Influence of Plasma Cholesterol on Erythrocyte Membrane Cholesterol in Type 2 Diabetic Subjects

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Abstract: Diabetes mellitus (DM) induced dyslipidemia results in various micro and macro vascular complications. It has been observed by many workers that the erythrocyte membrane cholesterol is elevated in DM which may lead to alterations in erythrocyte membrane characteristics. As there is no proper cholesterol synthetic mechanisms available in mature erythrocytes, the raised membrane cholesterol might have originated from plasma cholesterol, as erythrocytes are continuously bathing in plasma. Hence a study was undertaken to assess the impact of plasma cholesterol concentration on the erythrocyte membrane cholesterol content.

Diabetic as well as normal subjects of both sexes in the age group of 30-55 years were selected and a fasting heparinised blood sample was collected. Glucose and Cholesterol levels and the membrane cholesterol content of erythrocytes were estimated. The diabetic subjects were grouped into Group-1 (plasma cholesterol <200mg/dl) and Group-2 (plasma cholesterol ≥200mg/dl).

The results shows a significant elevation of erythrocyte membrane cholesterol (p<0.001) in diabetic subjects as well as a proportional raise in erythrocyte membrane cholesterol level in relation to plasma cholesterol concentration (Group-1: Plasma cholesterol 173.8±22.7mg/dl, Membrane cholesterol 8.0±3.57mg/cc; Group-2 Plasma cholesterol 341.1±14.43mg/dl, Membrane cholesterol 12.1±4.43mg/cc)

It is concluded from the present study that the plasma cholesterol level do influence erythrocyte membrane cholesterol concentration and higher the plasma cholesterol higher will be erythrocyte membrane cholesterol level.

Keywords: Diabetes Mellitus(DM), Erythrocyte membrane cholesterol, plasma cholesterol.

I. Introduction

Type-2 DM is a metabolic syndrome characterized by chronic hyperglycemia & disturbance of carbohydrate, lipid and protein metabolism due to underlying lack or subnormal functioning of insulin(1). Hyperglycemia, if not properly controlled, may cause dyslipidemia, which further leads to various life threatening complications including micro and macrovascular complications(2-4). It is observed that dyslipidemia accompanying diabetes causes two to four fold increase risk of cardiovascular disease (5). Insulin resistance contributes to “diabetic dyslipidemia” by favouring the hepatic production of lipoproteins and suppressing the peripheral uptake of circulating lipids. Since cholesterol forms a major constituent of lipoprotein, increase lipoproteins in blood leads to increase cholesterol concentration in the blood(6). It has been suggested that the ability of erythrocytes to change their shape is decreased in diabetic patients and such an impairment of erythrocyte deformability might be another contributing factor to the reduction of blood flow in the capillaries leading to micro and macro vascular diseases (6-9).

Membrane lipids are mostly unesterified cholesterol which are essential for maintenance of biological membranes, including, erythrocyte membrane in a bilayer matrix of intermediate fluid state(10). This fluid property is significant in regulation of membrane fragility for maintaining structural integrity, biconcave shape and also membrane permeability for transport of substances, specifically the glucose across the plasma and the cytoplasm of erythrocytes (11-13). The relative amounts of phospholipids and cholesterol are responsible for the fluid properties of erythrocyte membrane. Increase in the cholesterol content of the erythrocyte membrane alters the membrane behavior making it rigid(12,14-18). The erythrocytes are devoid of many sub-cellular organelles, including ribosomes, hence erythrocytes cannot synthesis the cellular as well as membrane components and has to be dependent on the media i.e., plasma for these components. Therefore, the membrane properties of erythrocytes are dependent on plasma lipid components, specifically, the cholesterol.
In diabetic dyslipidemia, normally there is a high concentration of cholesterol in the plasma in which the erythrocytes are continuously bathing, hence a study was planned to assess whether the high plasma cholesterol leads to addition of more cholesterol to the erythrocyte membrane in type 2 diabetics, making the erythrocyte membrane more rigid and subsequently altering its potential to deform as well as its normal utilization of glucose (19).

The Aim Of The Study Was To
1. Estimate the plasma and erythrocyte membrane cholesterol levels in Type 2 diabetic subjects.
2. Evaluate the influence of plasma cholesterol on erythrocyte membrane cholesterol concentration.

