



## The Plasma Phospholipids Exchange on to the Erythrocyte Membrane Lipids: With Different Age Groups and Anti Diabetic Therapy.

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### ABSTRACT

Dyslipidaemia is a major cause, observed in type 2 diabetic subjects with micro vascular complications and membrane functional alteration is very common. The relative amount of phospholipids and cholesterol is responsible entity for normal function and membrane fluidity and rigidity. Many research studies reported on plasma lipid anomaly even on anti diabetic agents and also our earlier laboratory study supports, influence of anti diabetic therapy on plasma lipids and its relation to lipid alteration in erythrocyte membrane of diabetic subjects. Now effort has been made to rule out an anti diabetic effect on plasma phospholipids levels and its exchange on to the RBC membrane phospholipids composition of diabetic subjects with different age groups and anti diabetic therapy. Blood samples from randomly selected type-2 diabetic subjects were collected after obtaining written consent and institutional ethical clearance was obtained those who were attending Sri B.M. Patil medical college hospital for routine glycaemic check up. The results of the study were statistically evaluated by Student 't' and Anova. The significant rise of Plasma and membrane TC, PL ( $P < 0.001$ ) at age of 45-55 years as compared to older age. On drug therapy, plasma and membrane TC, PL significant decrease ( $P < 0.001$ ) but ratio Increase, whereas on insulin treatment, plasma Glucose significantly decrease, But not much alteration of Plasma and membrane cholesterol However m PL  $P < 0.001$  Significantly Increase and m TC /m PL Ratio significantly decrease. Conclusion - In spite of treatment of diabetes, there is anomaly of lipid composition with age and anti diabetic therapy. Hence, the middle age group (45-55 years) diabetes subjects were more prone to risk of micro vascular complications than older age group (55 and above) and selection of anti diabetic treatment may play a significant role for glycaemic control and Diabetic complications .

**Key words-** Anti diabetic Therapy, phospholipids (PL), Total Cholesterol (TC) ,RBC Membrane



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## INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia due to decrease secretion or absence of insulin, which leads to Dyslipidaemia with micro vascular complications due to long standing diabetes Mellitus (1-3, 24, 25). The membrane phospholipids and cholesterol are asymmetrically arranged in a bilayers. The relative amount of phospholipids and cholesterol is responsible entity for normal function and membrane fluidity and rigidity (4). If any change in blood rheology affects the membrane deformability degenerative changes lead to its micro vascular complication and platelets aggregation also reduction of blood flow in the capillaries (5). The diabetes induced plasma Dyslipidaemia and its RBC membrane lipid changes might be effect by anti diabetic therapy, which in turns act on GLUT particles and membrane cholesterol or phospholipids transport protein (6) The various studies observed many diabetic complications, specifically cardiovascular disease and neurological symptoms common in the middle years of life,(7) which might be due to more family stress with less physical exercise. The typical pattern of dyslipidaemia seen in type 2 diabetes is normal or slightly elevated total cholesterol, elevated triglycerides and low levels of high density lipoprotein (HDL)-cholesterol levels. Low density lipoprotein (LDL)-cholesterol levels are variably elevated, but not significantly different from non-diabetic patients. Despite similar LDL levels, type 2 diabetic patients have a more atherogenic LDL phenotype than non-diabetic individuals. Diabetic patients have a shift to smaller, denser LDL particles (phenotype B). Phenotype B has been associated with a three- to seven-fold increased risk of MI. Interventions that lower blood glucose often lower triglyceride levels, primarily by reducing free fatty acid and glucose levels, which are substrates for triglyceride production. Despite this, glucose lowering alone rarely leads to a pronounced change in the major LDL- or HDLclasses. In recent years there has been an increase in the number and classes of medications available for the treatment of type 2 diabetes.

Given the implications of macrovascular disease in this population, an understanding of the alterations in the major lipid classes and subclasses that occur as a result of the broad range of anti diabetic medications may enhance our approach to drug selection for the treatment of type 2 diabetes.(8) Hence the present study was undertaken to rule out effect of anti diabetic drugs and insulin on plasma phospholipids levels and its exchange on to erythrocyte membrane phospholipids, Cholesterol, and ratio with different age groups of diabetic subjects.

