Diabetes Disease Burden by Platelet Indices As Possible Biomarkers in Evaluation of Initial Vascular Risks in Grading Diabetes Mellitus Part I: Correlation of Platelet Dysfunction Indices With Hematopoietic and Biochemical Parameters

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Abstract

Diabetes disease burden in Diabetes mellitus was explored for its possible association with hematopoietic indices and serum predictors of hypertension with dyslipidemia. Hypothesis was that disease burden of diabetes is correlated with change in serum levels of sugar, lipoproteins, urea, uric acid, creatinine and hematological parameters as consequence of platelet dysfunction manifested as dyslipidemia, nephrosis, thrombosis and diabetes mellitus. A total of 125 diabetic patients with different disease burden were analyzed for correlation and contribution of serum sugar, lipoproteins, urea, uric acid, creatinine and hematological parameters with platelet dysfunction as diabetes disease burden index’. Results showed that ‘diabetes disease burden index’ may reflect the initial trigger of dyslipidemia, hypertension followed by progressive renal complications with growing risk of atherothrombosis as increased burden and uncontrolled diabetes based on comparison of P values between diabetes groups. Males in their fifties age were at high risk of diabetes. Change in serum levels of sugar, lipoproteins, urea, uric acid, creatinine as contributing factors showed ‘diabetes disease burden index’ with increased risks in the order of sugar < lipids < platelet indices < urea < creatinine < hematological parameters < uric acid. Deranged platelet indices were closely associated with initial vascular complication and progressive diabetes. In conclusion, diabetes disease burden associated with platelet indices, biochemical and hematopoietic biomarkers served as better risk indicators of progressive vascular complications in diabetes mellitus patients. Key words: Diabetes disease burden, Platelet index, Diabetes mellitus grading, atherosclerosis, dyslipidemia, Diabetic kidney disease

I. Introduction

Diabetes mellitus disease burden and its grading is a current challenge to evaluate disease severity in both type 1 and type 2 diabetes mellitus forms. Type 2 diabetes ranges from insulin resistance with relative insulin deficiency to an predominantly insulin secretory defect with insulin resistance. The prevalence of type 2 was estimated to be 150 million people in year 2000 and set to rise 300 million in 2025(1). In last decade,
emphasized was focused to explore possible measures of disease burden and factors responsible to induce platelet dysfunction and insulin resistance with chronic development of cardiovascular risks among diabetic subjects with prothrombotic tendency (2).

Platelet physicochemical changes such as elevated platelet production measured by mean platelet volume (MPV), increased aged platelet heterogeneity measured by platelet distribution width (PDW), large platelet number measured by platelet large cell ratio (P-LCR), play a significant role in development of diabetes mellitus type 2 disease (3-5). Still, it is dilemma how platelet sub-physiological changes in hyperglycemia condition increase the MPV initially leading to osmotic swelling as a result of raised blood glucose level orglucose metabolites precipitated with metabolic syndrome, stroke and diabetes (6). From biochemical standpoint, it is established that increased insulin receptor number and poor affinity of insulin receptor on platelets cause the reduced insulin sensitivity and increased platelet volume or platelet hyperactivity (7). Hyperactive platelets show significantly increased MPV, PDW as a result of impaired thrombopoiesis in diabetes mellitus (8-9). However, elevated platelet size of hyperactive platelets during hyperglycemia serves as precursor in micro-vascular complications of diabetes mellitus and ultimately beginning of endothelium dysfunction and atherosclerosis (10).

Initial hyperglycemia is believed as major cause of non-enzymatic glycation of platelet membrane (10). Later, alteration in protein structure and conformation in platelet membrane causes rearrangement of platelet membrane lipids and lipid dynamics to lead hyperlipidemia. The hyperlipidemia derived changes were seen in platelet membrane dynamics to trigger the “vicious circle” to cause alterations in membrane fluidity, platelet activation, platelet release and platelet volume distribution (11-13). Rearranged lipid dynamics regulates the enhanced expression of crucial platelet receptors such as p-selection and GP IIb/IIIa receptors to elevate platelet activity (14-15). Authors believe that dyslipidemia and platelet protein changes both modulate platelet membrane dynamics to cause altered platelet indices with a consequence of thrombopoiesis stimulated by higher blood glucose levels in diabetes mellitus.

In advancing diabetes type 2 individuals, altered interaction of platelets with the walls ofsmall and large arteries cause the damage in parenchymal organs (retina, kidney, heart, brain etc) to enhance classical cardiovascular risk and vascular damage. In later stages, platelet aggregation and adhesion results intravascular thrombosis and vascular occlusion that might cause atherosclerosis plaque disruption to cause ischemic coronary and cerebrovascular events (16). Diabetic patients are unusually at risk of diabetic cardiomyopathy, microvascular disease, glycosylation of myocardial proteins, and autonomic neuropathy with final congestive heart failure (17).