II. Materials & Methods

Diabetic subjects of both sexes in the age group of (30-55 years) attending the medical Out Patient Department of Subbaiah Medical College and Hospital, Purale, Shimoga were randomly selected. A brief history of their ailment was taken. Normal subjects of both sexes in the age group of 30-35 years were selected as control from the staff of Subbaiah Institute of Medical Science. A fasting blood sample of 4-5 ml quantity from the selected subjects were collected with heparin as an anticoagulant after obtaining informed consent. Heparinised blood was centrifuged at 3500 rpm for 10 minutes to obtain plasma. The plasma samples were employed for total cholesterol estimation (20) and the plasma glucose (21).

The erythrocytes obtained on centrifugation were washed three times with normal saline of 5 ml quantity. Washed erythrocytes were mixed with equal amount of normal saline to give 50% saturated erythrocyte suspension. To 1 ml of 50% saturated erythrocyte suspension, 4 ml of distilled water was added and the mixture was stirred vigourously with a clean glass rod to lyse the erythrocytes. This was centrifuged at 3500 rpm for 5 minutes and the supernatant was discarded. The sedimented membranes were washed three times with 3 ml of normal saline. One part of washed erythrocyte membranes was homogenized with 9 parts of chloroform : methanol (1:1) mixture for 8 minutes using Remi tissue homogenizer. The extracts were used for estimation of membrane total cholesterol by Zak’s method (20).

III. Results

The study consists of 25 normal subjects and 61 diabetic subjects. Diabetic subjects were divided into two groups depending on their plasma cholesterol level.

**Group 1:** Diabetics having plasma cholesterol <200 mg/dl (normocholesterolemic diabetic subjects).

**Group 2:** Diabetics having plasma cholesterol ≥200 mg/dl (hypercholesterolemic diabetic subjects).

There were 30 diabetic subjects in group 1 & 31 diabetic subjects in group 2. The results obtained of the present study are given in table-1, table-2 as well as in bar graph-1. Table-1 narrates the values of fasting plasma glucose, plasma total cholesterol, erythrocyte membrane cholesterol levels in normal subjects as well as in diabetic subjects. It is evident from the table that fasting plasma glucose levels, plasma total cholesterol levels as well as erythrocyte membrane cholesterol levels are significantly elevated (p<0.001) in diabetic subjects as compared to normal subjects. Table-2 gives the membrane cholesterol levels in normal subjects, in group-1 diabetic subjects and in group-2 diabetic subjects. It is seen from the table that a significant raise in membrane cholesterol (p<0.001) is seen in both group-1 and in group-2 diabetic subjects as compared to normal subjects, suggesting that diabetes induces an increase in erythrocyte membrane cholesterol concentration. The proportional elevation of membrane cholesterol in relation to plasma cholesterol is depicted in bar graph-1(A&B). It is evident from the graph that a significant elevation (p<0.001) is seen in group-2 diabetic subjects as compared group-1 diabetic subjects indicating the raise in erythrocyte membrane cholesterol in diabetic subjects is proportional to the raised plasma cholesterol levels in diabetic subjects.

IV. Discussion

Cholesterol, a steroid alcohol and a normal constituent of human blood plasma has been shown by several workers to be elevated in DM. The elevation of cholesterol in diabetic subjects is attributed to increased availability of acetyl coenzyme-A, the starting substrate for cholesterol synthesis in human beings, due to increased fatty acid utilization as well as fatty acid oxidation for energy purposes because of non-availability of glucose due to either deficiency of insulin or due to sub-normal functioning of insulin (1). The increased level of cholesterol in diabetic subjects may cause micro or macro vascular changes leading to an array of diabetic cardio-vascular complications (22-26). The observed raise in plasma cholesterol in diabetic subjects (group-1 & group-2) as compared to normal subjects (p<0.001) as evident from the table-2 is in agreement with the earlier reports (27-29).
Influence of Plasma Cholesterol on Erythrocyte Membrane Cholesterol in Type 2 Diabetic Subjects.