## MATERIALS AND METHODS

The present study included a total number of 127 subjects consisting 31 normal subjects ( 25 male and 6 female) and 96 diabetic type 2 subjects( 62 male and 34 female) in the age group of 35 -65 years attending to Sri B.M. Patil Medical College, Hospital for routine glycemic checkup, were randomly selected and Institutional ethical certificate was obtained. The normal subjects were randomly picked among employees of college as well as Hospital. These diabetic subjects included 75 diabetics receiving oral anti diabetic drugs and 21 receiving insulin only. Blood samples (6-7ml) from the selected normal subjects and type 2 diabetic subjects were collected, in the fasting state with suitable anticoagulant by obtaining informed consent. Plasma was separated by centrifugation at 3500 rpm, for 10 minutes. Erythrocytes were washed three times with an aliquot of 5 ml normal saline and were preserved for use

### *Preparation of Erythrocyte Membrane*

To 1 ml of 50% saturated erythrocyte suspension 4 ml distilled water were added and the mixture was stirred vigorously with clean glass rod to lyses the erythrocytes. This was centrifuged at 3500 rpm for 5 minutes. Supernatant was discarded. The sediment of the membranes was washed 3 times with 3ml aliquots of normal saline. The washed erythrocyte membranes were employed for membrane cholesterol and phosphor lipid studies. The washed

membranes was homogenized with 9 parts of chloroform: methanol (1:1v/v) mixture for 8 minutes using Potter-Elvehjem tissue homogenizer. The extracts were used for the estimation of membrane mTC , mPL

(9,10 11). Groups 1 and 2 were statistically analyzed by Students't' and Statistical Significance mentioned between GP 1 & 2, 3,4 by - \*. GP 2 & 3 by -  $\alpha$  . GP 3 & 4 by -  $\beta$  by Anova.

**Table – 1**  
**showing the levels of plasma glucose, Total Cholesterol, total Phospholipids and Levels of m TC, m PL in Erythrocyte Membrane as well as Calculated Ratio of mPL to mTC in Normal and Diabetic Subjects with Different Age Groups:**

Parameters	Normal Subjects Group -1 35 - 65 years (31)	DiabetesSubjects Group 2 35-45 Years (22)	DiabetesSubjects Group 3 45-55 Years (24)	Diabetes Subjects Group 4 55&above Years (50)
Plasma glucose. mg/dl	84.54+12.55	180 .26+25.30***	195.50=20.25 $\alpha\alpha\beta\beta\beta$	165.60=23.80
Plasma total cholesterol.mg/dl	134.21+22.10	255.40+28.80**	285.52+36.30 $\alpha\alpha\beta\beta\beta$	252.48+23.80
Plasma phospho lipid. mg/dl	15.62+3.15	21.31+3.16***	26.42+3.10 $\alpha\alpha\beta\beta\beta$	23.80+3.18
Membrane Total Cholesterol mg/cc (mTC)	1.36 $\pm$ 1.78	1.66 $\pm$ 0.50***	1.78 $\pm$ 0.61 $\alpha\alpha\alpha$	1.76 $\pm$ 0.66
Membrane Total Phospholipid mg/cc (mPL)	7.36 $\pm$ 1.78	7.86 $\pm$ 0.90***	8.32 $\pm$ 0.88 $\alpha\alpha\alpha\beta$	8.10 $\pm$ 0.78
Membrane Phospholipid/Cholesterol Ratio mg/cc (mPL/mTC)	6.40 $\pm$ 0.64	5.02 $\pm$ 0.58	5.09 $\pm$ 0.56	4.81 $\pm$ 0.49

