum Triglycerides, lipoproteins and total lipoprotein are raised in diabetic control patients.
6. Serum cholesterol, triglycerides, Beta & Alpha lipoprotein and Total Lipids are within normal limits in diabetic offsprings.
7. There is no disturbance of lipid metabolism

**DISCUSSION**

The present study comprised of 60 diabetic offsprings. Simultaneously 10 control normal healthy individuals were also studied for their serum contents of cholesterol, triglycerides, lipoproteins & total lipids. A control study of 10 diabetic patients was also done, in order to compare these values with those of diabetic offsprings.

Idiopathic diabetes mellitus embraces a heterogeneous group of disorders having in common disordered carbohydrate fat and protein metabolism. Insulin is a major anabolic hormone in the body. Derangement of insulin function affects not only glucose metabolism but fat and protein metabolism as well. There is concomitant excessive breakdown of fat stores sometimes resulting in elevated levels of free fatty acids and hyperlipidemia. Oxidation of free fatty acids within the liver through acetyl Co-enzyme-A produces ketone bodies. Ketogenic amino acids aggravate the derangement in lipid metabolism.

The main aim to find out that whether there is disturbance of lipid metabolism in diabetic offsprings also because diabetes is a hereditary transmitted disease.

Diabetes causes an increase in total cholesterol and triglycerides level in the body. It has been observed that patients with Type 1 DM usually have concentrations of the major lipoproteins. LDL and VLDL are normal or subnormal, whereas HDL is normal or increased. Type 1 DM patients have a high free cholesterol/lecithin ratio in plasma and VLDL-LDL fractions. These abnormalities may interfere with lipid transport between lipoproteins and consequently the remodelling of lipoprotein particles. The concentration of phospholipids in HDL is abnormal.

In type 2 DM patients, moderate hypertiglyceridaemia with reduced levels of HDL cholesterol is common. Since glycemic control is often insufficient, serum triglycerides are elevated. The increase in triglyceride-rich lipoproteins induces a mild elevation in total serum cholesterol. In the case of poor glycemic control, total cholesterol is increased due to an accumulation of LDL, HDL particles contain an increased proportion of triglycerides, with a faster catabolic rate that leads to a lower number of circulating HDL.

In the present study it was found that Serum triglycerides, lipoproteins & total lipids were raised in diabetic control cases, which is in accordance with the observations made in the past, where as in diabetic offsprings lipids were within normal limits (Table 3).

Thus from above observation and discussion it can be concluded that probably there is no disturbance of lipid metabolism in diabetic offsprings as compared to patients of diabetes.

**References**

15. Dr. Suvadip Chatterjee Diabetes and Hypertension, MCNA, 2001; 3:205-209.
ABO Rh Blood Group and HbA1c: A Retrospective Study in The Urban Niddm Population of Indore

This retrospective study to a certain extent is not supported by the above references so this shows that the environment, dietary habits and lifestyle factors effect the study results. Due to a background of high prevalence of Type 2 diabetes, the study of the pattern of distribution of various blood groups within the NIDDM population is of great importance. These frequencies not only influence the use and availability of blood with various groups but also pattern of hemolytic disease of new born and hemolytic transfusion reactions in our population. There is a paucity of literature as far as the relationship between HbA1c, ABO Rh blood groups is concerned. Perusal of this relation shows promise in being a successful approach to the monitoring of diabetic patient and also provides a conceptual frame work for the pathogenesis of secondary sequelae of diabetes.

Dr. Meena Varma, Preetha Badi, Dr. Sangeeta Paneri

Described and treated since ancient times, diabetes is an incurable chronic disease that affects the body's ability to convert food into energy. NIDDM is a multi-factorial disease caused by both oligo and polygenic factors as well as non-genetic factors that result from a lack of balance between the energy intake and output and other life-style related factors. Type 2 diabetes, by far more common than Type 1, has spared few societies or ethnic groups. Studies in India in the last decade have highlighted that not only is the prevalence of Type 2 diabetes high but that it is increasing within the urban population. The Rh blood group system is one of the most polymorphic and immunogenic systems known in humans. Statistically significant association were found between NIDDM and Rh blood type in a study of 1237 Mexican Americans. In another cross-sectional study of 2312 confirmed diabetics carried out in Bangladesh, the data on statistical analysis showed no association between ABO blood groups and diabetes mellitus. In an American study the world wide blood group distribution was found to be as follows, A positive (28%), B positive (32%), O positive (33%) and AB positive (7%).

These and scores of other studies and investigations conducted in different parts of the world in the varied ethnic groups and diverse populations provide divergent and conflicting reports. The aim of the present study is to investigate the presence of an association between ABO Rh blood groups, HbA1c and NIDDM in the urban population of Indore.

Material and Method

Locus of Study:

This study was conducted in the Department of Biochemistry and Clinical Biochemistry of M.G.M. Medical College and O.P.D of M.Y. Hospital, Indore.

Clinical Material: Subjects:

The clinical material for the present study comprised of a total of 528 subjects. The study group subjects were established diabetics. These individuals were screened for the presence of diabetes based on the diagnostic criteria of the American Diabetes Association (ADA). Data on therapy of diabetes, HbA1c values, fasting blood glucose (FBG), post-prandial blood glucose (PPBG) and ABO Rh blood group was obtained by structured questionnaires and by clinical and laboratory assessments.

Collection of Material: Blood:

5-ml whole blood was collected from all subjects in the fasting state. 0.5 ml whole blood is mixed with EDTA reagent (anti-coagulant) and kept for HbA1c estimation. The remaining blood is kept at room temperature for 1 hour, after which the superantem clear fluid is pipetted out into another tube. This tube is then centrifuged for 10 min., the clear serum is pipetted out into a clean dry tube and used for estimation of blood sugar. Similarly, 0.5 ml blood is collected from the subjects, 2 hours after having food for the estimation of post-prandial blood sugar along with the urine sample.

Clinical Method: Estimation of HbA1c, FBG, PPBG, and ABO Rh Blood Typing:

Estimation of HbA1c, FBG and PPBG was done by using commercial kits on fully automated biochemistry analyzer (Selectra-E). ABO Rh(D) Blood grouping was done by Direct Agglutination Test.

Statistical Analysis:

The results were entered in a computer database and
statistically analyzed using Microsoft Excel Spread Sheet Program.

OBSERVATION:

Table No. 1
Analysis of 528 subjects under study with respect to their ABO Rh(D) Blood Group.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Blood Group</th>
<th>Rh(D)</th>
<th>Typing</th>
<th>n=528</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>A</td>
<td>38</td>
<td>6</td>
<td>44</td>
</tr>
<tr>
<td>2.</td>
<td>B</td>
<td>190</td>
<td>18</td>
<td>208</td>
</tr>
<tr>
<td>3.</td>
<td>AB</td>
<td>97</td>
<td>14</td>
<td>101</td>
</tr>
<tr>
<td>4.</td>
<td>O</td>
<td>165</td>
<td>10</td>
<td>175</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>490</td>
<td>38</td>
<td>528</td>
</tr>
</tbody>
</table>

n = number of subjects (sample size)

Table No.2
Status of mean values of HbA1c, fasting blood glucose and post-prandial blood glucose with respect to the ABO Rh Blood Groups (n=528)

<table>
<thead>
<tr>
<th>S.No</th>
<th>ABO Blood</th>
<th>Mean Blood</th>
<th>HbA1c(%)</th>
<th>FBG</th>
<th>PPBG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rh(D)</td>
<td>Group</td>
<td>Sugar(mg/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>A positive</td>
<td>160</td>
<td>240</td>
<td>7.4</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>A negative</td>
<td>138</td>
<td>198</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>B positive</td>
<td>164</td>
<td>248</td>
<td>7.4</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>B negative</td>
<td>152</td>
<td>218</td>
<td>6.2</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>AB positive</td>
<td>166</td>
<td>254</td>
<td>8.2</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>AB negative</td>
<td>178</td>
<td>278</td>
<td>7.8</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>O positive</td>
<td>194</td>
<td>292</td>
<td>6.8</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>O negative</td>
<td>182</td>
<td>286</td>
<td>8.1</td>
<td></td>
</tr>
</tbody>
</table>

FBG=Fasting Blood Glucose
PPBG=Post-Prandial Blood Glucose
HbA1c=Glycated Hemoglobin

RESULT:
The clinical and biochemical findings obtained from the urban NIDDM population of Indore in this particular study can be translated into the following results:
1. In the group of subjects with Rh positive blood groups, incidence of NIDDM is found to be higher in the B positive group and lower in the A positive group.
2. In the group of subjects with Rh negative blood groups, incidence of NIDDM is found to be higher in the B negative and lowest in AB negative group.
3. The highest level of fasting blood sugar is found in O positive patients and corresponding lowest level in A negative group.
4. The AB positive diabetic patients group had the highest value for the glycated hemoglobin level while the lowest value for the same was found in the B negative diabetic patients' group.

CONCLUSION:
In conclusion, it is very clearly evident from this retrospective study that the highest incidence of NIDDM is found in the subjects with B positive blood group and the lowest incidence of NIDDM is seen in the A positive blood group subjects. The AB positive individuals have a greater tendency to have high values for glycated hemoglobin level. The present study supports the hypothesis that diabetes mellitus Type 2 and blood groups are inter-related because of the broad genetic immunologic basis in both.

DISCUSSION:
It is a known fact that it is neither war nor a natural calamity but it is the lifestyle of man which is the largest cause of death and disease in the world today. Man now lives in an environment which is not conducive to his health and performs activities which his body is not conditioned to. The WHO estimates that there were 19.4 million persons with diabetes in India in 1995 and that this number is likely to be 57.2 million in 2025. Quantum of the problem is that the current prevalence rates are 1.1-12% in the urban Indian adult population. In a study of 520 proven cases of adult diabetes mellitus, a strong indication of an association of diabetes mellitus with blood groups especially with A, AB and Rh positive blood groups was found. The maximum differences was seen in the AB groups in the two series and minimum in the A group. In another study of 490 Type 2 diabetes mellitus cases, it was found that the frequency of blood groups B and O is significantly higher and lower, respectively in the diabetes mellitus Type 2 patients as compared to the general population. A major assumption in the interpretation of HbA1c as a measure of glycemic control is that the duration of hemoglobin exposure to glucose does not vary among patients who are hemotologically normal. Since glycated hemoglobin is found inside the red blood cells, the relationship between Rh genetic variability and HbA1c level suggest that RH proteins may influence glucose transport through red cell membrane and/or hemoglobin glycation.

Status of MDA and Antioxidant enzymes in hyperglycemic postmenopausal women

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**Department of Biochemistry, King George Medical College, Lucknow (U.P.)

Abstract

Diabetes mellitus is a chronic disease associated with serious complications and enhanced oxidation is the underlying abnormality responsible for some of these complications of diabetes. In postmenopause, the ovaries do not produce estrogen, a hormone which has got antioxidant properties and so the antioxidant enzyme system seems to be affected in the postmenopausal women. Therefore, the already imbalanced antioxidant system in the postmenopausal women is unable to effectively counteract the augmented oxidative stress due to hyperglycemia in diabetes mellitus type 2 and this manifests in the patients as a plethora of complications. The study comprised a total of 64 postmenopausal women who had attained menopause for average 5 years. Data regarding malondialdehyde (MDA), antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), glycated hemoglobin (HbA1c) and random blood glucose (RBG) values were analysed in all the subjects, who were divided into two groups. Group 1 comprised of 32 normal, non-diabetic postmenopausal women while Group 2 comprised of 32 postmenopausal women with diabetes mellitus type 2. The results obtained showed increase in plasma MDA indicating increased oxidative stress (OS) in the diabetic postmenopausal women with a corresponding increase in the antioxidant enzymes, SOD and CAT showing oxidative stress in the cells.

Key words: Diabetes mellitus type 2, postmenopausal women, antioxidant enzymes, malondialdehyde.

Introduction

Diabetes mellitus type 2 with an increasing incidence worldwide is characterized by an increased risk for the development of neuropathic, macro and macro-vascular complications. Menopause in women with diabetes mellitus type 2 compounds the situation by increasing a wide variety of physical and psychological problems. Several experimental, epidemiologic and clinical studies support the notion that oxidative stress plays a significant role in type 2 diabetes mellitus(2) and in the development of coronary vascular diseases(3). Diabetes is associated with more lipid peroxidation via free radical formation(4) and many disease states associated with free radicals are the same as those found with insulin resistance(5).

Free oxygen radicals have been proposed as important causative agents of aging(6) and menopause is a natural step in the process of aging. Hence, menopause women develop oxidative stress because of estrogen deficiency and advancing age(7) accompanied with oxidative stress due to hyperglycemia in postmenopausal women with diabetes mellitus type 2.

