Influence of Anti Diabetic Therapy on Plasma Lipid Profile and its Relation to Erythrocyte Membrane Lipid Levels in Type 2 Diabetic Subjects

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Abstract

The diabetes induced dyslipidemia may lead to an alteration in RBC membrane cholesterol/phospholipids ratio in diabetic subjects resulting in an alteration in RBC membrane properties. It has been observed in our laboratory that diabetes induced dyslipidemia causes a change in RBC membrane lipid composition in type 2 diabetic subjects. The effect of various oral anti diabetic drugs and or Insulin therapy on diabetes induced RBC membrane lipid alteration is not established. Hence the present work was undertaken to study the influence of anti diabetic drugs and or Insulin on RBC membrane lipid composition in type 2 diabetic subjects. Blood samples from randomly selected type 2 diabetic subjects were collected after obtaining written consent. The plasma lipids as well as RBC membrane lipids were estimated. The study group include normal subjects (group-1), control diabetics diabetic subjects (group-2), diabetic subjects receiving oral drugs (group-3), diabetic subjects receiving insulin (group-4) and diabetic subjects receiving both oral drugs and insulin (group-5). The study suggest an increase in plasma lipid levels with a parallel raise in RBC membrane lipid composition in diabetic subjects and hypoglycemic drugs -insulin combined therapy regime may help to control the diabetic dyslipidemia induced erythrocyte membrane lipid alterations.

Index terms— RBC membrane lipids, anti diabetic drugs, plasma Lipids.

1 Introduction

iabetes Mellitus is a metabolic syndrome with disturbances principally in carbohydrate ,protein and lipid metabolism due to insulin deficiency or subnormal insulin functions.

In diabetic subjects overproduction of FFA and impaired lipoprotein metabolism induces an increase in plasma lipid components. (1). The long-standing diabetes induces micro vascular complications due to oxidative damage of membrane poly unsaturated fatty acids (2,3). The membrane fluidity is directly related to the membrane phospholipids and cholesterol which are asymmetrically arranged in the membrane lipid bilayer. The relative amounts of phospholipids and cholesterol are responsible for basic structural integrity of the red cell membrane. There are conflicting results in the literature regarding variations of red cell membrane lipid levels and their relevance to plama lipid alterations in diabetic subjects (4). Our earlier report clearly indicates a direct relationship between plasma lipid profile and the erythrocyte membrane lipid composition (5). However no reports available to show the influence of plasma lipid changes on erythrocyte membrane lipid levels and its relationship with anti diabetic therapy. Hence an attempt being made in the present study to investigate the influence of anti diabetic therapy on the diabetes induced plasma lipid alterations and its effect on red blood cell membrane lipid levels in type2 diabetic subjects receiving various anti diabetic drugs and or insulin.

2 II. Materials & Methods

The type 2 diabetic subjects attending the medical OPD of Sri B M Patil Medical College and Hospital Bijapur were randomly selected and a brief diabetic history with the anti diabetic therapy was collected from each of these selected diabetic subjects. An informed consent was also taken from these subjects. Blood sample in fasting state was collected from these diabetic subjects using heparin as an anticoagulant. The blood samples were centrifuged at 3500 rpm for 8 minutes to separate plasma which was employed for estimation of glucose (6), total lipids (7), total cholesterol (8),triacyl glycerol (9), HDL cholesterol (10) & free fatty acids (FFA) (11). The RBCs were washed three times with 4 ml aliquot of normal saline and the washed erythrocytes were lysed by adding 3ml distilled water and stirring with a clean glass rod. The resultant mixture was centrifuged at 3500 rpm for 5 minutes. The supernatant was discarded and the membranes were washed three times with 3 ml aliquots of normal saline. One part of the erythrocyte membranes were homogenized with 9 parts of chloroform-methanol mixture (1: 1, v/v) for 7 minutes in a Potter-Elvejham tissue homogenizer. The resultant mixture was centrifuged at 3500 rpm for 5 mins and the clear supernatant was employed for the estimation of lipid profile:-total lipids (7), total cholesterol (8) and total phospholipids (12).