In spite of all above information, still less is known if diabetes disease burden index or predisposing factor(s) may reflect the initial disease progress of diabetes mellitus and its later consequences to evaluate the cardiovascular and renal risks. In this direction, authors suggest that cross sectional survey on the association of initial platelet membrane physicochemical changes with serum biomarkers may explain better disease burden of the emerging initial cardiovascular and renal risks in initial stages of diabetes type 2 developments. It may suggest evidence of a link between insulin resistance and platelet activation to progress cardiovascular and renal disease burden in initial diabetes.

The present study will explore the feasibility of platelet indices as initial predictors of disease burden of growing cardiovascular risk and renal complications in diabetes mellitus with or without presence of factors like hypertension and dyslipidemia.

To accomplish the above, comparison of platelet indices- mean platelet volume (MPV), platelet distribution width (PDW) and platelet large cell ratio (P-LCR)-with hematopoietic indices and serum biomarkers in healthy controls was proposed with biomarkers in diabetes mellitus type 1 patients suffering with predisposing factors of hypertension and dyslipidemia.

The novelty of this study is that association and/or contribution of hypertension or dyslipidemia as predisposing factors will be singled out if any or both factors together may initiate the disease burden or growing risk of platelet membrane physicochemical changes with possible complications of insulin resistance, serum biomarker alteration, vascular damage, vulnerable plaque and lately sudden cardiac death. The study has potentials to explain the mechanism of platelet dysfunction and role of platelet physicochemical changes occurred as a result of disease burden (endothelial dysfunction and insulin resistance) manifested as altered serum biomarkers and hematopoietic changes during activation in hypertension and dyslipidemia.

Platelet Activation and Diabetes Mellitus Disease Burden
The platelets are produced in bone marrow. The main physiological function of blood platelet membrane is to maintain hemostasis by initiating and forming a hemostatic plug. Simultaneously, secretion of active factors leads to the repair of vascular injuries (17).

The platelet cytoplasmic membrane is made of a bilayer of polarized phospholipids containing arachidonic acid. The external layer of the platelet membrane contains abundant platelet GPIIb/IIIa glycoprotein
as fibrinogen receptor. The poor regulation of these glycoprotein fibrinogen receptors during platelet activation seems a major player in diabetes disease progress and may cause vascular injury and tissue damage (18-19). Diabetes disease burden index (DBI) is proposed in this study as sequential changes in platelet parameters and associated biochemical, hematopoietic parameters due to platelet activation as manifestations of diabetes control level in patients as shown in Figure 1.

Figure 1: A systematic algorithmic approach is presented supporting the diabetes disease progress from good control to uncontrolled form indicated by platelet indices and biochemical/hematopoietic biomarkers (DBI) in prognosis of disease as risk levels: 1 self care; 2 medical attention; 3 supervised care; 4 hospital care.

Platelet activation abnormalities occur during diabetes mellitus at various levels of disease progress. Platelets in mild controlled diabetes, exhibit the increased reactivity (i.e. increased platelet aggregation) due to high glucose associated with endothelial dysfunction, oxidative stress and inflammation, platelet activation to reduce nitric oxide and thrombin formation (20-21). Further, advanced hyperglycemia triggers the glycation of platelet protein while dysfunction of whole coagulation cascade and inflammation (hematopoietic biomarkers) triggers the platelet activation in mild controllable diabetes (22). In addition, hypertriglyceridemia, insulin resistance, insulin deficiency also increases the platelet reactivity (23). In uncontrolled diabetes mellitus, disturbed carbohydrate and lipid metabolism lead to physicochemical changes in cell membrane dynamics. It includes altered phospholipid organization, adhesion and aggregation, hypersensitivity to agonist and increase the sequential events of endothelial dysfunction as prothrombotic states leading to atherosclerosis, nephrosis, retinopathy and neuropathy (24). It is believed that prothrombotic states are manifested as altered serum markers and hematopoietic markers. It results in altered exposure of surface membrane receptor with subsequent altered serum lipids and other serum biomarkers (24). The “primed” diabetic platelets respond more frequently to sub-threshold stimuli, and become exhausted, consumed and finally turn to ‘hyporesensitive’ in lesser time span. As a result, platelets accelerate the thrombopoiesis and release of ‘fresh’ hyperactive platelets. Improved metabolic control or improved insulin sensitivity preserves the pancreatic beta-cells function to decrease platelet reactivity or acts as anti-platelet factors. Another factor is also intensity of diabetic stimuli.