The RBC has an effective mechanism to prevent and neutralize the oxidative stress induced damage. This is accomplished by a set of antioxidant enzymes like CAT, SOD etc. Recently it has been reported that the gene expression of various antioxidant enzymes i.e. SOD, CAT was substantially lower in mouse pancreatic islets than in various other tissues. This fact suggests that pancreatic islets would be more vulnerable to oxidative stress than other tissues. Another paper reported that cytokines might damage islet cells by inducing oxygen free radical generation, lipid peroxidation and consequently the formation of aldehydes such as MDA in the islet cells. The accelerated oxygen radical production can have serious adverse effects on cell membrane protein and lipid resulting in thiol oxidation and lipid peroxidation.

In the midst of the above detailed reviews, it is thought worthwhile to investigate the status of MDA, SOD, CAT and to assess the effect of hyperglycemia on these parameters and compared with those in age matched normal postmenopausal women treated as control.

Material and methods

The study was conducted in the Department of Biochemistry and Clinical Biochemistry of MGM Medical...
The clinical material for the present study comprised of a total of 64 subjects. They were divided into two groups. The first group comprised of 32 normal postmenopausal women as control group while the second group comprised of 32 diabetes mellitus type 2 postmenopausal women. Data on therapy of diabetes, HbA1c, blood glucose levels, MDA, SOD, CAT and other parameters was obtained by structured questionnaires and by clinical and laboratory assessments.

1. Normal subjects (Group 1)

This group comprised of 32 normal, non-diabetic postmenopausal women without any prior family history of diabetes. These individuals were screened for the presence of diabetes based on the diagnostic criteria of the ADA12.

2. Hyperglycemic postmenopausal women (Group 2)

This group comprised of 32 postmenopausal diabetes mellitus type 2 women. Blood glucose was controlled by balanced diet and exercise. All the patients had normal hepatic and kidney functions. None of the subjects had received hormone replacement therapy or any supportive treatment for menopausal symptoms for at least 6-8 months prior to the study. The exclusion criteria also included other factors affecting blood sugar level.

Collection of material : blood

From all the above groups 5ml whole blood was collected along with 24 hrs. urine sample. 0.5ml whole blood was mixed with EDTA reagent (anticoagulant) and kept for the estimation of HbA1c. The remaining whole blood is kept at room temperature for 1 hour after which the supernatant clear fluid is pipetted out into another tube and the sample is used for estimation of blood sugar. The blood samples were also analysed for plasma lipid peroxidation (MDA) and antioxidant enzymes like superoxide dismutase and catalase.

Clinical method : estimation of HbA1c, blood sugar, MDA, SOD and CAT.

1. HbA1c estimation

10 microliter of whole blood + EDTA reagent is mixed with 1ml HbA1c reagent and direct reading is taken on the auto-analyzer (selectra E) the value recorded is in percent.

2. Blood glucose estimation

10 microliter of the clear serum is mixed with 1ml glucose reagent and incubated for 10 min. at 370c, then direct reading is taken on the autoanalyzer (selectra E). The value recorded is in milligram percent.

3. MDA, SOD, CAT estimation

The blood sample was also analysed for MDA13, SOD14 and CAT15 levels.

Statistical analysis

The statistical analysis was done by student 't' test. The values were expressed as Mean ± Standard Deviation (S.D.)

Observation

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n = 32)</th>
<th>Study (n = 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>50 ± 12.5</td>
<td>56 ± 13.6</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>26 ± 9.2</td>
<td>30 ± 8.2</td>
</tr>
<tr>
<td>Systolic B.P. (mm Hg)</td>
<td>126 ± 20.5</td>
<td>138 ± 18.2</td>
</tr>
<tr>
<td>Diastolic B.P. (mm Hg)</td>
<td>82 ± 8.8</td>
<td>92 ± 12.4</td>
</tr>
<tr>
<td>Pulse (per minute)</td>
<td>72 ± 4.4</td>
<td>74 ± 2.2</td>
</tr>
</tbody>
</table>

Results

The biochemical findings of the study can be expressed in the form of the following results:

1. Diabetic postmenopausal women have high blood pressure as compared to the postmenopausal women treated as control, as is evident from table 1.

2. The blood glucose values also showed a significant increase in the diabetic postmenopausal women along with a corresponding significant increase in the HbA1c values (table 2).

3. The levels of MDA is significantly increased in the study group subjects as compared to the control (table 2).

4. The level of antioxidant enzyme SOD showed a very significant increase in the diabetic post-menopausal women (table 2).

5. The level of CAT showed a significant increase in the study group subjects (table 2).

Discussion

The multi-faceted toxic effects that high sugar level inflicts through out the body has been profusely studied, with many studies showing that sugar damages cells via multiple mechanism and is a causative factor in common diseases of aging16-19. Menopausal phase, an important physiological phenomenon in a woman’s life, also is a natural step in the process of aging. The deficiency of estrogen in postmenopausal women develops oxidative stress, due to release of free radical or reactive oxygen species and becomes the cause of various pathologies like development of hypertension, as is evident from table 1. Estrogen is a powerful antioxidant, which prevents lipid peroxidation and change in lipid profile7.
Higher blood glucose also causes increased oxidative stress. The blood sugar levels were increased in the study group subjects (table 2). Accelerated generation of reactive oxygen species has been shown to occur in diabetes mellitus in association with hyperglycemia. Lipid peroxidation products impair insulin secretion induced by glucose probably through affecting both the glycolytic pathway and citric acid cycle. As is evident from table 2, the HbA1c levels were also increased in the study group subjects. Due to hyperglycemia increase in non-enzymatic glycation occurs accompanied with glucose oxidation and these reactions are catalysed by Cu2+ and Fe2+ resulting in formation of oxidized and hydroxyl radicals which further accelerates the risk of cardiac diseases.

Increased MDA levels in patients of study group compared with healthy controls suggests increased systemic oxidative stress. MDA levels are clinical indicators for an oxidative process linked to diabetes mellitus type 2, especially in women. The reaction of free radicals with membrane lipids leads to the formation of lipid peroxidation products like MDA. In postmenopausal women aging has been associated to a more atherosclerotic lipid profile.

In the diabetic postmenopausal women the level of SOD is very significantly increased as compared to control (table 2). This finding is consistent with the earlier finding of Turk HM et al for the level of SOD. Superoxide dismutase is irreversibly inactivated by its product H2O2 because exposure of intact erythrocyte to H2O2 resulted in inactivation of endogenous SOD activity in the concentration dependent manner. Catalase activity is enhanced in the red blood cells taking care of the disposal of H2O2 in the cells. This finding is consistent with the earlier studies of Turk HM et al, Sozmen EY et al and Kesavulu MM et al for the status of CAT.

It is therefore concluded that the oxidative stress due to estrogen deficiency as well as the oxidative stress due to hyperglycemia further compromises the already unbalanced antioxidant system in the postmenopausal diabetic women and predisposes their cells to potential oxidative injury and paves the way for development of associated complications.

References
Correlative Study of Duration of Type 2 Diabetes Mellitus with Glycated Hemoglobin, Insulin Resistance and Blood Glucose

Abstract

The present study was carried out to determine the correlation, if any between insulin resistance, blood glucose levels, glycated hemoglobin and duration of type 2 diabetes mellitus (DM).

The study comprised a total of 76 subjects out of which 30 were normal, non-diabetic persons and the rest 46 were diabetics with different duration of time in years, after being diagnosed diabetic. Data was analyzed after dividing the subjects into 4 groups: Group 1, comprised of 1 year old diabetics, Group 2 was made up of those, who had diabetes, for the past 2-5 years, Group 3 included patients who were diabetic since more than 5 years and Group 4 included non-diabetics as the normal control group. The results obtained indicated that the HbA1c levels showed a significant increase with the duration of diabetes as well as the insulin level showed a significant correlation after adjustment for age, sex and duration of diabetes.

Introduction

Diabetes mellitus is a lifelong disease, which makes many people worry about the quality and longevity of their life after diagnoses. The complications of diabetes are influenced not only by the duration of diabetes but also by the average level of chronic glycemia, which is measured most reliably with glycated hemoglobin assay. In normoglycemic subjects, a small proportion of hemoglobin A is attached to a carbohydrate moiety thus, creating what is called glycated hemoglobin. In conditions of sustained hyperglycemia, such as in diabetes mellitus, the proportion of hemoglobin that is glycated is increased substantially. Studies conducted by Arnetz, et al. and Kilpatrick, et al. in diabetic patients have shown a significant positive correlation between HbA1c and age as well as duration of diabetes. In contradiction to this, Kabadi found no significant relationship between age, duration
of diabetes and fasting blood glucose (FBG), glycosylated hemoglobin, glycated protein or glycated albumin. According to the results of many longitudinal and cross-sectional studies, it has been demonstrated that the earliest detectable abnormality in type 2 DM is an impairment in the body’s ability to respond to insulin. Studies have shown that insulin sensitivity correlated inversely with fasting insulin and the insulin levels increased with the duration of diabetes.

Though such detailed investigations have been carried out in different parts of the world to prove a correlation between the different parameters, the results were contradictory, blurring the diagnostic significance of some of these parameters. It was thought worthwhile to investigate the significance of such correlations in the Indian diaspora, where such a biochemical equation on the effect of these parameters for the progression of the diabetic sequelae have not yet been postulated. The aim of this study was to evaluate the correlation between the above detailed parameters so that they can be used as diagnostic or prognostic markers for the assessment of the degree of control of this lifestyle disease, to delay or prevent the multifaceted complications.

Materials and methods

The study was conducted in the Department of Biochemistry and Clinical Biochemistry of M.G.M. Medical College and OPD of M.Y. Hospital, Indore.

Subjects

The clinical material for the present study comprised of a total of 76 subjects. Three groups were formed on the basis of difference in duration of diabetes. The fourth group comprised of subjects who were normal and non-diabetic, as a control group. Data on therapy of diabetes, HbA₁c values, FBG and postprandial blood glucose (PPBG) was obtained by structured questionnaires and by clinical and laboratory assessments. Insulin-sensitive subjects were defined as having an insulin-sensitivity estimate as per the median in non-diabetic subjects participating in the study. Using this definition 88% of all the type 2 diabetics were insulin resistant. The study subjects were established diabetics.

Group 1: One-year duration diabetes

These subjects had been diagnosed with diabetes only one year before.

Group 2: Two to five years duration of diabetes

This group of patients was diabetic since the last two to five years.

Group 3: More than five years duration of diabetes

This group of patients was diagnosed with diabetes for more than five years. The maximum duration in this group was found to be of a patient with a diabetic history of 22 years.

Group 4: Normal subjects

These individuals were screened for the presence of diabetes based on the diagnostic criteria of the American Diabetes Association (ADA). They were found to be normal, healthy individuals without any prior family history of diabetes. The exclusion criteria also included hypertension, use of alcohol or cigarettes and other factors affecting blood sugar levels.

Collection of material: Blood

In all the above groups, 5 ml whole blood was collected in the fasting state. 0.5 ml whole blood was mixed with EDTA regent (anticoagulant) and kept for HbA₁c estimation. The remaining blood is kept at room temperature for 1 hour after which the supernatant clear fluid was pipetted out into another tube. This tube was then centrifuged for 10 minutes. The clear serum was pipetted into a clean dry test tube and used for estimation of blood sugar and insulin. Similarly, 0.5 ml blood was collected from the subjects 2 hours after having food for the estimation of postprandial blood sugar, along with the urine sample.

Clinical method

- HbA₁c estimation

For the estimation of HbA₁c, 10 µl of the whole blood + EDTA reagent, was mixed with 1 ml HbA₁c reagent and direct reading taken on the auto-analyzer (Selectra E). The value recorded is in percent.

- FBG and PPBG estimation

Ten microliters of the clear serum is mixed with 1 ml glucose reagent and incubated for 10 minutes at 37°C. Then,
direct reading was taken on the auto-analyzer (Selectra E). The value recorded is in mg%. The same process was repeated for the estimation of PPBG with the postprandial blood sample.

**Insulin level estimation**

The insulin level was estimated from the clear serum separated from the fasting whole blood sample by fully automated radioimmunoassay system. The value recorded is in μIU/ml.

**Statistical analysis**

The results were entered in a computer database and statistically analyzed using Microsoft Excel Spreadsheet Program and student’s 't' test and p value determined.

**Observations and results**

Table 1 shows the distribution of the normal control group and the diabetic groups of people with different durations of the disease. Out of the total subjects investigated, 39 (51.3%) were males and 37 (48.68%) were females. The control group included 13 (33.33%) males and 17 (45.94%), females while the number of males and females in the study group were 26 (56.52%) and 20 (43.48%), respectively. Of the total 76 cases studied, the control cases numbered 30 (39.5%) and the total number of study subjects numbered 46 (60.5%).