The results were statistically evaluated with student "t"test. The diabetic subjects (Group 2) are compared with normal subjects (Group 1) and groups 3, group 4 and group 5 were compared with one another for statistical evaluation.

3 III. Results

The present study included a total number of 166 subjects consisting 36 normal subjects (Group 1) and 130 type 2 diabetic subjects (Group 2). These diabetic subjects included 86 diabetics receiving oral anti diabetic drugs (Group 3), 28 diabetics receiving insulin (Group 4) and 16 diabetics receiving both oral anti diabetic drugs and insulin (Group 5).

The results obtained in the present study are given table 1-3. Table **??** narrates the plasma levels of glucose and lipid profile levels in normal subjects(Group 1), in diabetic subjects (Group 2), in diabetic subjects receiving oral anti diabetic drugs (Group 3), in diabetic subjects receiving insulin alone (Group 4) and in diabetic subjects receiving both oral anti diabetic drugs and insulin (Group 5). It is seen from the table that the parameters included in the lipid profile (TL, TAG, PL and FFA) are significantly elevated in group 2 as compared to group 1 whereas the TL and FFA are significantly lowered in group 3, group 4 and in group 5 as compared to group 2.

Table **??** depicts the plasma cholesterol profiletotal cholesterol, HDL cholesterol, LDL cholesterol and VLDL cholesterol in group1, group 2, group3, group 4 and in group 5 subjects. It is evident from the table that total cholesterol , LDL cholesterol and VLDL cholesterol levels were significantly raised in group 2 as compared to group 1 whereas the HDL cholesterol is significantly lowered. Further it is evident from the table that there is no much change in the parameters studied in group 3 and group 5 as compared to group 2 but a significant decrease is seen in total cholesterol , LDL cholesterol and in VLDL cholesterol as well as a significant raise in HDL cholesterol is seen in group 4 as compared to group 2.

Table **??** shows the erythrocyte membrane lipid levels -total lipids (mTL), total cholesterol (mTC), phospholipids (mPL) and the ratio mPL/mTC in group 1, group 2, group 3, group 4 and in group 5. As it evident from the table mTL and mPL were significantly raised whereas the ratio mPL/mTC is significantly lowered in group 2 as compared to group 1. No much alterations observed in group 3, group 4 and in group 5 as compared to group 2 whereas a significant raise seen in group 4 as compared to group 2.

4 IV. Discussion

Diabetes mellitus is a chronic syndrome involving not only disturbance in glucose metabolism, protein but also there is disturbances in lipid and purin metabolism, resulting in varied life threatening complications like nephropathy, cardiopathy, retinopathy etc. (3,13,14). Apart from hyperglycemia and glucoseuria in diabetes mellitus, lipid alteration has been observed by many workers **??**15 -17, 19). A significant raise was observed in serum total lipids ($p<0.001$), serum total cholesterol ($p<0.001$), serum phosholipids. ($p<0.001$) and in serum total free fatty acids. ($p<0.001$) in diabetic subjects as compared to normal subjects. This in agreement with earlier studies **??**10, 18 -26). As well as with our earlier report (5).

The observed elevation in TL, TC, may be due to an increase in availability of more acetyl CoA, the starting substance for the synthesis of fatty acids and cholesterol (22). This is in part due to non availability of glucose for energy purpose and tissues do depend on fatty acid oxidation and increased fatty acid oxidation is responsible to increase cellular acetyl CoA concentration, hence favoring fatty acid, and cholesterol synthesis. The elevated serum TL, and serum TC in diabetic subjects as compared to normal subjects (Ref Table **??** and Table 2), may be in part due to decreased suppression of tissue lipolysis in diabetes mellitus, due to lack of Insulin. As insulin is known to suppresses tissue lipolysis (23,24,25).