II. Materials And Methods
The study was conducted at the Department of Pathology, Hindu Rao Hospital Delhi sponsored by National Board of Medical Specialties, New Delhi funded post-graduate medical education program in the year 2013.

A total of 222 cases (male 111 and female 111) included in our study comprising of 125 diabetic patients and 97 non-diabetic control subjects. Patients were categorized into four groups i.e. group I (uncontrolled diabetes mellitus positive) with no atherosclerosis risk (n=29), group II (mild diabetes)
with one or more atherosclerosis risk (n=96), group III (mild controllable diabetes) with no atherosclerosis risk factors (n=22) and group IV (mild controlled diabetes) with one or more risk factor (n=75).

All four groups were analyzed for various hematological parameters like Hb, HCT, RBC COUNT, MCV, MCH, MCHC, RDW-SD, TLC,PC, MPV, PDW and P-LCR. RBC parameters were evaluated by auto analyzer.

Disease burden was evaluated by % fold changes (significant different P values) in platelet parameters and associated biochemical, hematopoietic parameters predicting the severity and control of diabetes disease in different diabetes groups. For it, Comparative analysis was done by Mann-Whitney test for biochemical parameters including serum fasting and postprandial glucose, glycated Hb A1c as indicator of diabetes control, serum cholesterol, triglycerides, HDL, LDL, serum creatinine, urea levels of subjects in group I with groups II, III, and IV. P values were calculated. Comparative analysis was done by Mann-Whitney test for various hematological parameters like Hb, HCT, RBC COUNT, MCV, MCH, MCHC, RDW-SD, TLC,PC, MPV, PDW and P-LCR. RBC parameters of subjects in group I with groups II, III, and IV. P values were calculated. Multivariate analysis was done for evaluation of contributory factor(s) to demonstrate association of platelet indices (PC, MPV, PDW and P-LCR) with, biochemical or hematological factor.

III. Results And Observations

Group I diabetic patients without one or more other risk factors for atherosclerosis and renal disease (n=29), group II consisted of diabetic patients with one or more other risk factor than atherosclerosis and renal disease (n=96) among which 61 cases had altered body mass index, 37 cases were smokers, 52 cases were hypertensive at time of study, 78 cases were dyslipidemic and lastly eight cases had diabetic vascular complications. Group III consisted of non-diabetic (control) subjects without one or more risk factors for atherosclerosis (n=22) and group IV consisted of non-diabetic (control) subjects with one or more risk factors for atherosclerosis (n=75), among which 41 cases had altered body mass index, 35 cases of smokers, 32 cases with hypertension at time of study, 52 cases with dyslipidemia and one case of coronary artery disease.

The mean age of patients in our diabetic group without risk factors and diabetic group with other risk factors was 50.86 ± 12.56 years and 51.59 ± 10.21 years. In control group without risk factors and control group with risk factors, mean age was 41.55 ± 12.44 years and 44.19 ± 11.19 years.

Mean ± SD values of platelet parameters were observed in group I (n=29); group II (n=96); group III (n=22); and group IV (n=75) for different platelet indices to evaluate disease burden as shown in Figure 2. DBI is shown in Table 1 based on significant differences in P values.

![Figure 2](https://example.com/figure2.png)

**Figure 2**: Diabetes disease burden by platelet parameters between various groups (Y-axis represents values of platelet parameters in conventional units): mean platelet count (PC) 2.01 ± 0.72 lac/cumm (range 1.37 to 5.20 lac/cumm in group I), 2.09 ± 0.86 lac/cumm (range 1.00 to 5.80 lac/cumm in group II), 2.28 ± 0.88 lac/cumm (range 1.50 to 4.97 lac/cumm in group III) and 2.27 ± 0.81 lac/cumm (range 1.00 to 5.21 lac/cumm in group IV) with no significant statistical difference in PC value in groups I and II (P=0.923), groups I and III (P=0.254), groups II and III (P=0.229), and groups III and IV (P=0.823); mean platelet volume (MPV) 12.46 ±1.36 fL (range 9.1 to 14.9 fL in group I), 11.97 ±1.37 fL (range 8.5 to 14.5 fL in group II), 10.98 ± 1.10 fL (range 8.2 to 13.7 fL in group III) and 11.42 ± 1.42 fL (range 8.5 to 14.8 fL in group IV) with no significant difference in MPV value in group I and group II (P=0.128) but strong statistical difference in MPV value in group I and group II (P<0.001) with strong statistical difference in MPV value in group I and group II (P<0.001).
Diabetes Disease Burden by Platelet Indices As Possible Biomarkers in Evaluation of Initial... III (P=0.000), groups II and III (P=0.001) as well as in group III and group IV (P=0.233); platelet distribution width (PDW) 19.78 ± 3.85 fl (range in 11.4 to 26.20 fl in group I, 18.27 ± 5.13 fl (range 9.9 to 36.1 fl in group II, 15.60 ± 3.81 fl (range 10 to 23.2 fl in group III), and 16.42 ± 4.15 fl (range 10.6 to 25.9 fl in group IV) with significant difference in PDW value in groups I and II (P=0.045) but strong statistical difference in groups I and III (P=0.000) as well as in groups II and III (P=0.026), groups III and IV (P=0.502); platelet large cell ratio (P-LCR) 44.71±10.15 % (range 22.3 to 62.7 % in group I, 39.81±10.16 % (range 14.8 to 59.9 % in group II, 33.42 +11.09 % (range 13.7 to 54.9 % in group III) and 36.38 +11.34 (range 15.2 to 61.1 % in group IV) with significant difference in PDW value in groups I and II (P=0.034), in groups I and III (P=0.001) but strong statistical difference in groups II and III (P=0.010), groups III and IV (P=0.283).