Table 2 shows the status of mean ± standard deviation of fasting insulin level, HbA1c, fasting and postprandial blood glucose in the males and females of the study and control group.

Table 3 shows the correlation between the duration of diabetes with the various parameters, like HbA1c, fasting insulin level, FBG and PPBG.

- The clinical and biochemical findings of this study can be summarized as follows:
  1. Insulin level increases with the duration of diabetes though the increase is found to be within the normal limit. The insulin level shows a very significant correlation with the duration of diabetes.
  2. Type 2 DM occurs more frequently in females at a more advanced age as compared to the males as was found in the study group.
  3. The fasting and postprandial blood glucose also showed a very significant increase with the duration of diabetes.

**Discussion**

Type 2 DM is a chronic disease of epidemic proportions and is one of the major challenges to public health. India has the dubious distinction of being home to the largest number of people suffering from diabetes in any country. In theory, treating diabetes should be simple, just prevent hyperglycemia from causing damage to organs and not allow hypoglycemia to cause coma as energy supply to brain fails. In practice, it does not work that way. Glucose fluctuations occur all the time and one effective way is to monitor the HbA1c, which gives the average blood glucose level of the preceding 2-3 months. In a study of 178 Libyan men it was found that the patients having a poorly controlled diabetes showed a significant correlation between HbA1c and duration of diabetes. Glycosylated hemoglobin will be a valuable adjunct to blood glucose determinations in epidemiological studies. In another study of 500 diabetic patients it was found that in the group of patients with HbA1c > 8%, there was a significant relation to the duration of diabetes. Various studies prove that the amount of carbohydrate attached to the HbA1c increases with increasing duration of the disease.

Normal levels of insulin are healthy and necessary. But too much of a good thing, in this case, insulin can be deadly. To be healthy our body needs to...
produce the right amount of insulin and respond to the insulin appropriately. A confounding factor is that hyperglycemia and hyperinsulinemia in themselves can impair insulin secretion and insulin sensitivity\textsuperscript{16-18}. The body becomes more resistant to insulin with increasing duration of diabetes, so that insulin level is high or normal in the body but the available insulin is insufficient\textsuperscript{19}. As recently pointed out in a study, because of the feedback between glucose concentration (the major stimulus for insulin release) and \(\beta\)-cell insulin secretion, it is virtually impossible to develop diabetes due to severity of insulin resistance found in most type 2 diabetic patients unless the capacity to secrete additional amounts of insulin to compensate for the insulin resistance is impaired\textsuperscript{20}. The present study shows that females suffer from diabetes at an older age as compared to males. Various other studies also proves that the disease shows a little gender preference, although diabetes becomes slightly more frequent in women with advancing age\textsuperscript{21}. Females have estrogen hormone which is protective for developing diabetes\textsuperscript{22}, estrogen makes the body cells more receptive or sensitive to insulin. Estrogen seems to contribute to glucose homeostasis in women\textsuperscript{23}. Poor glycemic control and age-related pathology with duration of diabetes are thought to accelerate degenerative changes in a cooperative manner\textsuperscript{24-26}. The correlation analysis carried out in another study suggests that the variables like sex, age at onset of disease, duration of diabetes and age of patients influence glycemia directly and HbA\(_1c\) indirectly\textsuperscript{27}.

References

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5. Trivelli LA, Ranney HM and Lai...


Effect of Oral Administration of Antioxidant Vitamins on Insulin Sensitivity and Lipid Profile in Obese Adults with Type 2 Diabetes Mellitus

Abstract

Aims and objects: To find out the effect of oral administration of antioxidant vitamins on lipid profile and insulin sensitivity in obese patients with recently diagnosed type 2 diabetes mellitus.

Materials and methods: Forty-five adult obese with recently diagnosed type 2 diabetes mellitus and 20 (sex, age, weight and match) control subjects were examined for lipid profile, blood glucose and insulin sensitivity.

Result: Result show significant reduction in total cholesterol, triglyceride, LDL, VLDL and blood glucose significant increase in HDL, slight significant increase in insulin sensitivity by taking antioxidant therapy.

Conclusion: Antioxidant vitamin definitely reduces oxidative stress and help in improving lipid profile blood glucose and possibly increased insulin sensitivity.

Introduction

Globally, diabetes mellitus is assuming an epidemic proportion due to increase in the population and increased life span. In India, the prevalence of the disease especially of type 2 diabetes mellitus in above 40 years old obese persons is very high.

In type 2 diabetes mellitus, hyperinsulinemia occurs due to β-cell exhaustion, which leads to glucose intolerance. It is well proven that patient with upper body obesity (android obesity) have increased lipolytic activity leading to increased levels of free-fatty acids in the portal system which in turn leads to disturbances in various metabolic process in the liver, like lipid metabolism, insulin extraction and gluconeogenesis.

In this study, antioxidant vitamin therapy in obese adult with recently diagnosed type 2 diabetes mellitus patients showed beneficial effect in improving lipid profile, blood glucose level and insulin sensitivity.
2 contributing in obese adult type 2 diabetes mellitus are insulin resistance, hyperlipidemia and hyperglycemia, and these factors increase chances of vascular diseases by many folds. In type 2 diabetes mellitus, the following changes are involved in lipid profile:

- Increased triglyceride levels with increased VLDL production and decreased clearance of VLDL and defect in apoprotein.
- Increased cholesterol with primary genetic defect along with insulin resistance.
- Increased LDL synthesis and turnover.
- On enzymatic glycolysis of the LDL-receptor altering the receptor binding site and increased LDL, VLDL, TG with decreased or normal HDL.

Recent studies have shown the benefits of antioxidants in the prevention of diabetes and its complications. Ascorbic acid, α-tocoferol and β-carotene are natural antioxidants having the quality of helping the body to use glucose and enhancing the synthesis of glutathione which is a major antioxidant within our cells responsible for mopping up all type of toxins and free radicals. In diabetes, many of the complications have been attributed to oxidative stress or increased free radical formation. The antioxidant vitamins, e.g., vitamin A (β-carotene), vitamin C (ascorbic acid) and vitamin E (α-tocoferol) have been shown to have a protective effect against oxidative stress. Antioxidant vitamin therapy improves serum lipid profile and insulin sensitivity, by decreasing lipid peroxidation and suppressing free radical chain reaction and protect the individual against cardiovascular events.

Materials and methods

This prospective study was conducted in 45 recently diagnosed obese patients with type 2 diabetes mellitus coming to the diabetic clinic and medicine OPD wards of Maharaja Yashwantrao Rano Hospital and M.G.M. Medical College, Indore (M.P.), between 2001 to 2002. The mean age of the patients was 51.2 ± 7.2 years in males and 48.2 ± 6.8 in females and the duration of type 2 diabetes mellitus was 0-1 year.

Exclusion criteria

a) Type 2 diabetes mellitus patients of more than 1 year.
b) Associated cardiac and respiratory disease.
c) Associated hypertension, cancer and renal failure.
d) Subjects with history of smoking, alcoholism or oral contraceptive use were also excluded.
e) Subjects taking any type of drugs, specially lipid lowering.

The study subjects had a BMI of 30 or more. The lipid profile, glucose levels and insulin sensitivity were estimated and compared with control. Then, a daily dose of 1,000 mg vitamin C, 800 IU vitamin E and 25,000 IU vitamin A, was given to every study subject continuously for 6 months along with normal but low fat vegetarian diet.

After every 2 months, the lipid profile, glucose and insulin sensitivity of study group were checked. The serum total cholesterol, HDL-cholesterol and triglyceride were estimated by enzymatic method, LDL and VLDL were calculated by formula while glucose was estimated by glucose oxidase method. Insulin sensitivity was estimated by the chemiluminescence method.

Statistical analysis of the results were calculated and results were denoted by mean, standard deviation, student 't' test and p values.

Results and observations

Mean values of studied biochemical parameters in control subjects are shown in Table 1.

The mean age of study group subjects was 51.2 years in males and 48.2 years in females.

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>172 ± 17.2</td>
</tr>
<tr>
<td>HDL</td>
<td>53.4 ± 7.4</td>
</tr>
<tr>
<td>TG</td>
<td>126.2 ± 16.5</td>
</tr>
<tr>
<td>VLDL</td>
<td>26.3 ± 7.0</td>
</tr>
<tr>
<td>LDL</td>
<td>102.6 ± 22.7</td>
</tr>
<tr>
<td>Glucose (F)</td>
<td>98 ± 8.8</td>
</tr>
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</table>
The mean BMI of study subjects was 37.6 kg/m².

Mean values of studied serum biochemical parameters in study subject before and after antioxidant vitamin therapy are shown in Table 3.

The mean value of studied blood biochemical parameters in studied subjects before antioxidant therapy were as follows: Triglyceride 275.3 ± 57 mg/dl; cholesterol 278 ± 33.9 mg/dl; HDL 39.2 ± 8.4 mg/dl; VLDL 51.9 ± 7.3 mg/dl; LDL 142 ± 43.7 mg/dl; glucose (F) 158 ± 44.2 mg/dl while after 6 months of antioxidant therapy the levels were as follows: Triglyceride 202.4 ± 59.2 mg/dl; cholesterol 220.8 ± 35.2 mg/dl; HDL 48.2 ± 10.2 mg/dl; VLDL 32.2 ± 7.2 mg/dl; LDL 112.3 ± 45.2 mg/dl and glucose (F) 132.8 mg/dl.

The study shows significant changes in studied blood biochemical parameter after 6 months of antioxidant therapy. There was significant (p < 0.001) decrease in cholesterol with 57.2 mg/dl, triglyceride 72.9 mg/dl, VLDL 19.7 mg/dl, LDL 29.7 mg/dl, glucose 35.2 mg/dl and increase in HDL level was 9.6 mg/dl, after 6-month of antioxidant therapy. In addition, a slight increase in insulin sensitivity was seen.

Discussion

According to Mishra, et al., obesity causes increased lipolytic activity of lipocytes leading to increased free fatty acids, which in turn, lead to increased free triglycerides and total cholesterol. In obese individuals, increase in the level of free fatty acids result in increase apo-β-100 secretion by the liver. The long-chain free fatty acids would divert apo-β away from degradation in the endoplasmic reticulum and towards secretion. This would explain the increased level of VLDL and LDL.

According to Manocha, et al., obesity is associated with low HDL because increase in level of triglycerides increases transfer of triglyceride particle from HDL and this leads to decrease in HDL levels.

According to Mishra, et al., in adults, obesity increases free fatty acids which leads to insulin resistance.

The present study showed percentage of obese adult females with type 2 diabetes mellitus to be 60% and obese males with type 2 diabetes mellitus to be 40%. Clearly mean percentage of obese female patients were more than male patients. According to Arone, et al., postmenopausal women have low level of estrogen which causes insulin resistance and hyperlipidemia.

The mean age of study group was 51.2 years in males and 48.2 years in females. Type 2 diabetes mellitus is more prevalent in adult persons with age above 45.

After 2 and 4 months of antioxidant therapy, the changes in studied blood biochemical parameter was not significant (p > 0.05), however, after 6 months of antioxidant therapy, there was significant (p < 0.001) decrease in triglycerides, total cholesterol, LDL, VLDL, glucose as well as a significant increase (p < 0.05) in HDL and insulin sensitivity was seen.

According to Shah, oxidative stress and free radicals have been reported to be responsible for various complications of diabetes, and hyperlipidemia is one of them. The results of this study are supported by Stane, et al. They have reported antioxidant therapy to have a protective effect against hyperlipidemia. Shah study concluded that antioxidants have the quality of helping the body to use glucose by increasing insulin sensitivity. William, et al. reported that antioxidants did not modify the insulin sensitivity. According to Martinez-Abundis, et al., antioxidant vitamins have
Table 3
Mean values of studied serum biochemical parameters in study subject before and after antioxidant vitamin therapy

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Before antioxidant therapy</th>
<th>2 months after antioxidant therapy</th>
<th>4 months</th>
<th>6 months</th>
<th>Increase or decrease after 6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mg/dl)</td>
<td>278 ± 33.9</td>
<td>272.4 ± 34.8</td>
<td>260.2 ± 32.1</td>
<td>220.8 ± 35.2</td>
<td>-57.2</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>39.2 ± 8.4</td>
<td>39.2 ± 7.2</td>
<td>42.8 ± 9.1</td>
<td>46.8 ± 10.2</td>
<td>+9.6</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>275.3 ± 57</td>
<td>262 ± 58.2</td>
<td>240 ± 52.8</td>
<td>202.4 ± 59.2</td>
<td>-72.9</td>
</tr>
<tr>
<td>VLDL</td>
<td>51.9 ± 7.3</td>
<td>49.2 ± 6.4</td>
<td>39.9 ± 7.0</td>
<td>32.2 ± 7.2</td>
<td>-19.7</td>
</tr>
<tr>
<td>LDL</td>
<td>142 ± 43.7</td>
<td>139 ± 44.1</td>
<td>130.4 ± 40.2</td>
<td>112.3 ± 45.1</td>
<td>-29.7</td>
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<tr>
<td>Glucose (F)</td>
<td>158.9 ± 44.2</td>
<td>150 ± 48.2</td>
<td>142.2 ± 40.2</td>
<td>132.8 ± 48.2</td>
<td>-35.2</td>
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</tbody>
</table>

protective effects against oxidative stress. Antioxidant vitamin therapy improves serum lipid and insulin sensitivity by decreasing lipid peroxidation and suppressing free radical chain reactions. According to Sigel, et al., antioxidant vitamins have metal chelating activity. They scavenge hydroxyl radicals, singlet oxygen and may chelate transition metals.