Cholesterol is the principle sterol present in human plasma and its concentration in fasting serum amounts to 150-200 mg/dl in adults. This cholesterol is principally transported in plasma by lipoproteins. It is evident from the Table **??** total cholesterol ($p< 0.001$) VLDL-C ($p<0.01$) and LDL-C ($p< 0.001$) are significantly raised in diabetic subjects as compared to normal subjects, suggesting cholesterol synthesis as well as transport may be abnormal in diabetes mellitus. Lipoprotein lipase, a lipase different from other lipases, catalyses hydrolysis

of triacylglycerol (TAG) part of lipoproteins. TAG are transported in plasma mainly in the form of chylomicron and VLDL, these circulatory chylomicron, VLDL are acted by lipoprotein lipase, which also known as clearing factor. The plasma enzyme, LP lipase, is insulin sensitive and activity enhanced by insulin favoring the clearance of chylomicron, VLDL from circulating plasma. The result observed as shown in table 1 clearly indicates a elevation in serum TAG levels in diabetes as compared to normal subjects ($P < 0.\ 001$) may be in part due to non availability of insulin as insulin is essential for lipoprotein lipase activity.

In diabetes mellitus high incident of microvascular atherosclerotic disease has been associated with abnormality of erythrocyte composition and rheological function and with increased oxidative stress among many other factors. The increased blood viscosity seen in diabetes mellitus (31) is more in patients with established complications (32) and has been ascribed to decrease in erythrocyte deformability (31) and changes in erythrocyte membrane fluidity.

It is now well established that phospholipids distribution across erythrocyte membrane, bilayer is asymmetrical (32) Sphingomyelin and phosphatedylcholin, and most phosphatidylethhanolamine are present in inner side of the bilayer membrane. The presence of phosphatidylserine and phosphatidylethhanolamine on the inner side of the erythrocyte membrane has a biological significance. Phosphatidylserine plays a very significant role as a rate enhancing cofactor in blood coagulation cascade (33,34,37). And alteration in the levels of lipid components specifically cholesterol and phospholipids do effect the transport of glucose thus causing a subnormal glucose utilization leading to hyperglycemia (25).

An increase in the erythrocyte membrane lipid levels as well as the mPL/mTC ratio as evident from the table —in group 2 as compared to group 1 is in agreement with our earlier findings (5) and may be due to diabetes induced dislipidemia. Any such alteration in the erythrocyte membrane lipid composition may alter the glucose transport by altering the orientation of the membrane transport particles thereby affecting glucose uptake and utilization (39). A significant decrease in the mPL/mTC ratio is observed in Group 4 and Group 5 as compared to group 3 (ref table **??**) . Such an alteration in group 4 and in group 5 diabetics may be assumed due to a lipoprotein mediated exchange of lipid components from the plasma on to the erythrocyte membrane which may be due to insulin induced altered lipoprotein function and metabolism as it is known that insulin has a role in lipoprotein metabolism (30). Altered mPL/mTC in part which may be due to an exchange of fatty acids between plasma and erythrocyte membrane lipids. The plasma fatty acid levels as well as plasma lipid levels is under the influence of not only dietary fats but also on insulin amount and action. A change in fatty acid type and content of erythrocyte membrane lipid may alter the fluidity of membrane, hence may bring about an alteration in cell surface receptors (37).

The present study suggests that there is a definite change in the erythrocyte membrane lipid composition in type 2 diabetic subjects inducing a change in the cholesterol-phospholipids composition thereby inducing changes in the membrane behaviors whereas the insulin therapy or oral ant diabetic drugsinsulin combined therapy has a definite beneficial effect in controlling the diabetes induced lipid alterations in erythrocyte membranes thus controls any possible changes in the membrane properties. The present study suggests that insulin may have a role in phospholipids addition on to the membrane inducing more flexibility in the membrane as well as suggests that the oral hypoglycemic drugs -insulin combined protocol therapy may help regulation of normalcy of erythrocyte membrane lipid composition favoring better glucose utilization by the cells.