The disease burden and level of diabetes control was evaluated by fasting blood sugar levels 149.93 ± 63.65 mg/dl (range 92 to 367 mg/dl) in uncontrolled diabetes mellitus positive group I, 179.16 ± 88.26 mg/dl (range 82 to 142 mg/dl) in mild diabetes group II, 89.77 ± 8.52 mg/dl (range 69 to 106 mg/dl) in mild controllable diabetes group III, 89.03 ± 11.31 mg/dl (range 69 to 109 mg/dl) in mild controlled diabetes group IV as shown in Figure 3. Postprandial sugar levels 227.85 ± 93.95 mg/dl (range 108 to 542 mg/dl in uncontrolled diabetes mellitus positive group I), 259.28 ± 107.03 mg/dl (range 110 to 727 mg/dl in mild diabetes group II), 128 ± 25.62 mg/dl (range 103 to 162 mg/dl in mild controllable diabetes group III), 119.94 ± 15.05 mg/dl (range 89 to 140 mg/dl in mild controlled diabetes group IV) with no significant statistical difference in groups I and II (P=0.152), groups III and IV (P=0.493), but significant difference in groups I and III (P=0.000), and groups II and III (P=0.000) as shown in Figure 4. Mean HbA1c values 7.42 ± 1.12 % (range 6.6 to 11.5 % in ) in uncontrolled diabetes mellitus positive group I, 7.98 ± 2.06 % (range 4.3 to 13.7 % in mild diabetes group II), 4.83 ± 0.34 % (range 4.10 to 5.50 % in mild controllable diabetes group III) and 5.67 ± 0.59 % (range 4.0 to 6.40 % in mild controlled diabetes group IV) with no significant statistical difference in groups I and II (P=0.071) but significant difference in groups I and III (P=0.000), groups II and III (P=0.000), and groups III and IV (P=0.000) as shown in Figure 5. Platelet associated serum dyslipidemia mean ± SD values in different groups are shown in Figures 6-9. Platelet associated renal function biomarkers mean ± SD in different groups are shown in Figures 10-11.

Figure 3: Diabetes disease burden by fasting blood sugar values between various groups is shown as no significant statistical difference in groups I and II (P=0.054), groups III and IV (P=0.274), but significant difference in groups I and III (P=0.000), and groups II and III (P=0.000).

Figure 4: Diabetes disease burden by postprandial blood sugar values between various groups is shown as no significant statistical difference in groups I and II (P=0.152), groups III and IV (P=0.493), but significant difference in groups I and III (P=0.000), and groups II and III (P=0.000).
Figure 5: Diabetes disease burden by Hb A1c values between various groups is shown as no significant statistical difference in groups I and II (P=0.071), but significant difference in groups III and IV (P=0.000), in groups I and III (P=0.000), and groups II and III (P=0.000).

Figure 6: Diabetes disease burden by serum cholesterol values between various groups is shown as no significant statistical difference in groups I and III (P=0.189), groups III and IV (P=0.014) but significant difference in groups I and II (P=0.000), groups II and III (P=0.000).

Figure 7: Diabetes disease burden by serum triglyceride values between various groups is shown as no significant statistical difference in groups I and III (P=0.270) but significant difference in groups I and II (P=0.001), in groups II and III (P=0.000), groups III and IV (P=0.006).
Figure 8: Diabetes disease burden by serum HDL values between various groups is shown as statistical difference in groups I and III (P=0.036) but significant difference in groups I and II (P=0.046), groups II and III (P=0.025), and groups III and IV (P=0.022).