Conclusion
The incidence of type 2 diabetes mellitus is very high in obese adult individuals. In this condition, hyperlipidemia is very common. Majority of the obese adult with type 2 diabetes mellitus have hypertension and are at higher risk of future myocardial infarction and other vascular complications.

It is very important to correct the lipid levels in obese type 2 diabetes mellitus patients. Recently, researchers highlighted role of antioxidant therapy in prevention of recently diagnosed type 2 diabetes mellitus and its complications. The emerging scenario in our region also emphasizes that the treating physician be aware of the importance of antioxidant in recently diagnosed type 2 diabetes mellitus patients.

As we mentioned earlier, our study subjects were not taking any type of drugs and they were only on antioxidant vitamin therapy with a balanced vegetarian diet, it may be inferred that all the changes are due to antioxidant vitamin therapy.

Therefore, we conclude that antioxidant vitamin therapy in obese adult with recently diagnosed type 2 diabetes mellitus patients showed beneficial effect in improving lipid profile, glucose level and insulin sensitivity, and protect the individual from future myocardial infarction and other complications.

References


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contributing in obese adult type 2 diabetes mellitus are insulin resistance, hyperlipidemia and hyperglycemia, and these factors increase chances of vascular diseases by many folds. In type 2 diabetes mellitus, the following changes are involved in lipid profile:

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Recent studies have shown the benefits of antioxidants in the prevention of diabetes and its complications. Ascorbic acid, α-tocopherol and β-carotene are natural antioxidants having the quality of helping the body to use glucose and enhancing the synthesis of glutathione which is a major antioxidant within our cells responsible for mopping up all type of toxins and free radicals. In diabetes, many of the complications have been attributed to oxidative stress or increased free radical formation. The antioxidant vitamins, e.g., vitamin A (β-carotene), vitamin C (ascorbic acid) and vitamin E (α-tocopherol) have been shown to have a protective effect against oxidative stress. Antioxidant vitamin therapy improves serum lipid profile and insulin sensitivity, by decreasing lipid peroxidation and suppressing free radical chain reaction and protect the individual against cardiovascular events.

Materials and methods

This prospective study was conducted in 45 recently diagnosed obese patients with type 2 diabetes mellitus coming to the diabetic clinic and medicine OPD wards of Maharaja Yashwant Rao Hospital and M.G.M. Medical College, Indore (M.P.), between 2001 to 2002. The mean age of the patients were 51.2 ± 7.2 years in males and 48.2 ± 6.8 in females and the duration of type 2 diabetes mellitus was 0-1 year.

Exclusion criteria

a) Type 2 diabetes mellitus patients of more than 1 year.

b) Associated cardiac and respiratory disease.

c) Associated hypertension, cancer and renal failure.

d) Subjects with history of smoking, alcoholism or oral contraceptive use were also excluded.

e) Subjects taking any type of drugs, specially lipid lowering.

The study subjects had a BMI of 30 or more. The lipid profile, glucose levels and insulin sensitivity were estimated and compared with control. Then, a daily dose of 1,000 mg vitamin C, 800 IU vitamin E and 25,000 IU vitamin A, was given to every study subject continuously for 6 months along with normal but low fat vegetarian diet.

After every 3 months, the lipid profile, glucose and insulin sensitivity of study group were checked. The serum total cholesterol, HDL-cholesterol and triglyceride were estimated by enzymatic method and LDL were calculated by formula while glucose was estimated by glucose oxidase method. Insulin sensitivity was estimated by the chemiluminescence method.

Statistical analysis of the results were calculated and results were denoted by mean, standard deviation, student 't' test and p values.

Results and observations

Mean values of studied biochemical parameters in control subjects are shown in Table 1.

The mean age of study group subjects was 51.2 years in males and 48.2 years in females.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>172 ± 17.2</td>
</tr>
<tr>
<td>HDL</td>
<td>53.4 ± 7.4</td>
</tr>
<tr>
<td>TG</td>
<td>126.2 ± 16.5</td>
</tr>
<tr>
<td>VLDL</td>
<td>26.3 ± 7.0</td>
</tr>
<tr>
<td>LDL</td>
<td>102.6 ± 22.7</td>
</tr>
<tr>
<td>Glucose (F)</td>
<td>98 ± 8.8</td>
</tr>
</tbody>
</table>

Table 1

Mean values of studied biochemical parameters in control subjects
(Table 2). The mean BMI of study subjects was 37.6 kg/m².

Mean values of studied serum biochemical parameters in study subject before and after antioxidant vitamin therapy are shown in Table 3.

The mean value of studied blood biochemical parameters in studied subjects before antioxidant therapy were as follows: Triglyceride 275.3 ± 57 mg/dl; cholesterol 278 ± 33.9 mg/dl; HDL 39.2 ± 8.4 mg/dl; VLDL 51.9 ± 7.3 mg/dl; LDL 142 ± 43.7 mg/dl; glucose (F) 158 ± 44.2 mg/dl while after 6 months of antioxidant therapy the levels were as follows: Triglyceride 202.4 ± 59.2 mg/dl; cholesterol 220.8 ± 35.2 mg/dl; HDL 48.2 ± 10.2 mg/dl; VLDL 32.2 ± 7.2 mg/dl; LDL 112.3 ± 45.2 mg/dl and glucose (F) 132.8 mg/dl.

The study shows significant changes in studied blood biochemical parameter after 6 months of antioxidant therapy. There was significant (p < 0.001) decrease in cholesterol with 57.2 mg/dl, triglyceride 72.9 mg/dl, VLDL 19.7 mg/dl, LDL 29.7 mg/dl, glucose 35.2 mg/dl and increase in HDL level was 9.6 mg/dl, after 6-month of antioxidant therapy. In addition, a slight increase in insulin sensitivity was seen.

**Discussion**

According to Mishra, et al. obesity causes increased lipolytic activity of lipocytes leading to increased free-fatty acids, which in turn, lead to increased free triglycerides and total cholesterol. In obese individuals, increase in the level of free-fatty acids result in increase apo-β-100 secretion by the liver. The long-chain free-fatty acids would divert apo-β away from degradation in the endoplasmic reticulum and towards secretion. This would explain the increased level of VLDL and LDL.

According to Manocha, et al. obesity is associated with low HDL because increase in level of triglycerides increases transfer of triglyceride particle from HDL and this leads to decrease in HDL levels.

According to Mishra, et al. in adults, obesity increases free-fatty acids which leads to insulin resistance.

The present study showed percentage of obese adult females with type 2 diabetes mellitus to be 60% and obese males with type 2 diabetes mellitus to be 40%. Clearly mean percentage of obese female patients were more than male patients. According to Arone, et al. postmenopausal women have low level of estrogen which causes insulin resistance and hyperlipidemia.

The mean age of study group was 51.2 years in males and 48.2 years in females. Type 2 diabetes mellitus is more prevalent in adult persons with age above 45.

After 2 and 4 months of antioxidant therapy, the changes in studied blood biochemical parameter was not significant (p > 0.05), however, after 6 months of antioxidant therapy, there was significant (p < 0.001) decrease in triglycerides, total cholesterol, LDL, VLDL, glucose as well as a significant increase (p < 0.05) in HDL and insulin sensitivity was seen.

According to Shah, oxidative stress and free radicals have been reported to be responsible for various complications of diabetes, and hyperlipidemia is one of them. The results of this study are supported by Stane, et al. They have reported antioxidant therapy to have a protective effect against hyperlipidemia. Shah study concluded that antioxidants have the quality of helping the body to use glucose by increasing insulin sensitivity. William, et al. reported that antioxidants did not modify the insulin sensitivity. According to Martinaz-Abundis, et al., antioxidant vitamins have
Table 3

Mean values of studied serum biochemical parameters in study subject before and after antioxidant vitamin therapy

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Before antioxidant therapy</th>
<th>2 months after antioxidant therapy</th>
<th>4 months</th>
<th>6 months</th>
<th>Increase or decrease after 6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mg/dl)</td>
<td>278 ± 33.9</td>
<td>272.4 ± 34.8</td>
<td>260.2 ± 32.1</td>
<td>220.8 ± 35.2</td>
<td>-57.2</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>39.2 ± 8.4</td>
<td>39.2 ± 7.2</td>
<td>42.8 ± 9.1</td>
<td>48.8 ± 10.2</td>
<td>+9.6</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>275.3 ± 57</td>
<td>262 ± 58.2</td>
<td>240 ± 52.8</td>
<td>202.4 ± 59.2</td>
<td>-72.9</td>
</tr>
<tr>
<td>VLDL</td>
<td>51.9 ± 7.3</td>
<td>49.2 ± 6.4</td>
<td>39.9 ± 7.0</td>
<td>32.2 ± 7.2</td>
<td>-19.7</td>
</tr>
<tr>
<td>LDL</td>
<td>142 ± 43.7</td>
<td>139 ± 44.1</td>
<td>130.4 ± 40.2</td>
<td>112.3 ± 45.1</td>
<td>-29.7</td>
</tr>
<tr>
<td>Glucose (F)</td>
<td>158.9 ± 44.2</td>
<td>150 ± 48.2</td>
<td>142.2 ± 40.2</td>
<td>132.8 ± 48.2</td>
<td>-35.2</td>
</tr>
</tbody>
</table>

The protective effects against oxidative stress. Antioxidant vitamin therapy improves serum lipid and insulin sensitivity by decreasing lipid peroxidation and suppressing free radical chain reactions. According to Sigel, et al.16, antioxidant vitamins have metal chelating activity. They scavenge hydroxyl radicals, singlet oxygen and may chelate transition metals.

**Conclusion**

The incidence of type 2 diabetes mellitus is very high in obese adult individuals. In this condition, hyperlipidemia is very common. Majority of the obese adult with type 2 diabetes mellitus have hypertension and are at higher risk of future myocardial infarction and other vascular complications.

It is very important to correct the lipid levels in obese type 2 diabetes mellitus patients. Recently, researchers highlighted role of antioxidant therapy in prevention of recently diagnosed type 2 diabetes mellitus and its complications. The emerging scenario in our region also emphasizes that the treating physician be aware of the importance of antioxidant in recently diagnosed type 2 diabetes mellitus patients.

As we mentioned earlier, our study subjects were not taking any type of drugs and they were only on antioxidant vitamin therapy with a balanced vegetarian diet, it may be inferred that all the changes are due to antioxidant vitamin therapy.

Therefore, we conclude that antioxidant vitamin therapy in obese adult with recently diagnosed type 2 diabetes mellitus patients showed beneficial effect in improving lipid profile, glucose level and insulin sensitivity, and protect the individual from future myocardial infarction and other complications.

**References**


Correlative Study of Duration of Type 2 Diabetes Mellitus with Glycated Hemoglobin, Insulin Resistance and Blood Glucose

Abstract

The present study was carried out to determine the correlation, if any between insulin resistance, blood glucose levels, glycated hemoglobin and duration of type 2 diabetes mellitus (DM).

The study comprised a total of 76 subjects out of which 30 were normal, non-diabetic persons and the rest 46 were diabetics with different duration of time in years, after being diagnosed diabetic. Data was analyzed after dividing the subjects into 4 groups: Group 1, comprised of 1 year old diabetics, Group 2 was made up of those, who had diabetes, for the past 2-5 years, Group 3 included patients who were diabetic since more than 5 years and Group 4 included non-diabetics as the normal control group. The results obtained indicated that the HbA1c levels showed a significant increase with the duration of diabetes as well as the insulin level showed a significant correlation after adjustment for age, sex and duration of diabetes.