Note: 1) The number in parenthesis shows the number of subjects.2) Values are expressed as their Mean  $\pm$  SD  
3) p value \* / $\alpha/\beta$ , p<0.02, \*\*  $\alpha\alpha/p$  <0.01, \*\*\*/  $\alpha\alpha\alpha / \beta\beta\beta$  p<0.001. Statistical Significance mentioned between GP 1 & 2,3,4 by - \* GP 2 & 3 by -  $\alpha$  GP 3 & 4 by -  $\beta$

**Table – 2**  
**Table showing the levels of plasma TC, TPL and levels of m TC, m PL also ratio in Erythrocyte Membrane of Normal and Diabetic Subjects with Anti diabetic therapy.**

Parameters	Group-1 Normal Subjects (31)	Group -2 Diabetes Subjects (96)	Group- 3 Diabetes,Receiving,Oral Drugs alone (75)	Group- 4 Diabetes Receiving Insulin alone (21)
Plasma Glucose mg/dl	84.54+12.55	166.56+30.28***	135.58+22.32 $\alpha\alpha\alpha$	126.38+10.82 $\beta\beta\beta$
Plasma total cholesterol.mg/dl	134.21+22.10	264.24+25.34***	227.81+20.62 $\alpha\alpha\alpha$	259.48+19.90 $\beta\beta\beta$
Plasma phosphor lipid.mg/dl	16.62	28.23+ 5.10***	26.12+4.10 $\alpha\alpha$	28.10 +6.16 $\beta\beta$
Total membrane Cholesterol mg/cc (mTC)	1.20 $\pm$ 0.32	1.76 $\pm$ 0.10***	1.58 $\pm$ 0.63 $\alpha\alpha\alpha$	1.66 $\pm$ 0.36
Membrane Total Phospholipid mg/cc (mPL )	7.36 $\pm$ 1.78	8.18 $\pm$ 0.88***	7.51 $\pm$ 0.66 $\alpha\alpha\alpha$	8.10 $\pm$ 0.71 $\beta\beta\beta$
Membrane Phospholipid/Cholesterol Ratio.mg/cc (mPL/mTC)	6.40 $\pm$ 0.64	4.73 $\pm$ 0.28***	5.09 $\pm$ 0.53 $\alpha\alpha\alpha\beta\beta\beta$	4.45 $\pm$ 0.28

Note: 1) The number in parenthesis shows the number of subjects.2) Values are expressed as their Mean  $\pm$  SD  
3) p value \* / $\alpha/\beta$ , p<0.02, \*\*  $\alpha\alpha/p$  <0.01, \*\*\*/  $\alpha\alpha\alpha / \beta\beta\beta$  p<0.001 Statistical Significance mentioned between GP 1 & 2 by - \* GP 2 & 3 by -  $\alpha$  GP 3 & 4 by -  $\beta$

## RESULTS

The result shows in table 1- The blood glucose and plasma cholesterol, phospholipids and membrane TC, PL significant increase ( $p < 0.001$ ) in diabetic subjects with groups G-2, G-3 and G-4 as compared to G-1 but ratio decrease in all groups as compared G-1. However very significant rise of Plasma and membrane TC, PL ( $p < 0.001$ ) in G-3, at age of 45-55 years as compared to other groups G-2 at below 45 years and G - 4, at above 55 years. Table -2 shows the levels of plasma TC, PL and membrane TC. PL was significantly increased ( $p < 0.01$ ) in G-2, G-3, and G-4 as compared G-1. On drug therapy, plasma and membrane TC, PL in G-3 showed significant decrease ( $p < 0.001$ ) as compared to G-2, G-4. However, TC/PL ratio in G-3 ( $P < 0.01$ ) increase as compared to G-2, G-4. On Insulin treatment G -4., plasma glucose significantly decrease as compared to G-2 and G-3. But not much alteration of plasma and membrane cholesterol as compared to G-2. However G-4, m PL m TC  $p < 0.001$  significantly increase as compared to G-3. But m TC /m PL ratio significantly decrease in G-4, when compared to G-2 and G-3.