Figure 9: Diabetes disease burden by serum LDL values between various groups is shown as significant statistical difference in groups I and II (P=0.000), groups I and III (P=0.0180), groups II and III (P=0.000), groups III and IV (P=0.000).

Figure 10: Diabetes disease burden by serum urea values between various groups is shown as significant statistical difference in groups I and II (P=0.000), groups I and III (P=0.0180), groups II and III (P=0.000), groups III and IV (P=0.000).
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Figure 11: Diabetes disease burden by serum creatinine values between various groups is shown as no significant difference in group I and III (P=0.081) but significant difference in groups I and II (P=0.003), in groups II and III (P=0.000), in groups III and IV (P=0.002).

HEMATOLOGICAL PARAMETERS
Hematological parameters Hb, HCT, RBC COUNT, MCV, MCH, MCHC, RDW-SD, TLC,PC and RBC parameters were evaluated in all four groups as shown in Figure 12.

TOTAL LEUCOCYTE COUNT (TLC)
Mean + SD were observed in group I(n=29); group II(n=96); group III(n=22); and group IV(n=75) of TLC: 8173.45±1650.33 (range 4800-12400/cumm in group I), 7828.96+1816.77 (range 3960-12400/cumm in group II), 7828.96±1816.77 (range 3960-12400/cumm in group II), 1634.08± 145.45(range 4000-11000/cumm. In group III), 7324.3±1989.42 (range 4100-15800/cumm in group IV) with no significant difference in groups I and II(P=0.341), in groups III and IV (p=0.111) but significant difference in groups I and III (p=0.002) and II and III(P=0.005).

Figure 12: Diabetes disease burden by RBC parameters between various groups (Y-axis represents values of red cell parameters in conventional units) is shown different biochemical parameters. Hb shows no significant difference in groups I and II, groups I and III, groups II and III, groups III and IV. Hematocrit value shows no significant difference in groups I and III, groups II and III but significant difference in groups I and II (p=0.042), groups III and IV (p=0.007). RBC shows no significant difference in groups I and II, I and III, II and III, III and IV. MCV reflects anisocytosis with no significant difference in groups I and II (p=0.050) and other groups I and III, II and III, III and IV, MCH shows no significant difference in groups I and III (p=0.011), I and II, III and IV but significant difference in groups II and III(p=0.032), MCHC shows no significant difference in groups I and III but significant difference in groups I and II (p=0.007), group II and III( p=0.000), groups III and IV (p=0.001). RDW-SD shows no significant difference in groups I and II, I and III, II and III, III and IV.
Diabetes Disease Burden Evaluation for Grading Diabetes

Different diabetes groups with different HbA1c control levels were graded based upon comparison of platelet parameter P values and associated biochemical and hematopoietic biomarkers. Uncontrolled diabetes group showed higher significant difference in P values of MCV, PDW, HbA1c, serum glucose, biochemical and hematopoietic parameters while mild controlled diabetes group showed least or no significant difference in P values of said parameters as shown in Table 1.

Table 1: Different P value significant levels are shown in different diabetes groups to visualize as quick guideline to evaluate different prognostic levels of diabetes control and diabetes associated factors as cardiovascular and renal risks. Diabetes disease burden index is shown in brackets in each group. *Highly significant different P values indicated the level of poor diabetic control and high risk of cardiovascular, renal and neural diseases.

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IV. Discussion

Diabetes is a growing health problem, associated with increased risk of atherosclerosis and its thromboembolic complications affecting retina, brain, kidney, muscles with life threatening states of inflammation, stroke, cardiac arrest and obesity.

In recent years, it has been a dilemma on subphysiological cause of insulin resistance in beta cells under the influence of circulating blood high glucose and possible role of anuclear megakaryocyte fragments released from bone marrow so called ‘platelets’ during subphysiological stages of adhesion, aggregation, secretion of fibrinogen alpha granules and dense bodies leading to visible physiological stages of platelet activation, thrombin formation for plasma coagulation, clot retraction and support to injured endothelium to correct the vascular smooth muscle contraction(25). During subphysiological stages of dense bodies, epinephrine, adenine nucleotides, serotonin molecules play significant role perhaps in the event of high circulating glucose in blood.