Introduction

Diabetes mellitus is a lifelong disease, which makes many people worry about the quality and longevity of their life after diagnoses. The complications of diabetes are influenced not only by the duration of diabetes but also by the average level of chronic glycemia, which is measured most reliably with glycated hemoglobin assay. In normoglycemic subjects, a small proportion of hemoglobin A is attached to a carbohydrate moiety thus, creating what is called glycated hemoglobin. In conditions of sustained hyperglycemia, such as in diabetes mellitus, the proportion of hemoglobin that is glycated is increased substantially. Studies conducted by Arnetz, et al. and Kilpatrick, et al., in diabetic patients have shown a significant positive correlation between HbA1c and age as well as duration of diabetes. In contradiction to this, Kabadi found no significant relationship between age, duration
of diabetes and fasting blood glucose (FBG), glycosylated hemoglobin, glycated protein or glycated albumin. According to the results of many longitudinal and cross-sectional studies, it has been demonstrated that the earliest detectable abnormality in type 2 DM is an impairment in the body’s ability to respond to insulin. Studies have shown that insulin sensitivity correlated inversely with fasting insulin and the insulin levels increased with the duration of diabetes.

Though such detailed investigations have been carried out in different parts of the world to prove a correlation between the different parameters, the results were contradictory, blurring the diagnostic significance of these parameters. It was thought worthwhile to investigate the significance of such correlations in the Indian diaspora, where such a biochemical equation on the effect of these parameters for the progression of the diabetic sequelae have not yet been postulated. The aim of this study was to evaluate the correlation between the above detailed parameters so that they can be used as diagnostic or prognostic markers for the assessment of the degree of control of this lifestyle disease, to delay or prevent the multifaceted complications.

Materials and methods

The study was conducted in the Department of Biochemistry and Clinical Biochemistry of M.G.M. Medical College and OPD of M.Y. Hospital, Indore.

Subjects

The clinical material for the present study comprised of a total of 76 subjects. Three groups were formed on the basis of difference in duration of diabetes. The fourth group comprised of subjects who were normal and non-diabetic, as a control group. Data on therapy of diabetes, HbA₁c values, FBG and postprandial blood glucose (PPBG) was obtained by structured questionnaires and by clinical and laboratory assessments. Insulin-sensitive subjects were defined as having an insulin-sensitivity estimate as per the median in non-diabetic subjects participating in the study. Using this definition 88% of all the type 2 diabetics were insulin resistant. The study subjects were established diabetics.

Group 1: One-year duration of diabetes

These subjects had been diagnosed with diabetes only one year before.

Group 2: Two to five years duration of diabetes

This group of patients was diabetic since the last two to five years.

Group 3: More than five-years duration of diabetes

This group of patients was diagnosed with diabetes for more than five years. The maximum duration in this group was found to be of a patient with a diabetic history of 22 years.

Group 4: Normal subjects

These individuals were screened for the presence of diabetes based on the diagnostic criteria of the American Diabetes Association (ADA). They were found to be normal, healthy individuals without any prior family history of diabetes. The exclusion criteria also included hypertension, use of alcohol or cigarettes and other factors affecting blood sugar levels.

Collection of material:

Blood

In all the above groups, 5 ml whole blood was collected in the fasting state. 0.5 ml whole blood was mixed with EDTA regent (anticoagulant) and kept for HbA₁c estimation. The remaining blood is kept at room temperature for 1 hour after which the supernatant clear fluid was pipetted out into another tube. This tube was then centrifuged for 10 minutes. The clear serum was pipetted into a clean dry test tube and used for estimation of blood sugar and insulin. Similarly, 0.5 ml blood was collected from the subjects 2 hours after having food for the estimation of postprandial blood sugar, along with the urine sample.

Clinical method

- HbA₁c estimation

For the estimation of HbA₁c, 10 μl of the whole blood + EDTA reagent, was mixed with 1 ml HbA₁c reagent and direct reading taken on the auto-analyzer (Selectra E). The value recorded is in percent.

- FBG and PPBG estimation

Ten microlitres of the clear serum is mixed with 1 ml glucose reagent and incubated for 10 minutes at 37°C.
Clinical Study

Direct reading was taken on the auto-analyzer (Selectra E). The value recorded is in mg%. The same process was repeated for the estimation of PPBG with the postprandial blood sample.

- Insulin level estimation
  The insulin level was estimated from the clear serum separated from the fasting whole blood sample by fully automated radioimmunoassay system. The value recorded is in μIU/ml.

Statistical analysis

The results were entered in a computer database and statistically analyzed using Microsoft Excel Spreadsheet Program and student’s ‘t’ test and p value determined.

Observations and results

Table 1 shows the distribution of the normal control group and the diabetic groups of people with different durations of the disease. Out of the total subjects investigated, 39 (51.3%) were males and 37 (48.68%) were females. The control group included 13 (33.33%) males and 17 (45.94%), females while the number of males and females in the study group were 26 (56.52%) and 20 (43.48%), respectively. Of the total 76 cases studied, the control cases numbered 30 (39.5%) and the total number of study subjects numbered 46 (60.5%).

Table 2 shows the status of mean ± standard deviation of fasting insulin level, HbA1c, fasting and postprandial blood glucose in the males and females of the study and control group.

Table 3 shows the correlation between the duration of diabetes with the various parameters, like HbA1c, fasting insulin level, FPG and PPBG.

The clinical and biochemical findings of this study can be summarized as follows:

1. Insulin level increases with the duration of diabetes though the increase is found to be within the normal limit. The insulin level shows a very significant correlation with the duration of diabetes.
2. Type 2 DM occurs more frequently in females at a more advanced age as compared to the males as was found in the study group.
3. The fasting and postprandial blood glucose also showed a very significant increase with the duration of diabetes.

Discussion

Type 2 DM is a chronic disease of epidemic proportions and is one of the major challenges to public health. India has the dubious distinction of being home to the largest number of people suffering from diabetes in any country. In theory, treating diabetes should be simple, just prevent hyperglycemia from causing damage to organs and not allow hypoglycemia to cause coma as energy supply to brain fails. In practice, it does not work that way. Glucose fluctuations occur all the time and one effective way is to monitor the HbA1c, which gives the average blood glucose level of the preceding 2-3 months. In a study of 178 Libyan men it was found that the patients having a poorly controlled diabetes showed a significant correlation between HbA1c and duration of diabetes. Glycosylated hemoglobin will be a valuable adjunct to blood glucose determinations in epidemiological studies. In another study of 500 diabetic patients it was found that in the group of patients with HbA1c >8%, there was a significant relation to the duration of diabetes. Various studies prove that the amount of carbohydrate attached to the HbA1c increases with increasing duration of the disease.

Normal levels of insulin are healthy and necessary. But too much of a good thing, in this case, insulin can be deadly. To be healthy our body needs to

<table>
<thead>
<tr>
<th>Analysis of the 76 cases under study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
</tr>
<tr>
<td>--------------------------------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Age (yr) 54.4 ± 12</td>
</tr>
<tr>
<td>Duration of diabetes 2-5 yrs</td>
</tr>
<tr>
<td>Duration of diabetes More than 5 yrs</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

n = number of subjects (sample size).
produce the right amount of insulin and respond to the insulin appropriately. A confounding factor is that hyperglycemia and hyperinsulinemia in themselves can impair insulin secretion and insulin sensitivity. The body becomes more resistant to insulin with increasing duration of diabetes, so that insulin level is high or normal in the body but the available insulin is insufficient. As recently pointed out in a study, because of the feedback between glucose concentration (the major stimulus for insulin release) and β-cell insulin secretion, it is virtually impossible to develop diabetes due to severity of insulin resistance found in most type 2 diabetic patients unless the capacity to secrete additional amounts of insulin to compensate for the insulin resistance is impaired.

The present study shows that females suffer from diabetes at an older age as compared to males. Various other studies also prove that the disease shows a little gender preference, although diabetes becomes slightly more frequent in women with advancing age. Females have estrogen hormone which is protective for developing diabetes, estrogen makes the body cells more receptive or sensitive to insulin. Estrogen seems to contribute to glucose homeostasis in women.

Poor glycemic control and age-related pathology with duration of diabetes are thought to accelerate degenerative changes in a cooperative manner. The correlation analysis carried out in another study suggests that the variables like sex, age at onset of disease, duration of diabetes and age of patients influence glycemia directly and HbA1c indirectly.

References

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5. Trivelli LA, Ranney HM and Lai...


Status of MDA and Antioxidant enzymes in hyperglycemic postmenopausal women

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**Department of Biochemistry, King George Medical College, Lucknow (U.P.)

Abstract
Diabetes mellitus is a chronic disease associated with serious complications and enhanced oxidation is the underlying abnormality responsible for some of these complications of diabetes. In postmenopause, the ovaries do not produce estrogen, a hormone which has got antioxidant properties and so the antioxidant enzyme system seems to be affected in the postmenopausal women. Therefore, the already imbalanced antioxidant system in the postmenopausal women is unable to effectively counteract the augmented oxidative stress due to hyperglycemia in diabetes mellitus type 2 and this manifests in the patients as a plethora of complications. The study comprised a total of 64 postmenopausal women who had attained menopause for average 5 years. Data regarding malondialdehyde (MDA), antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), glycated hemoglobin (HbA1c) and random blood glucose (RBG) values were analysed in all the subjects, who were divided into two groups. Group 1 comprised of 32 normal, non-diabetic postmenopausal women while Group 2 comprised of 32 postmenopausal women with diabetes mellitus type 2. The results obtained showed increase in plasma MDA indicating increased oxidative stress (OS) in the diabetic postmenopausal women with a corresponding increase in the antioxidant enzymes, SOD and CAT showing oxidative stress in the cells.

Key words: Diabetes mellitus type 2, postmenopausal women, antioxidant enzymes, malondialdehyde.

Introduction
Diabetes mellitus type 2 with an increasing incidence world wide is characterized by an increased risk for the development of neuropathic, micro and macro-vascular complications(1). Menopause in women with diabetes mellitus type 2 compounds the situation by increasing a wide variety of physical and psychological problems. Several experimental, epidemiologic and clinical studies support the notion that oxidative stress plays a significant role in type 2 diabetes mellitus(2) and in the development of coronary vascular diseases(3). Diabetes is associated with more lipid peroxidation via free radical formation(4) and many disease states associated with free radicals are the same as those found with insulin resistance(5).

Free oxygen radicals have been proposed as important causative agents of aging(6) and menopause is a natural step in the process of aging. Hence, postmenopausal women develop oxidative stress because of estrogen deficiency and advancing age(7) accompanied with oxidative stress due to hyperglycemia in postmenopausal women with diabetes mellitus type 2.

The RBC has an effective mechanism to prevent and neutralize the oxidative stress induced damage7. This is accomplished by a set of antioxidant enzymes like CAT, SOD etc. Recently it has been reported that the gene expression of various antioxidant enzymes i.e. SOD, CAT was substantially lower in mouse pancreatic islets than in various other tissues. This fact suggests that pancreatic islets would be more vulnerable to oxidative stress than other tissues. Another paper reported that cytokines might damage islet cells by inducing oxygen free radical generation, lipid peroxidation and consequently the formation of aldehydes such as MDA in the islet cells. The accelerated oxygen radical production can have serious adverse effects on cell membrane protein and lipid resulting in thiol oxidation and lipid peroxidation.

In the midst of the above detailed reviews, it is thought worthwhile to investigate the status of MDA, SOD, CAT and to assess the effect of hyperglycemia on these parameters and compared with those in age matched normal postmenopausal women treated as control.

Material and methods
The study was conducted in the Department of Biochemistry and Clinical Biochemistry of MGM Medical
College and OPD of M.Y. Hospital, Indore.

Clinical material : subjects

The clinical material for the present study comprised of a total of 64 subjects. They were divided into two
groups. The first group comprised of 32 normal
postmenopausal women as control group while the
second group comprised of 32 diabetes mellitus type 2
postmenopausal women. Data on therapy of diabetes,
HbA1c, blood glucose levels, MDA, SOD, CAT and other
parameters was obtained by structured questionnaires
and by clinical and laboratory assessments.

1. Normal subjects (Group 1)

This group comprised of 32 normal, non-diabetic
postmenopausal women without any prior family history
of diabetes. These individuals were screened for the
presence of diabetes based on the diagnostic criteria of
the ADA12.

2. Hyperglycemic postmenopausal women (Group 2)

This group comprised of 32 postmenopausal
diabetes mellitus type 2 women. Blood glucose was
controlled by balanced diet and exercise. All the patients
had normal hepatic and kidney functions. None of the
subjects had received hormone replacement therapy or
any supportive treatment for menopausal symptoms for
at least 6-8 months prior to the study. The exclusion
criteria also included other factors affecting blood sugar
level.