Lipoprotein in addition to the transport function of non polar lipid, particularly cholesterol, recently has been shown to impart an important role in the metabolism of the four major categories of lipo proteins (28). The low density lipo protein (LDL) and high density lipo protein (HDL) along with transport function cholesterol are also known to be involved in exchange of certain protein, apo protein and phospholipids with VLDL as well as it favors conversion of VLDL to LDL (29). The results of serum lipid profile levels in diabetic subjects receiving oral drugs (Group 3), receiving insulin (Group 4) or receiving both oral drug and insulin (Group 5) are depicted in (table **??** & 2). It is evident from tables no much difference is seen between group 3, 4 and 5 except TAG and TL T PL and HDLC, LDLC levels ($P < 0.001$) where as a significant alteration was observed in group 3, 4, 5 as compared to group 2 in the levels of TL, TFA, T PL, ($P < 0.001$) and HDLC, LDLC ($P < 0.001$). This may be in part due to alterations in the lipoprotein or its apo protein metabolism as diabetes mellitus may induce changes in the synthesis of apo proteins or over all metabolism of lipoproteins (30). It is also known that there exists a symmetrical bilayer distribution of lipid in biological membrane including erythrocyte membrane. Normally amine-rich lipids are on the inner side (cytoplasmic side) of the membrane where as cholinerich spingolipid is on outer surface. 39. Rudrappa G, Basavaraj Aski, Kashinath R T (2011).

Erythrocytes membrane Lipid Alteration In Type 2

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Table : 1 Table : 3 [1]

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Figure 1: Note: 1)

showing

Parameters	Group 1 Normal Subjects (36)	Group 2 Diabetic Subjects (130)	Group 3 Diabetics Receiving Oral Dr
Fasting plasma Glucose mg/dl Total Lipids mg/dl	85.54 ± 13.65 705.62 ± 128.80 108.95 ±	156.20 ± 35.31*** 1348.96 ± 103.58*** 235.29 ±	
Triacylglycerol mg/dl	20.14 16.62 ± 3.18 166.51 ± 8.28 Note : 1) The number in parenthesis shows the number 31.66*** Total Ph		

Gp 3 & 5 by -? Gp 5 & 3 by -?

Figure 2: Table showing plasma

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LDL Cholesterol mg/dl	117.41 ± 23.90	123.83 ± 39.20. ***	120.61 ± 12.81	136.23 ± 16.58??? ??	130.30 ± 15.18?
VLDL Cholesterol mg/dl	22.13 ± 5.61	46.25 ± 8.51. ***	45.50 ± 6.16	47.33 ± 8.18	44.38 ± 6.78
Parameters	Group 1 Normal Subjects (36)	Group 2 Diabetic Subjects (130)	Group 3 Diabetics Receiving Oral Drugs alone (86)	Group 4 Diabetics Receiving Insulin alone (28)	Group 5 Diabetics Receiving Both Oral Drugs & Insulin (16)
Total Cholesterol mg/dl	144.22 ± 26.12	253.58 ± 35.90. ***	228.81 ± 20.62	257.72 ± 22.18???	242.48 ± 19.90 ??
HDL Cholesterol mg/dl	41.38 ± 9.36	37.37 ± 5.65	37.18 ± 4.40	41.21 ± 5.50???	38.25 ± 6.23

Figure 3: Table : 2

Showing

Membrane Phospholipid/Cholesterol Ratio (mPL/mTC)	6.40 ± 0.64	4.73 ± 0.28***	5.09 ± 0.53??? ???	4.45 ± 0.2	4.66 ± 0.31??
Parameters	Group-1 Normal subjects (36)	Group-2 Diabetic Subjects (130)	Group 3 Diabetics Receiving Oral Drugs alone (86)	Group 4 Diabetics Receiving Insulin alone (28)	Group5 Diabetics Receiving Both Oral Drugs & Insulin (16)
Membrane Total Lipid mg/dl (mTL)	5.02 ± 1.62	5.35 ± 1.53	5.08 ± 0.88	5.22 ± 0.76	5.64 ± 0.94 ???
Membrane Total Cholesterol mg/dl (mTC)	1.16 ± 0.32	1.72 ± 0.10***	1.68 ± 0.63	1.76 ± 0.36	1.69 ± 0.48
Membrane Total Phospholipids mg/dl (mPL)	7.36 ± 1.78	8.18 ± 0.88**	7.61 ± 0.66	7.94 ± 0.71? ???	7.38 ± 0.44

Figure 4: Table Showing m

[Varley et al.] , H Varley , H Alan , Maurice . London: Heineman Professional publishing Ltd. p. .