To solve the said paradox of subphysiological platelet changes during pre-diabetes, study is an attempt in the direction of possibility if biochemical and hematological biomarkers can predict the association or role of platelets in progressive diabetes burden (diabetes without factors) or established uncontrolled diabetes mellitus burden (with factors) leading to cardiovascular, renal and hematopoietic complications. Major complications were dyslipidemia, glycation and vascular changes in diabetes initiated with platelet physiological dysfunction in present study. Present study also reflects a sequential disease burden measurement index of progressive diabetes if visible by platelet parameter values to distinguish controlled, controllable and uncontrolled diabetic population from non-diabetic population for possible link between platelet reactivity with biochemical and hematological parameters as risk predictors or insulin deficiency and/or insulin resistance biomarkers in order to figure out contributory factor(s) likely to enhance the metabolic and/or cardiovascular events. However, association of prethrombotic state and inflammation could not be concluded.

Scattered reports are available on platelet reactivity and associated insulin resistance, insulin deficiency and dyslipidemia, renal dysfunction in progressive diabetes in different control levels. Major focus was on to identify initial lipid abnormalities to cause vascular damage in advancing diabetes and its poor control. Initially in type 2 diabetes with obesity/insulin-resistant metabolic disarray leads to lipid abnormalities (initial release of fatty acids from adipose tissue, increased production of VLDL,TG , and cholesterol)due to hyperglycemia and insulin resistance in the fat cells in liver. In late stages, increased plasma TG levels are the major “driving force”
for low HDL-C and abnormal, small dense LDL. Insulin-resistance fat cells further breakdown of their stored triglycerides and subsequent release of free fatty acid into the circulation in both obese and non-obese insulin-resistant subjects and those with type 2 diabetes. Increased fatty acid in the plasma leads to elevated fatty acid uptake by the liver to synthesize them back into triglycerides. As a result of the presence of increased triglycerides, it further stimulates the assembly and release of the apolipoproteins (apo) B and very low density lipoproteins. This results in an increased number of VLDL particles and increased level of triglycerides in the plasma, which eventually leads to the rest of the diabetic dyslipidemia picture. Moreover, in population studies and small clinical studies, small, dense LDL was reported with high triglycerides and low HDL cholesterol in the plasma. Hypertriglyceridemia is related with the accumulation of chylomicron remnants and VLDL remnants, both possibly atherogenic contents. Hypertriglyceridemia was also seen associated with increased coagulability, decreased fibrinolysis and increased levels of plasminogen activator inhibitor 1 (PAI-1) and factor VII leading to activation of prothrombin to thrombin (26).

To establish diabetes disease burden, it is important to understand impaired platelet membrane fluidity changes due to glycation of proteins and changes in lipid composition in DM along with increased fibrinogen binding, prostanoid metabolism, phosphoinositide turnover and calcium mobilization. From biochemical standpoint, glycation of circulating low-density lipoproteins (LDL) may render platelets as hypersensitive. Glycated LDL causes an elevation in intracellular calcium levels and platelet nitric oxide (NO) production, as inhibition of the platelet membrane Na’/K’-adenosine triphosphatase (Na’/K’-ATPase) activity. Simultaneously, other lipid abnormalities such as increased levels of triglyceride, small dense LDL and low high-density lipoproteins (HDL) also affect platelet function by interfering with membrane fluidity and intracellular systems in type 2 diabetes (27). The presence of elevated triglycerides and decreased HDL levels are the best predictors of cardiovascular disease in patient with type 2 diabetes (28). Thus, larger platelets are more reactive and contribute to vaso-occlusive events in patients with dyslipidemia. Hence, P-LCR may be used as indicator of thromboembolic ischemic events. Poor glycemic management worsens abnormalities as diabetic nephropathy and obesity and contributes to unfavorable changes in the plasma lipid pattern (29).

Diabetic macroangiopathy and microangiopathy cause a variety of severe late complications of DM. Nephropathy coupled with type 2 DM is the most frequent cause of end-stage renal vascular disease in most countries (30). Enhanced platelet activation and adhesiveness are thought to be involved in diabetic angiopathies (31).

Hyperglycemia is the main determinant in progress of both types of DM but patho-physiological mechanisms differ between type 1 and type 2 DM. The interaction of insulin resistance and inflammation are mainly seen in insulin dependent type. Type 2 DM is vital for the development of macrovascular complication (32). Hypertension (without/with microalbuminuria) and dyslipidemia are more frequent in type 2 than in type 1 DM and may antedate the emergence of overt type 2 DM. In type 1 DM, microalbuminuria and nephropathy both cause hypertension as endothelial dysfunction. Endothelial dysfunction is also strongly associated with diabetic retinopathy, nephropathy and atherosclerosis in both type 1 DM and type 2 DM.

Recently, evidences from cellular, physiological, clinical, and epidemiologic studied strongly suggest a reciprocal link between insulin resistance and endothelial dysfunction. Authors suggest a possible link between insulin resistance and platelet activation which may precipitate as disorders of metabolic and cardiovascular homeostasis assessable by biochemical and hematological biomarkers.