It has been observed by many workers that the erythrocyte membrane cholesterol is elevated in diabetic population (13,30). As there are no cholesterol synthetic mechanisms available in mature erythrocytes, the only possibility for raise in erythrocyte membrane cholesterol is the raised plasma cholesterol in diabetic subjects. In order to establish the inter-relationship of erythrocyte membrane cholesterol with plasma total cholesterol levels as well as to study the impact of plasma total cholesterol on erythrocyte membrane cholesterol, the diabetic subjects were divided into group-1 diabetics(plasma total cholesterol<200mg/dl) and group-2 diabetics(plasma cholesterol ≥200mg/dl).

It is clear from the results depicted in graph-1 that more the plasma cholesterol more is the cholesterol inclusion to the erythrocyte membrane, indicating that the plasma cholesterol is the principle source for the erythrocyte membrane cholesterol. Such an over addition of cholesterol on the erythrocyte membrane may induce rigidity into the membrane as cholesterol tends to increase the membrane rigidity as well as such an increase in membrane cholesterol may alter the membrane transport mechanism including glucose transport, thus altering glucose utilization. Such an alteration in tissue cell membrane cholesterol levels may add to the abnormal glycemic conditions in diabetic subjects. This study suggests a proper regulation of plasma cholesterol level in diabetic subjects is helpful in regulation of their glycemic status.

V. Figures and Tables

Table-1

<table>
<thead>
<tr>
<th></th>
<th>Fasting plasma glucose(mg/dl)</th>
<th>Plasma total cholesterol(mg/dl)</th>
<th>Erythrocyte membrane cholesterol(mg/cc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal subjects(25)</td>
<td>79.4±11.2</td>
<td>121.8±17.8</td>
<td>1.25±0.31</td>
</tr>
<tr>
<td>Diabetic subjects(61)</td>
<td>152.61±32.38***</td>
<td>257.4±21.2***</td>
<td>10.05±0.81***</td>
</tr>
</tbody>
</table>

Note:
1. Number in parentheses indicate the number of subjects.
2. The values are expressed as their mean ± SD.
3. Statistical evaluation- probability level *p<0.05, **p<0.01, ***p<0.001.

Table-2

<table>
<thead>
<tr>
<th>Membrane cholesterol (mg/cc)</th>
<th>Normal subjects (25)</th>
<th>Group-1 (30)</th>
<th>Group-2 (31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma total cholesterol (mg/dl)</td>
<td>121.8±17.88</td>
<td>173.8±22.75***</td>
<td>341.10±14.43***</td>
</tr>
<tr>
<td>1.25±0.31</td>
<td>8.0±1.57***</td>
<td>12.1±0.31***</td>
<td></td>
</tr>
</tbody>
</table>

Note:
4. Number in parentheses indicate the number of subjects.
5. The values are expressed as their mean ± SD.
6. Statistical evaluation- probability level *p<0.05, **p<0.01, ***p<0.001.

Graph-1

Graph-1 (A & B) shows plasma total cholesterol and erythrocyte membrane cholesterol in normal subjects, diabetic Group-1 and diabetic Group-2. It is evident from the charts that the raise in erythrocyte membrane cholesterol is proportional to raised plasma cholesterol level in diabetic subjects.
VI. Conclusion

Dyslipidemia is a major complication of type 2 diabetes mellitus which results in abnormal plasma cholesterol levels leading to a possible variation of erythrocyte membrane cholesterol concentration. The results obtained in present study in type 2 diabetic subjects confirms a raise in erythrocyte membrane cholesterol concentration in relation to plasma cholesterol level. It has been further confirmed that addition of cholesterol to erythrocyte is influenced by plasma cholesterol levels. The erythrocytes membrane cholesterol level of diabetic erythrocytes bathing in plasma having a cholesterol level ≥ 200mg/dl(group-2) is significantly higher(p<0.001) as compared to membrane cholesterol of diabetic erythrocytes bathing in plasma cholesterol less than 200mg/dl(group-1) which is evident from the results given in table-2 and graph-1. It can be concluded from the present study that the raise in erythrocyte membrane cholesterol level is related to plasma cholesterol level and higher the plasma cholesterol levels more the cholesterol addition to the membrane. Hence positively responsible for alteration in membrane properties. A prompt regulation of plasma cholesterol level may assist in maintaining normal membrane functions in erythrocytes as well as in tissue cell membranes.

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