Collection of material : blood

From all the above groups 5ml whole blood was
collected along with 24 hrs. urine sample. 0.5ml whole
blood was mixed with EDTA reagent (anticoagulant) and
kept for the estimation of HbA1c. The remaining whole
blood is kept at room temperature for 1 hour after which
the supernatant clear fluid is pipetted out into another
tube and the sample is used for estimation of blood
glucose. The blood samples were also analysed for plasma
lipid peroxidation (MDA) and antioxidant enzymes like
superoxide dismutase and catalase.

Clinical-method : estimation of HbA1c, blood sugar,
MDA, SOD and CAT

1. HbA1c estimation

10 microliter of whole blood + EDTA reagent is mixed
with 1ml HbA1c reagent and direct reading is taken
on the auto-analyzer (selectra E) the value recorded is in
percent.

2. Blood glucose estimation

10 microliter of the clear serum is mixed with 1ml
glucose reagent and incubated for 10 min. at 370c, then
direct reading is taken on the autoanalyzer (selectra E).
The value recorded is in milligram percent.

3. MDA, SOD, CAT estimation

The blood sample was also analysed for MDA13,
SOD14 and C15 levels.

Statistical analysis

The statistical analysis was done by student 't' test.
The values were expressed as Mean ± Standard
Deviation (S.D.)

Observation

Table 1 : Physical Analysis of Subjects (n = 64)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n = 32)</th>
<th>Study (n = 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>50 ± 12.5</td>
<td>56 ± 13.6</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>26 ± 9.2</td>
<td>30 ± 8.2</td>
</tr>
<tr>
<td>Systolic B.P. (mm Hg)</td>
<td>125 ± 20.5</td>
<td>138 ± 18.2</td>
</tr>
<tr>
<td>Diastolic B.P. (mm Hg)</td>
<td>82 ± 8.8</td>
<td>92 ± 12.4</td>
</tr>
<tr>
<td>Pulse (per minute)</td>
<td>72 ± 4.4</td>
<td>74 ± 2.2</td>
</tr>
</tbody>
</table>

Table 2 : RBG, HbA1c, MDA, SOD, CAT values in control and Study Groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n = 32)</th>
<th>Study (n = 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBG (mg %)</td>
<td>112± 22</td>
<td>154± 28*</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.2± 0.4</td>
<td>7.8± 0.8*</td>
</tr>
<tr>
<td>MDA (mmol/mL)</td>
<td>5.47± 0.8</td>
<td>8.8± 1.2*</td>
</tr>
<tr>
<td>SOD (U/mg protein/mL)</td>
<td>6.5± 0.632</td>
<td>8.2± 1.7**</td>
</tr>
<tr>
<td>CAT (U/mg protein/mL)</td>
<td>7.0± 0.8</td>
<td>8.6± 1.4*</td>
</tr>
</tbody>
</table>

* P < 0.05 (significant)
** P < 0.001 (very significant)

Results

The biochemical findings of the study can be expressed
in the form of the following results :

1. Diabetic postmenopausal women have high blood
pressure as compared to the postmenopausal
women treated as control, as is evident from table 1.

2. The blood glucose values also showed a significant
increase in the diabetic postmenopausal women
alongwith a corresponding significant increase in the
HbA1c values (table 2).

3. The levels of MDA is significantly increased in the
study group subjects as compared to the control
(table 2).

4. The level of antioxidant enzyme SOD showed a very
significant increase in the diabetic post-menopausal
women (table 2).

5. The level of CAT showed a significant increase in the
study group subjects (table 2).

Discussion

The multi-faceted toxic effects that high sugar level
inflicts through out the body has been profusely
studied, with many studies showing that sugar
damages cells via multiple mechanism and is a
causative factor in common diseases of aging16-19.
Menopausal phase, an important physiological
phenomenon in a woman's life, also is a natural step in
the process of aging. The deficiency of estrogen in
postmenopausal women develops oxidative stress.
Due to release of free radical or reactive oxygen
especies and becomes the cause of various
pathologies like development of hypertension, as is
evident from table 1. Estrogen is a powerful
antioxidant, which prevents lipid peroxidation and
change in lipid profile7.
Higher blood glucose also causes increased oxidative stress. The blood sugar levels were increased in the study group subjects (table 2). Accelerated generation of reactive oxygen species has been shown to occur in diabetes mellitus in association with hyperglycemia\(^{20,21}\). Lipid peroxidation products impair insulin secretion induced by glucose probably through affecting both the glycolytic pathway and citric acid cycle\(^9\). As is evident from table 2, the HbA1c levels were also increased in the study group subjects. Due to hyperglycemia increase in non-enzymatic glycation occurs accompanied with glucose oxidation and these reactions are catalysed by Cu\(^{2+}\) and Fe\(^{2+}\) resulting in formation of oxime and hydroxide radicals which further accelerates the risk of cardiac diseases\(^{22}\).

Increased MDA levels in patients of study group compared with healthy controls suggest increased systemic oxidative stress\(^{23}\). MDA levels are clinical indicators for an oxidative process linked to diabetes mellitus type 2, especially in women\(^{24}\). The reaction of free radicals with membrane lipids leads to the formation of lipid peroxidation products like MDA\(^{9}\). In postmenopausal women aging has been associated to a more pro-oxidant lipid profile\(^{25}\).

In the diabetic postmenopausal women the level of SOD is very significantly increased as compared to control (table 2). This finding is consistent with the earlier finding of Turk HM\(^{26}\) et al for the level of SOD. Superoxide dismutase is irreversibly inactivated by its product H\(^{2}\)O\(^2\) because exposure of intact erythrocyte to H\(^{2}\)O\(^2\) resulted in inactivation of endogenous SOD activity in the concentration dependent manner. Catalase activity is enhanced in the red blood cells taking care of the disposal of H\(^{2}\)O\(^2\) in the cells\(^{27}\). This finding is supported by the earlier studies of Att\(^{28}\) et al, Sozmen \(^{29}\) et al and Kesavulu MM\(^{30}\) et al for the status of CAT.

It is therefore concluded that the oxidative stress due to estrogen deficiency as well as the oxidative stress due to hyperglycemia further compromises the already imbalanced antioxidant system in the postmenopausal diabetic women and predisposes their cells to potential oxidative injury and paves the way for development of associated complications.

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(23)
ABO Rh Blood Group and HbA1c: A Retrospective Study in The Urban NIDDM Population of Indore

This retrospective study to a certain extent is not supported by the above references so this shows that the environment, dietary habits and lifestyle factors affect the study results. Due to a background of high prevalence of Type 2 diabetes, the study of the pattern of distribution of various blood groups within the NIDDM population is of great importance. These frequencies not only influence the use and availability of blood with various groups but also pattern of hemolytic disease of new born and hemolytic transfusion reactions in our population. There is a paucity of literature as far as the relationship between HbA1c, ABO Rh blood groups is concerned. Perusal of this relation shows promise in being a successful approach to the monitoring of diabetic patient and also provides a conceptual frame work for the pathogenesis of secondary sequelae of diabetes.

DR. MEENA VARMA, PREETHA BADI, DR. SANGEETA PANERI

Described and treated since ancient times, diabetes is an incurable chronic disease that affects the body's ability to convert food into energy. NIDDM is a multi-factorial disease caused by both oligo and polygenic factors as well as non-genetic factors that result from a lack of balance between the energy intake and output and other life-style related factors. Type 2 diabetes, by far more common than Type 1, has spared few societies or ethnic groups. Studies in India in the last decade have highlighted that not only is the prevalence of Type 2 diabetes high but that it is increasing within the urban population. The Rh blood group system is one of the most polymorphic and immunogenic systems known in humans. Statistically significant association were found between NIDDM and Rh blood type in a study of 1237 Mexican Americans. In another cross-sectional study of 2312 confirmed diabetics carried out in Bangladesh, the data on statistical analysis showed no association between ABO blood groups and diabetes mellitus. In an American study the world wide blood group distribution was found to be as follows, A positive (28%), B positive (32%), O positive (33%) and AB positive (7%).

These and scores of other studies and investigations carried out in different parts of the world in the varied ethnic groups and diverse populations provide divergent and conflicting reports. The aim of the present study is to investigate the presence of an association between ABO Rh blood groups, HbA1c and NIDDM in the urban population of Indore.

MATERIAL AND METHOD

LOCUS OF STUDY:

This study was conducted in the Department of Biochemistry and Clinical Biochemistry of M.G.M. Medical College and OPD of M.Y. Hospital, Indore.

CLINICAL MATERIAL: SUBJECTS:

The clinical material for the present study comprised of a total of 528 subjects. The study group subjects were established diabetics. These individuals were screened for the presence of diabetes based on the diagnostic criteria of the American Diabetes Association (ADA). Data on therapy of diabetes, HbA1c values, fasting blood glucose (FBG), post-prandial blood glucose (PPBG) and ABO Rh blood group was obtained by structured questionnaires and by clinical and laboratory assessments.

COLLECTION OF MATERIAL: BLOOD:

5-ml whole blood was collected from all subjects in the fasting state. 0.5 ml whole blood is mixed with EDTA reagent (anti-coagulant) and kept for HbA1c estimation. The remaining blood is kept at room temperature for 1 hour, after which the supernatant clear fluid is pipetted out into another tube. This tube is then centrifuged for 10 min., the clear serum is pipetted out into a clean dry tube and used for estimation of blood sugar. Similarly, 0.5 ml blood is collected from the subjects, 2 hours after having food for the estimation of post-prandial blood sugar along with the urine sample.

CLINICAL METHOD: ESTIMATION OF HbA1c, FBG, PPBG, AND ABO Rh BLOOD TypING:

Estimation of HbA1c, FBG and PPBG was done by using commercial kits on fully automated biochemistry analyzer (Selectra-E). ABO Rh(D) Blood grouping is done by Direct Agglutination Test.

STATISTICAL ANALYSIS:

The results were entered in a computer database and
statistically analyzed using Microsoft Excel Sheet program.

OBSERVATION:

Table No. 1
Analysis of 528 subjects under study with respect to their ABO Rh(D) Blood Group.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Blood Group</th>
<th>Rh(D) Typing</th>
<th>Positive</th>
<th>Negative</th>
<th>n=528</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>A</td>
<td>38</td>
<td>6</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>B</td>
<td>190</td>
<td>18</td>
<td>208</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>AB</td>
<td>97</td>
<td>14</td>
<td>101</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>O</td>
<td>165</td>
<td>10</td>
<td>175</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TOTAL</td>
<td>490</td>
<td>38</td>
<td>528</td>
<td></td>
</tr>
</tbody>
</table>

n = number of subjects (sample size)

Table No. 2
Status of mean values of HbA1c, fasting blood glucose and post-prandial blood glucose with respect to the ABO Rh Blood Groups (n=528)

<table>
<thead>
<tr>
<th>S.No</th>
<th>ABO Blood Rh(D)</th>
<th>Mean Blood Fasting Blood Glucose (mg/dL)</th>
<th>Mean Blood Post-Fasting Blood Glucose (mg/dL)</th>
<th>HbA1c(%)</th>
<th>Sugar(mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>A positive</td>
<td>160</td>
<td>240</td>
<td>7.4</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>A negative</td>
<td>138</td>
<td>198</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>B positive</td>
<td>164</td>
<td>248</td>
<td>7.4</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>B negative</td>
<td>152</td>
<td>218</td>
<td>6.2</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>AB positive</td>
<td>166</td>
<td>254</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>AB negative</td>
<td>178</td>
<td>278</td>
<td>7.8</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>O positive</td>
<td>194</td>
<td>292</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>O negative</td>
<td>182</td>
<td>286</td>
<td>8.1</td>
<td></td>
</tr>
</tbody>
</table>

FBG=Fasting Blood Glucose
PPBG=Post-Prandial Blood Glucose
HbA1c=Glycated Hemoglobin

RESULT:
The clinical and biochemical findings obtained from the urban NIDDM population of Indore in this particular study can be translated into the following results:

1. In the group of subjects with Rh positive blood groups, incidence of NIDDM is found to be higher in the B positive group and lower in the A positive group.
2. In the group of subjects with Rh negative blood groups, incidence of NIDDM is found to be higher in the B negative and lowest in the AB negative group.
3. The highest level of fasting blood sugar is found in the O positive patients and corresponding lowest level in the A negative group.
4. The AB positive diabetic patients group had the highest value for the glycated hemoglobin level while the lowest value for the same was found in the B negative diabetic patients' group.

CONCLUSION:
In conclusion, it is very clearly evident from this retrospective study that the highest incidence of NIDDM is found in the subjects with B positive blood group and the lowest incidence of NIDDM is seen in the A positive blood group subjects. The AB positive individuals have a greater tendency to have high values for glycated hemoglobin level. The present study supports the hypothesis that diabetes mellitus type 2 and blood groups are inter-related because of the broad genetic immunologic basis in both.