[Conathy and Povic ()] , M C Conathy , WJ , Alu Povic . *Reviews of Biochemistry* 1976. 15 p. . (Biochem. 2 nd edition Page 215)

[Tall and Small ()] , A Tall , D Small . *Journal of Medicine* 1978. p. 1232.

[Karnieli et al. ()] , E Karnieli , M J Zarnowski , P J Hissin , T A Simpson , L B Salans , S W Cushman . 1981.

[Brown et al. ()] , M S Brown , P T Kowanin , J L Goldstein . *Science* 1981. 212 p. 628.

[Brown et al. ()] , M S Brown , P T Kowanin , J L Goldstein . *Science* 1981. 212 p. 628.

[Aq and Fass ()] , Dang Aq , F H Fass , CorterW . *J. -lipids* 1984. 19 p. .

[Steiner and Dyslipoproteinemias ()] , G Steiner , Dyslipoproteinemias . *diabetes atherosclerosis* 1994. 110 p. . (suppl)

[Robert and Murray ()] , K Robert , Murray . *Harpers Biochemistry* 2000. Appleton and Lange Publication. p. . (25th Edition)

[Vague and Dris ()] *Abnormalities of myth membrane lipids in insulin dependent diabetics are improved by shorter term control of diabetes. C/in Haematology*, Juhan Vague , J Dris . 1984. 4 p. . (Roul cedel)

[American Diabetes Association management of dyslipidemia in adults with diabetes Diabetes care (200)] 'American Diabetes Association management of dyslipidemia in adults with diabetes'. *Diabetes care* 200. 23 (1) p. . (suppl)

[Harper et al. ()] *Biochemistry 2 nd edn*, Robert K Harper , Peter A Murray , Daryl K Mayes , Grannes Victor , W Rodwell . 2000.

[Harper et al. ()] *Biochemistry 2 nd edn*, Robert K Harper , Peter A Murray , Daryl K Mayes , Grannes Victor , W Rodwell . 2000.

[Nehal et al. (1990)] 'Changes in lipid composition of red blood cells in hyperglycemic rats'. M Nehal , P Venugopal , Z Najma , Baquer . *Biochemistry international* 1990. Oct. 22 p. .

[Choudhary ()] K Choudhary . *Biochemical Techniques', Japeee Brothers*, (New Delhi) 1989. p. .

[Richard and Henry ()] 'Clinical chemistry principles and practice 2 nd edn'. J Richard , Henry . *Herper row publishers*, (New York, Chapter) 1974a. 28 p. .

[Herbert et al. ()] *Clinical chemistry: Theory, Analysis, correlation*, K Herbert , ; A Lipid , Kaplan , J P Amadeo , Stevenc Kazmierczak . 2003 Mosby. 1182-1230.

[Zwaal] *Comfurius and LLM van Deenen. 1977 membrane asymmetry and blood coagulation, nature (lord) 268*, Rfa P Zwaal . p. .

[Grant et al. ()] 'Differences in the nature of the interaction of insulin and proinsulin with zinc'. P T Grant , T L Coombs , B H Frank . *Biochem J* 1972. 126 p. .

[Grove ()] 'Effect of reagent pH on determination of high density lipoprotein cholesterolby precipitation with sodium phosphor tungstate magnesium'. T H Grove . *Clin Chem* 1979. 25 p. .

[Henary ()] Richard Henary . *Clinical Chemistry Principle and Practice 2 nd edition. Row publishers New York. Chapter 25, Carbohydrates*, 1974. p. .