## DISCUSSION

Dyslipidaemia plays an important role in the development of complications in diabetes mellitus. The lipid alteration in RBC membrane leads to function degenerative changes through red cell aggregation, change in shape, increased adhesion of red blood cells to endothelial cells (12). There are some reports regarding changes in membrane lipids in diabetes mellitus showing decrease in membrane cholesterol, total phospholipid ratio (13). Some others show decrease in membrane cholesterol and membrane phospholipids, and membrane fatty acids in diabetes mellitus (14), while other reports suggest increase in membrane cholesterol, decrease in membrane phospholipids with increase in cholesterol, total phospholipid ratio (14). And decrease in cholesterol with no change in phospholipids (16) In recent years there has been an increase in the number and class of

medications available for the treatment of type -2 diabetes. Given the implications of macro vascular disease in this population, an understanding of alterations in the major lipid classes and subclasses that occur as a result of the broad range of anti diabetic medications may enhance our approach to drug selection for the treatment of type -2 diabetes (17). Earlier our laboratory observations reported the Influence of anti diabetic drugs of plasma lipids levels on its relation to erythrocyte membrane lipid composition (18) and erythrocyte membrane lipid alteration in type 2 diabetic subjects (19) In diabetes mellitus, there are many changes in erythrocyte membrane. They are decreased insulin binding, abnormal lipid composition, altered membrane phospholipids asymmetry, altered membrane fluidity (20). Alterations in organization of phospholipids of erythrocyte membranes causes 'increased red cell- red cell aggregation (21) and increased in cholesterol, increased rigidity and slight change in RBC shape. (22) or altered phospholipids asymmetry causes platelet activation and aggregation. (23) It is evident from the results narrated in this table 1. It clearly indicates that there is a significant raise in mTC and mPL in diabetic subjects in middle age between G-3 45-55 as compared to age between G-2, G-4. This indicates probably there is diabetes induced alteration in diabetic subjects. These alterations may be reciprocal to alteration in plasma lipid level in diabetic subjects as there is a possibility to change in membrane lipid composition, may be due to an alteration in plasma lipid composition, which in turn is under dietary fat intake as well as diabetes induced lipid changes and more family stress at middle age with less physical exercise might be the cause for more diabetic early cardiovascular and neurological symptoms. It is further evident from the table 2 that, the levels of mTC, mPL, and ratio are significantly altered in diabetes subjects as compared to normal. The values show a significant alteration in mPL/mTC ratio ( $P < 0.001$ ) and mPL ( $P < 0.02$ ) in group 3 compared to group 4. This may be due to drug interferers with phospholipids absorption or transportation mechanism to

the membrane. Whereas Insulin promotes phospholipids transport by stimulation of transport protein for exchange, this clearly suggests, drug interferes with transport process either blocking the active site protein/receptors of the membrane but insulin activates receptor sites for more uptakes. This increase in the ratio may be due to diabetes induced alteration in erythrocyte mPL levels, which in turn may be due to increase in Plasma phospholipids levels in diabetic subjects as compared to normal, Indicating probably insulin may have a role in phospholipids addition on to the membrane causing more flexibility in the membrane.

## CONCLUSION

Our results suggest that middle age group is more prone to risk for complications due to stress with less physical exercise as compared to other age groups. Further our study Shows anti Diabetic drug interferes with the transport of lipid probably on the

phospholipids transport protein molecule, where as Insulin might promote the more plasma PL transportation to RBC membrane by activation of protein transport molecules. This study concludes that the only anti diabetic drugs decrease transport mechanism of phospholipids but very specifically Insulin favors more Phospholipids transport as compared to other lipids- In spite of treatment on diabetes, there is anomaly of lipid composition with age and anti diabetic therapy. Hence, the middle age group (45-55 years) diabetes subjects were more at risk of microvascular complications than older age group (55 and above) and selection of anti diabetic treatment plays a significant role for presentation of complications . Research is needed to be undertaken to rule out the exact role of type of Drug/Insulin mechanism action and combined therapy of drug and insulin effect on controlling PL and TC transport mechanism on the membrane for prevention of early complications.

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