DISCUSSION:
It is a known fact that it is neither war nor a natural calamity but it is the lifestyle of man which is the largest cause of death and disease in the world today. Man now lives in an environment which is not conducive to his health and performs activities which his body is not conditioned to. The WHO estimates that there were 19.4 million persons with diabetes in India in 1995 and that this number is likely to be 57.2 million in 2025. Quantum of the problem is that the current prevalence rates are 1.1-12% in the urban Indian adult population. In a study of 520 proven cases of adult diabetes mellitus, a strong indication of an association of diabetes mellitus with blood groups especially with A, AB and Rh positive blood groups was found. The maximum differences was seen in the AB groups in the two series and minimum in the A group. 3

In another study of 490 Type 2 diabetes mellitus cases, it was found that the frequency of blood groups B and O is significantly higher and lower, respectively, in the diabetes mellitus Type 2 patients as compared to the general population. 10 A major assumption in the interpretation of HbA1c as a measure of glycem control is that the duration of hemoglobin exposure to glucose does not vary among patients who are hemato logically normal. 11 Since glycated hemoglobin is found inside the red blood cells, the relationship between Rh genetic variability and HbA1c level suggest that RH proteins may influence glucose transport through red cell membrane and/or hemoglobin glycation. 12

References:
Estimation of Serum Lipoproteins and Glucose in Diabetic Offsprings

Diabetes Mellitus has always been regarded primarily as a disturbance of carbohydrate metabolism. It is only in the last few decades that the disturbances in the lipid metabolism have been described in association with the disease. The clinical material for the present study comprised of a total of 60 patients. They were the off springs of diabetic patients attending the diabetic out-door of M.Y. Hospital & M.G.M. medical College, Indore. 20 Individuals were studied as control cases of which 10 were healthy subjects and 10 were diabetic patients. 30 Cases were such whose parents were diabetic. 30 Cases were such whose one of the parent was diabetic. Estimation of Total lipids, lipoproteins, triglycerides, cholesterol and blood sugar was done in these patients. The study revealed that Serum triglycerides, lipoproteins & total lipids were raised in diabetic control cases and were within normal limits in diabetic offsprings.

Dr. Sangita Paneri, Dr. Meena Varma

Diabetes Mellitus has been known to man since time immemorial. It has always been regarded primarily as a disturbance of carbohydrate metabolism. It is only in the last few decades that the disturbances in the lipid metabolism have been described in association with the disease.

It has now been proved that Insulin in also related to lipid metabolism disturbances of insulin will equally derange the lipid metabolism. Secondly, carbohydrate metabolism will lead to alterations in the lipid metabolism 1). The hereditary nature of diabetes mellitus is generally accepted, although the mode of inheritance is controversial. Also, the high prevalence of diabetes in families of diabetic patients is well known 2). In recent years, detail studies have been made in clinics & laboratories all over the world in an attempt to discover some abnormality which would enable one to identify those persons who are labeled prediabetic on genetic grounds & who will in reality develop diabetes later on3,4).

Investigations on prediabetics were begun some years ago in Elliott P. Joslin research laboratory under the direction of several doctors. A wide variety of suitably controlled serial studies have been carried out in an attempt to detect any anatomic or biochemical abnormality in this earliest stage of diabetic state5, 6, 7, 8).

The present study has been carried out with an aim to find out that whether there is a disturbance of lipid metabolism in diabetic offsprings. Also an attempt has been made to evaluate the diagnostic significance of these investigations.

Material & Methods
The clinical material for the present study comprised of a total of 60 patients. They are the off springs of diabetic patients attending the diabetic out-door of M.Y. Hospital & M.G.M. medical College, Indore.

1. 20 Individual were studied as control cases; of which 10 were healthy subjects and 10 were diabetic patients.
2. 30 Cases are such whose both parents are diabetic.
3. 30 Cases are such whose one of the parents is diabetic.

Care was taken that none of the subjects of the study selected should be suffering from hypertension or taking medications such as β blockers which may alter their lipid profile. All of the subjects were non-smokers and non-obese with normal Basal metabolic Index (BMI) and Waist to Hip ratio (W/H). Estimation of total lipids, lipoproteins, triglycerides, cholesterol and blood sugar was done in these patients.

1. Normal Individuals
All these persons were clinically examined and found to be normal healthy individuals not suffering from any disease. They have no family history of diabetes and their fasting blood sugar and post prandial urine & blood examination did not reveal any sign of hyperglycemia.
2. Diabetic Controls
These persons were proven diabetics receiving treatment from diabetic out-door clinic of M.Y. Hospital.
3. Diabetic Offsprings
They are the off springs of diabetic parents and between the ages 10-40 yrs. Their Glucose tolerance tests were normal.

A detailed history of the patients was taken and through physical examination was carried out. Family history regarding diabetes was taken & following investigations were carried out:

1. Blood Sugar
2. Serum cholesterol

Department of Biochemistry, MGM Medical College, Indore (MP)
3. Total lipids
4. Serum triglycerides
5. (Alpha) Lipoproteins
6. (Beta) Lipoproteins

**COLLECTION OF MATERIAL**

In all cases blood was collected in fasting state. The blood was kept for 2 hrs at room temperature after which the supernatant clear fluid was collected with a pipette in another tube. This tube was centrifuged for 10 minutes. The clear serum was pipetted into a clean dry test tube & this was used for estimation of cholesterol, triglycerides, lipoprotein & total lipids. For blood sugar 2 ml blood was collected in an oxalated bulb.

**LOCUS OF STUDY**

The study was conducted in the department of Biochemistry & Pathology of M.Y. Hospital and M.G.M. Medical College, Indore.

**Observations**

**Table No. 1**

<table>
<thead>
<tr>
<th>Analysis of 80 cases under study</th>
<th>Control Cases</th>
<th>Diabetic offspring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Cases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. %</td>
<td>No. %</td>
<td></td>
</tr>
<tr>
<td>Males 5</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Females 5</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Total 10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Table No. 1 shows distribution of control cases and cases of diabetic offsprings. Out of 10 normal cases, 50% were males and 50% were females. Ten diabetic cases were selected for study so as to see any of the lipid metabolic derangements in them. In offsprings, 40%(66%) were males and 20%(34%) were females.

**Table No. 2**

Relationship of blood sugar, cholesterol, triglycerides, lipoproteins & total lipids in diabetic control cases.

**a) Males**

<table>
<thead>
<tr>
<th>S. Case No.</th>
<th>B.Sugar (mgm %)</th>
<th>Cholesterol (mgm %)</th>
<th>Triglycerides mgm %</th>
<th>Lipoproteins Beta</th>
<th>Lipoproteins Alpha</th>
<th>Total Lipids mgm %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11</td>
<td>200</td>
<td>180</td>
<td>72</td>
<td>28</td>
<td>890</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>140</td>
<td>200</td>
<td>160</td>
<td>76</td>
<td>288</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>280</td>
<td>200</td>
<td>1188</td>
<td>73.5</td>
<td>265</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>170</td>
<td>222</td>
<td>125</td>
<td>75</td>
<td>282</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>160</td>
<td>231</td>
<td>140</td>
<td>70</td>
<td>850</td>
</tr>
<tr>
<td>Mean</td>
<td>190</td>
<td>218.6</td>
<td>156.6</td>
<td>73.30</td>
<td>26.70</td>
<td>808</td>
</tr>
<tr>
<td>S.D.</td>
<td>48.98</td>
<td>16.21</td>
<td>23.66</td>
<td>2.13</td>
<td>2.13</td>
<td>70.8</td>
</tr>
</tbody>
</table>

**b) Females**

<table>
<thead>
<tr>
<th>S. Case No.</th>
<th>B.Sugar (mgm %)</th>
<th>Cholesterol (mgm %)</th>
<th>Triglycerides mgm %</th>
<th>Lipoproteins Beta</th>
<th>Lipoproteins Alpha</th>
<th>Total Lipids mgm %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>200</td>
<td>120</td>
<td>70</td>
<td>30</td>
<td>820</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>160</td>
<td>120</td>
<td>69</td>
<td>31</td>
<td>650</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>180</td>
<td>180</td>
<td>72</td>
<td>28</td>
<td>580</td>
</tr>
<tr>
<td>4</td>
<td>19</td>
<td>185</td>
<td>195</td>
<td>76</td>
<td>24</td>
<td>675</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>190</td>
<td>185</td>
<td>71</td>
<td>29</td>
<td>680</td>
</tr>
<tr>
<td>Mean</td>
<td>183</td>
<td>195.4</td>
<td>144</td>
<td>71.60</td>
<td>28.40</td>
<td>681</td>
</tr>
<tr>
<td>S.D.</td>
<td>13.26</td>
<td>25.85</td>
<td>31.52</td>
<td>2.41</td>
<td>2.4</td>
<td>78.12</td>
</tr>
</tbody>
</table>

In diabetic control cases, beta lipoproteins were raised with hypertriglyceridemia in 4 cases. Total lipids were also high as compared to non-diabetic control levels, but serum cholesterol was within normal limits.

**Table No. 3**

The mean value of serum cholesterol, triglycerides, lipoproteins and total lipids in diabetic offsprings according to age group and presence of diabetics in parents are as follows:

<table>
<thead>
<tr>
<th>Cholesterol (mgm %)</th>
<th>Triglycerides mgm %</th>
<th>Beta Lipoproteins mgm %</th>
<th>Alpha Lipoproteins mgm %</th>
<th>Total lipids mgm %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both parents</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>diabetic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10-25 yrs</td>
<td>1710</td>
<td>123.4</td>
<td>73.6</td>
<td>26.4</td>
</tr>
<tr>
<td>25-40 yrs</td>
<td>1785</td>
<td>123.4</td>
<td>73.6</td>
<td>26.4</td>
</tr>
<tr>
<td>One of the parents</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>diabetic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10-25 yrs</td>
<td>182.0</td>
<td>120.2</td>
<td>73.0</td>
<td>27.0</td>
</tr>
<tr>
<td>25-40 yrs</td>
<td>171.6</td>
<td>112.6</td>
<td>72.0</td>
<td>28.0</td>
</tr>
</tbody>
</table>

**RESULTS**

From the study undertaken following information is concluded.

1. a) Serum cholesterol value in non-diabetic control cases was between 120-275 mgm %, average of 199.40 mgm %.
   b) In diabetic control cases it was between 160-240, average of 205 mgm between.
   c) In diabetic offsprings it was found to be between 90-200 mgm %, average of 180 mgm %.

2. a) Serum triglycerides value in non-diabetic control cases was between 80-200 mgm % average of 128 mgm %
   b) In diabetic control cases it was between 115-118 mgm % average of 150.3%
   c) In diabetic offsprings it was found to be 90-200 mgm % average of 130 mgm %

3. a) Serum total lipid value in non-diabetic control cases was between 450 to 710 mgm % average of 545 mgm %
   b) In diabetic control cases it was between 420 to 890 mgm % average of 796 mgm %
4. a) Serum lipoprotein Beta & Alpha % in non-diabetic control cases was found to be 60:40 to 70:24, mean value of 68.32.
   b) In diabetic control cases it was between 69.31 to 76:24, mean value of 69.32.
5. Serum Triglycerides, lipoproteins and total lipoprotein are raised in diabetic control patients.
6. Serum cholesterol, triglycerides, Beta & Alpha lipoprotein and Total Lipids are within normal limits in diabetic offsprings.
7. There is no disturbance of lipid metabolism

**DISCUSSION**

The present study comprised of 60 diabetic offsprings. Simultaneously 10 control normal healthy individuals were also studied for their serum contents of cholesterol, triglycerides, lipoproteins & total lipids. A control study of 10 diabetic patients was also done, in order to compare these values with those of diabetic offsprings (9,10).

Idiopathic diabetes mellitus embraces a heterogeneous group of disorders having in common disordered carbohydrate fat and protein metabolism. Insulin is a major anabolic hormone in the body. Derangement of insulin function affects not only glucose metabolism but fat and protein metabolism as well. There is concomitant excessive breakdown of fat stores sometimes resulting in elevated levels of free fatty acids and hyperlipidemia. Oxidation of free fatty acids within the liver through acetyl Co-enzyme-A produces ketone bodies. Ketogenic amino acids aggravate the derangement in lipid metabolism (11,12).

The main aim to find out that whether there is disturbance of lipid metabolism in diabetic offsprings also because diabetes is a hereditary transmitted disease. 13.

Diabetes causes an increase in total cholesterol and triglycerides level in the body. It has been observed that patients with Type-1 DM usually have concentrations of the major lipoproteins, LDL and VLDL are normal or subnormal, whereas HDL is normal or increased. Type 1 DM patients have a high free cholesterol/lecithin ratio in plasma and VLDL-LDL fractions. These abnormalities may interfere with lipid transport between lipoproteins and consequently the remodelling of lipoprotein particles. The concentration of phospholipids in HDL is abnormal (14,15).