[Abbott et al. ()] 'High density Lipoprotein cholesterol screening and myocardial Infraction. The Framingham study'. R D Abbott , P Wilson , W B Kannel , W P Cashelli . *Arteriosclerosis* 1988. p. .

[Sushil and Jain ()] 'In vivo externalization of phosphotidyl choline and phosphotidyl ethanolamine in membrane bilayer and hyperactive coagulability by lipid peroxidation of erythrocytes of rats'. K Sushil , Jain . *J Grin Investigation* 1985. 76 p. .

[Aski et al. ()] 'Influence of Serum Lipid Levels on Erythrocyte Membrane Lipid Composition in Diabetes Mellitus'. Basavaraj Aski , G Rudrappa , R T Kashinath . *Journal of Advance Researches In Biological Sciences* 0975-3923. 2010. 2 (1) p. .

[James et al. ()] 'Insulin -regulatable tissue express unique insulin -sensitive glucose transport protein'. D E James , R Brown , J Navarro , P F Pilch . *Nature* 1988. 333 p. .

[Insulin stimulated translocation of glucose transport systems in adipose cell J. Biol. Chem] 'Insulin stimulated translocation of glucose transport systems in adipose cell'. *J. Biol. Chem* 256 p. .

[Calderhead and Lienhard ()] 'Labeling of glucose transporter at the cell surface in 3T3-LI adipocytes'. D M Calderhead , G E Lienhard . *J. Biol. Chem* 1988. 263 p. .

[Varly ()] *Lipid and lipoproteins. Chapter 21*, H Varly . 1980. (Inpractical chemical biochemistry)

[Locke and Tarsy ()] S Locke , D Tarsy . *The nervous system and diabetes in Joslin's diabetes mellitus. K.M. Varghese Company (India), 12 th Edition*, 1985. p. .

[Nath ()] *Practical Biochemistry in clinical medicine 2 nd edn*, R Nath . 1990. Academic Publisher Calcutta fattyacid page.

[Nath ()] *Practical Biochemistry in clinical medicine 2 nd edn. Academic Publisher Calcutta*, R Nath . 1990. p. .

[Agutter et al. ()] 'Properties of mammalian nuclearenvelope nucleoside triphosphatase'. P S Agutter , J B Cockrill , J E Lavine , B Mc Caldin , R B Sim . *Biochem. J* 1979. 181 p. .

[Prisco et al. ()] 'Red blood cell lipid alterations in type II diabetes mellitus'. D Prisco , R Paniccia , M Coppo . *Thrombus Res* 1989. 54 p. .

[O'brien and Granner ()] 'Regulation of gene expression by insulin'. R M O'brien , D K Granner . *Biochem. J* 1991. 278 p. .

[Hamid Zargar et al. ()] 'Serum lipid profile in non insulin dependent diabetes mellitus dissociated with obesity'. Abdul Hamid Zargar , Farooq Ahmad Wandroo , Bashir Mool Brahman Wadhwa , Ahmad . *INT. J DIAB DEU* 1995. 15. (Countries)

[Aiello et al. ()] 'The Eye and Diabetes'. L M Aiello , L I Rand , J G Sebastyen , J N Weiss , M J Bradbury , M Z Wafai , J C Briones . *Joslin's Diabetes Mellitus. K. M. Verghese Company (India)*, 1985. p. 12.

[Stubbs and Smith ()] 'the modification of mammalian membrane polyunsaturated fatty acid composition in relation to membrane fluiding and function -Biochem'. C D Stubbs , A D Smith . *Biophys. Acta* 1984. (779) p. .

[UK prospective diabetes study 27: plasma lipid and lipo proteins at diagnosis of NIDDM by age and sex. Diabetic care ()] *UK prospective diabetes study 27: plasma lipid and lipo proteins at diagnosis of NIDDM by age and sex. Diabetic care*, 1997. 20 p. . UKDDS Group

[World Health Organization; Diabetes mellitus ()] *World Health Organization; Diabetes mellitus*, Series Na. 727. 1985. Geneva, World Health Organization. WHO study group (Technical Report)