We believe that accelerated athero-thrombosis late outcome of advancing platelet activation unable to improve endothelial dysfunction, inflammation, thrombosis, renal oxidative stress, dyslipidemia and hemodynamic shear stress, in the long-term development of vascular disease in patients with type 2 diabetes. Hence, it may increase the mortality in the majority of diabetic patients presenting risk of acute coronary and renal syndromes (33). Acute coronary syndromes are precipitated atherosclerotic effects of an occlusive intracoronary thrombus which forms over a ruptured atherosclerotic plaque (34). The abnormal metabolic state that accompanies insulin resistance makes arteries susceptible to atherosclerosis and renal changes, by changing the functional properties of multiple cell types, including platelets (35).

Platelet hyperactivity in combination with abnormalities in coagulation and fibrinolysis contributes to cardiovascular complications. Platelets initiate and sustain thrombi within vessels. However, other factors including hypertension, smoking, hypercholesterolemia and physical inactivity also aggravate prothrombotic diabetes disease burden (36). Platelet size determines the platelet function. Larger platelets are seen as more reactive and display quick platelet aggregation. An association was suggested between increased MPV and increased risk of both myocardial and cerebral infarction (37). An abnormal increase in mean platelet volume and low platelet counts found in chronic renal disease patients with coronary heart disease may also serve as indicators for a pre-thrombotic state and the risk of myocardial infarction and kidney disease (38). MPV is associated with a poor outcome in acute ischemic cerebrovascular events. Higher MPV with low creatinine clearance values and high protein excretion in
diabetic cases may reinforce the above possibility(39). Platelet volume indices may serve to identify larger more active platelets during development of coronary thrombosis leading to myocardial infarction. Authors suggest that platelet volume indices by hematological method, is a simple, effortless and cost effective test that should be used extensively for predicting impending acute cardiovascular and renal disease attacks.

Platelet hypersensitivity in DM also predicts the enhanced risk of thromboembolic macroangiopathy, and consequently increased morbidity and mortality. It also validates the use of antiplatelet agents in diabetic individuals. Platelet hyper-reactivity may be ameliorated with variouansi-platelet drugs. Moreover, clinical and experimental survey shows that platelet hypersensitivity may not be efficient in people with diabetes(40).

Higher levels of HbA1c are found in people with persistently elevated blood sugar in diabetes mellitus. The International Diabetes Federation and American College of Endocrinology recommend HbA1c values below 6.5% while American Diabetes Association recommends that the HbA1c be below 7.0% for most patients in corroboration in present study (41).

Critical analysis and major focus of study

- Two hundred and twenty two patients in the age group 25-75 years over a period one year into four diabetic groups on risk of atherosclerosis and chronic kidney disease represents significant sample size for clinical observation on platelet activation to define disease burden. However, advancing age and elevated glucose levels were major risks among females while BMI, blood pressure and life style factors were noticeable unpublished secondary risks. In spite, mean age of diabetic subjects were not true representative for lab test predictability in risk evaluation of platelet dysfunction.
- Mild to moderate glucose control by HbA1c values, significant elevation of lipid parameters (serum cholesterol, triglyceride and LDL) were significantly elevated in controllable and uncontrolled diabetic groups. However, it does not specify the synergy of sub-physiological changes in platelet membrane changes and other associated factors.
- Diabetic group with altered values of Hct, MCHC, Hb, MCV and RDW-SD seem to be trivial and empirical. However, mild controlled diabetic group (without other risk factors) with significant decrease in MCH and MCHC values also suggested the possibility of other risk factors as possible cause for biochemical changes. TLC donot seem of any value as marker of risk.
- PC, MPV, PDW and P-LCR platelet indices poorly signify the possible correlation of sequentially altered cardiovascular, renal biochemical and hematological biomarkers to predict the diabetes disease burden and possible risk level of renal dysfunction to influence pre-diabetes progress to uncontrolled diabetes.

What are new lessons on diabetes and chronic kidney disease?