In type 2 DM patients, moderate hypertriglyceridaemia with reduced levels of HDL cholesterol is common. Since glycaemic control is often insufficient, serum triglycerides are elevated. The increase in triglyceride-rich lipoproteins induces a mild elevation in total serum cholesterol. In the case of poor glycemic control, total cholesterol is increased due to an accumulation of LDL, HDL particles contain an increased proportion of triglycerides, with a faster catabolic rate that leads to a lower number of circulating HDL.

In the present study it was found that Serum triglycerides, lipoproteins & total lipids were raised in diabetic control cases, which is in accordance with the observations made in the past, where as in diabetic offsprings lipids were within normal limits (Table 3).

Thus from above observation and discussion it can be concluded that probably there is no disturbance of lipid metabolism in diabetic offsprings compared to patients of diabetes.

**References:-**

15. Dr. Suvadip Chatterjee Diabetes and Hypertension, MCNA, 2001; 3:205-209.
Evaluation of hypoglycemic effect of Eugenia jambulana (jamun) on obese diabetic patients

The present study is conducted on 32 obese patients with recently diagnosed diabetes mellitus type 2 and 30 sex and age matched normal control subjects. Subjects were selected from Medicine OPD and staff of M.Y. Hospital & MGM Medical College, Indore. The patients with coronary heart disease, respiratory diseases and renal diseases were excluded from the study. The exclusion criteria also included use of cigarettes, alcohol and oral contraceptives.

DR. SANGEETA PANERI, DR. MEENA VARMA, PREETHA BADI

Described and treated since ancient times, diabetes is an incurable chronic disease that affects almost every organ and system of the body. In recent years emphasis is on the development of drugs from plants for the treatment of various diseases including diabetes mellitus, the incidence of which is very high all over the world, especially in India. A number of plants have been found to be useful in diabetes mellitus. Jambulana fruit, seeds, leaf and bark have been reported to possess antimicrobial, antihistimetic, immunomodulatory, hypoglycemic and a number of other effects. The Jambulana seeds contain beta-sitosterol, essential oil, limonene, alpha and beta pinene, cisocimene, alpha humulene and bornylacetate which are effective in reducing fasting blood sugar. The aim of the present study is to evaluate the hypoglycemic effect of Jambulana seeds on obese patients with type-2 diabetes mellitus.

Material and method:

The present study is conducted on 32 obese patients with recently diagnosed diabetes mellitus type 2 and 30 sex and age matched normal control subjects. Subjects were selected from Medicine OPD and staff of M.Y. Hospital & MGM Medical College, Indore. The patients with coronary heart disease, respiratory diseases and renal diseases were excluded from the study. The exclusion criteria also included use of cigarettes, alcohol and oral contraceptives.

The glucose levels and lipid profile was estimated before administration of Jambulana seed powder to each subjects and compared with the control. Then daily does of 6 to 9 grams of Jambulana seed powder was given to each study subjects continuously for 3 months along with balanced vegetarian diet. After each month the glucose levels and lipid profile were estimated. The estimation of glucose was done by Glucose oxidase Peroxidase method, cholesterol, triglycerides and HDL were estimated by Kinetic method.

Statistical analysis:

The statistical analysis was done by student 't' test. The values were expressed as Mean ± Standard Deviation (S.D.).

Results and discussion:

The results shown in table 1 and 2 reveal that there is a significant increase in glucose, cholesterol, HDL and

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n = 30)</th>
<th>Study (n = 32)</th>
<th>'p' Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>96.8 ± 9.8</td>
<td>188.2 ± 11.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>172.2 ± 13.2</td>
<td>282 ± 21.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>52 ± 8.2</td>
<td>38.2 ± 13.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>128.4 ± 18</td>
<td>268 ± 20.2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Department of Biochemistry, M.G.M. Medical College, Indore (M.P.)
Table 2
Status of parameters estimated after administration of Jambulana seed powder in study group and its comparison with control.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n = 30)</th>
<th>Control (n = 32) After administration of Jambulana seed powder</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 month</td>
<td>2 month</td>
</tr>
<tr>
<td>Glucose [mg/dl]</td>
<td>86 ± 8.5</td>
<td>75 ± 7.1</td>
</tr>
<tr>
<td>Total cholesterol [mg/dl]</td>
<td>170 ± 8.6</td>
<td>168 ± 4</td>
</tr>
<tr>
<td>HDL [mg/dl]</td>
<td>52 ± 13</td>
<td>50 ± 11</td>
</tr>
<tr>
<td>Triglyceride [mg/dl]</td>
<td>120.4 ± 8.2</td>
<td>129 ± 7.8</td>
</tr>
</tbody>
</table>

DECREASE IN GLUCOSE LEVEL AFTER ADMINISTRATION OF JAMBULANA SEED POWDER
triglycerides in obese patients with recently diagnosed diabetes mellitus type 2. But after treatment with Jambulana seed extract there was significant lowering of serum glucose, cholesterol and triglycerides and these values were close to normal. A slight increase in HDL level is also seen. This indicates that the Jambulana seed powder has favourable effect on the lipid and glucose metabolism of obese diabetic patients.

The increased level of fatty acids and fats in obese patients causes the insulin resistance which leads to the hyperglycemia. Hypolipidemic* and hypoglycemic property of Jambulana seed powder improve the lipid and glucose level in obese patients.

From the above results, it can be concluded that Jambulana seed powder which contains beta-sitosterol, essential oil, bornylacetate, ciscimene, alpha and beta pinene can be an useful antidiabetic agent and have the ability to cure the hyperglycemia and hyperlipidemia.

References:
“Impact of smoking on lipid profile:
With Sp. Ref. to urban M.P.”
Dr. Mrs. Meena Varma, Smt. Sangita Paneri

Smoking is injurious to health as this caption is
found everywhere even on the packet of cigarette and
bids. Smoking in different forms disturbs the lipids
and other Blood Biochemical parameter and Like wise
responsible for many diseases like atherosclerosis,
CHD, liver cirrhosis and Hepatic, mouth, sound box
cancers.1 There is a close relationship between the
number of Bidi/Cigarette smoked and lipid metabolism.2
Smoking leads to increased concentration of Serum
Triglycerides, Total cholesterol, LDL, VLDL, and fall in
the HDL-level.3 There are various mechanisms which
causes Lipid alteration by smoking. Nicotine Stimulets
Sympathetic adrenal system leading to increased se-
tretion of Catecholamine resulting in increased lipoly-
sis and increased Concentration of plasma free fatty
acids which further resulting increased synthesis of
Total cholesterol, triglyceride, VLDL and LDL in blood
stream.4 Insulin resistance found in smokers due to
increase FFA level which leads to increased lipid level.5
Consumption of diet rich in fat cholesterol as well as
diet low in fibre and cereals Contents by smokers as
compared to non smokers.6 Smoking causes impaired
synthesis of fat from liver cells which results in lever
cirrhosis which leads to increased lipid level.7 The aim
of the study was to find out the effect of smoking on
lipid profile in adult person of urban part of M.P.

Material and Methods :- The present study was con-
ducted on fasting serum samples of 55 adult persons
of the age between 30 to 70 years having the habit of
smoking cigarette daily since last 3 to 20 years Se-
lected from patient attendants M.Y. Hospital Staff Indore
and personal contact from 2001 to 2002. The chain
smokers with Diabetes, Renal failure and CHD, were
excluded. Same age weight matched 30 normal healthy
non smokers control subjects were taken for study.
After over night fast peripheral venous blood samples
were collected from control and smoker subjects. The
serum separated with in 1 to 2 hours of collection. The

Samples were analysed for total cholesterol, Triglyc-
eride, HDL, VLDL and LDL. Triglyceride, total choles-
terol and HDL-Cholesterol were estimated by kinetic
methods and LDL, VLDL calculated by formula.
The statistical analysis was done by mean, standard
deviation, student’s t test and p values.

Observation

Table -1-Distribution of Smokers in relation to duration
and number of Cigarettes smoked / day

<table>
<thead>
<tr>
<th>Duration</th>
<th>Mean No of cigarettes</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>In year</td>
<td>smoked /day</td>
<td></td>
</tr>
<tr>
<td>1-5</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>6-10</td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>11-15</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>55</td>
</tr>
</tbody>
</table>

Table II - Mean values of Studied blood biochemical
parameters in non-smokers and smokers.

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Non smokers</th>
<th>Smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride</td>
<td>184 ± 50</td>
<td>192 ± 65.6</td>
</tr>
<tr>
<td>Total choles</td>
<td>152.4 ± 20.2</td>
<td>186 ± 2</td>
</tr>
<tr>
<td>HDL-C</td>
<td>72.2 ± 5.3</td>
<td>40.5 ± 6.8</td>
</tr>
<tr>
<td>LDL-C</td>
<td>78.4 ± 4.8</td>
<td>108.3 ± 8.8</td>
</tr>
<tr>
<td>VLDL-C</td>
<td>16.6 ± 1.8</td>
<td>28.7 ± 3.8</td>
</tr>
</tbody>
</table>

Table IV : Mean values of Studied blood biochemical
parameters in relation to number of cigarettes smoked
per day as compared to non-smokers.
<table>
<thead>
<tr>
<th>Particulars</th>
<th>Non smoker (Control)</th>
<th>1 - 10 (n = 31)</th>
<th>11 - 20 (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride</td>
<td>118.1 ± 30.2</td>
<td>162.2 ± 30.2</td>
<td>186.8 ± 60.2</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>152.4 ± 20.2</td>
<td>173.4 ± 29.2</td>
<td>199.4 ± 32.1</td>
</tr>
<tr>
<td>HDL-C</td>
<td>47.2 ± 5.1</td>
<td>42.8 ± 5.1</td>
<td>37.2 ± 4.4</td>
</tr>
<tr>
<td>LDL - C</td>
<td>78.4 ± 14.8</td>
<td>96.4 ± 18.3</td>
<td>116.1 ± 28.4</td>
</tr>
<tr>
<td>VLDL - C</td>
<td>16.6 ± 1.8</td>
<td>22.4 ± 2.1</td>
<td>34.3 ± 3.9</td>
</tr>
</tbody>
</table>

**Results and Discussion:** It is revealed that triglycerides, total cholesterol, LDL and VLDL were significantly higher in Smokers as compared to non smokers. The mean serum total cholesterol in non smokers was 152.4 ± 20.2 mg/dl while it was significantly higher in smokers 186.2 ± 29.2 mg/dl. These findings are supported by other workers. Cigarettes smoking substantially increases the risk of CHD. The total cholesterol values in subjects smoking 1-10 cigarettes per day was 173.4±29.2 mg/dl and those smoking 11 to 20 cigarettes per day was 199.4±32.1 mg/dl. These findings are very close to the other workers the mean serum triglyceride levels in non smokers was 118.1 ± 30.2 mg/dl and in smokers was 174 ± 54.6 mg/dl. Nicotine stimulates sympathetic adrenal system leading to increased secretion of catecholamin resulting increased free fatty acid by increased lipolysis. Which leads to increased triglyceride levels. The values of serum triglyceride and total cholesterol were significantly higher in those subjects smoked 11 to 20 cigarette per day as compared to those smoked 1 to 10 cigarettes per day. These findings are similar to those of Rustogi et al. The mean LDL-C and VLDL-C values in non-smokers were 78.4 ± 14.8 mg/dl 16.6 ± 1.8 mg/dl and in smokers were 106.3 ± 26.2 mg/dl 28.7 ± 3.8 mg/dl respectively. But these values were significantly higher in subjects smoked 11-20 cigarettes per day as compared to those smoked 1-10 cigarettes per day. These findings are similar to Rastogi et al. The mean HDL-C in non-smokers was 47.2 ± 5.1 and in smokers 40.6 ± 5.8 respectively. According to Anoop Mishra et al the increase level of triglycerides causes transfer of triglyceride particles from HDL, which results decrease in HDL level. This decrease was very high in subjects smoked 11 to 20 cigarettes per day as compared to subjects smoked 1 to 10 cigarettes per day. Similar findings have been reported by Brischetto et al.

The results of our study suggest that the smoking affect the lipid metabolism and alter the lipid profile. This condition known as hyperlipidemia, which is suppose to be independent factor for chronic heart disease.

**Conclusion:**

The hyper triglyceridemia and low HDL-cholesterol level are the main risk factors of the Diabetes, Myocardial infarction and Vascular diseases in future. Findings of our study show both of these conditions found in chronic smokers. The study conclude that smokers having hyperlipidemia which increases chances of future myocardial infarction many folds.

**References:**