- Chronic hyperglycaemia is a fundamental cause of renal complications in patients with diabetes and possibly chronic kidney disease through the induction of renal glucotoxicity and adverse renal haemodynamic effects.
- The pathophysiological changes of diabetic kidney disease in type 2 diabetes involve complex interactions between metabolic and haemodynamic factors on a background of genetic predisposition.
- Chronic hyperglycaemia has a central role in the development and progression of possible diabetic kidney disease, whereas a cluster of cardiometabolic abnormalities (including obesity, systemic hypertension, glomerular hyperfiltration, albuminuria, and dyslipidaemia) also contribute early in the disease course.
- Multifactorial therapy may improve renal outcome in patients with type 2 diabetes such as lifestyle interventions (eg, diet and exercise to achieve weight loss, right attitude and behavior, smoking cessation) and pharmacological management of glucose, blood pressure, antithrombic therapy and lipids.
- Renin-angiotensin-aldosterone system (RAAS) inhibitors have shown renoprotective effects along with decreasing blood pressure. RAAS inhibition has shown to attenuate the progression of diabetic kidney disease in trials, in which renal risk is high and/or the associated treatable cancers. However, residual risk of chronic diabetic kidney disease remains in patients with type 2 diabetes and microalbuminuria. It certainly alerts for novel strategies or new therapeutic drugs to reduce this residual renal risk in patients with type 2 diabetes.
- UKPDS, VADT trials suggest the value of intensive glycaemic HbA1c control, BMI on renal outcome in patients with type 2 diabetes in the form of recovery of reduced microalbuminuria, macroalbuminuria and doubled serum creatinine with reduction of microvascular disease (renal failure, retinal photoacoagulation, or vitreous haemorrhage) to justify “legacy effect”.
- ADVANCE, ACCORD trials define a renoprotective effect of glycaemic control on diabetic kidney disease based on albuminuria reduction achieved with metformin, sulfonylureas, and insulin with possible for microvasculature cardio-protection.
- New proposal from authors on renoprotection beyond glycemic control in type 2 diabetes is possible by correction of pleiotropic effects such as obesity (hyperlipidemia) to reduce weight, hormonal, inflammatory factors, BMI, microalbuminuria, diet modification with exercise (life style interventions similar with AHEAD trial) to save diabetic kidney.

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● Safe use of antihypertensive strategy as suggested by KDIGO/JNC8 (correct BP measurement and safe use of CCB, ACEi or ARB in diabetic CKD treatment) along with stepwise LOW ALCOHOL INTAKE-NO SALT-HEALTHY WEIGHT lifestyle modifications and drug therapy (similar with BENEDICT, ROADMAP trials).

● Safe management of glomerular hyperfiltration and elevated albuminuria in diabetes type 2 (similar with RENNAL and ACE-inhibitor trials) by corticosteroid treatment, protein restrictive diet, RAAS blockade limited benefits, thiazolidinedione rosiglitazone, GLT-1 peptide, DPP-4 inhibitors (saxagliptin and linagliptin), SGLT-2 inhibitors.

● Careful treatment and management dyslipidaemia (hypertriglyceridaemia, decreased HDL cholesterol concentrations, high LDL cholesterol concentrations, obesity and insulin resistance) in patients with type 2 diabetes with or without chronic kidney disease, for reduction of microalbuminuria and cardiovascular risk and mortality by statins (similar with PLANET 1, FIELD trial guidelines).

● Safe supervised use of thiazolidinedione, metformin+sulfonylurea, glargine to reduce cholesterol, triglycerides (similar with ORIGIN trial).

LIMITATIONS AND FUTURE PROSPECTS OF STUDY

● Platelet dysfunction evaluation in present study appears insufficient without information of endothelial dysfunction, inflammation, thrombosis, oxidative stress, circulating atherogenic proteins and hemodynamic shear stress data in different diabetes subjects.

● Detailed studies and said data bank most likely will answer better the biochemical basis of disease burden and its progress with its prognostic control to develop more efficient treatment strategy.

● The antihyperglycaemic drug therapy might reduce pleiotropic risk factors and pleiotropic effects for diabetic kidney disease beyond their ability to lower glucose.

● The multifactorial treatment (GLP-1 receptor agonists, controversial SGLT-2 inhibitors and thiazolidinediones-rosiglitazone and DPP-4 inhibitors) will reduce the residual renal risk or renoprotective potential beyond glucose lowering in patients with type 2 diabetes if advantages are proven.

● Management of renal and pancreas oxidative stress and/or inflammation must be considered as renoprotection(similar with BEACON, RADAR, SONAR and ASCEND trials).

Therefore, future comparative renoprotection studies sufficiently powered with long duration are needed on investigation of renal outcome. The non-proteinuric pathway must be investigated for progressive renal function loss with no or negligible albuminuria in diabetic kidney disease. The option of lifestyle modification, exercise, behavior, attitude, healthy foods, environment, socio-economic uplift measures needs attention and specific government policies (42).

V. Conclusion

During the last decade, renal risk factors and pathophysiological mechanisms of diabetic kidney disease were major focus to design treatment strategies to reduce diabetic kidney disease risk in patients with type 2 diabetes. Present study suggests the diagnostic value of platelet indices and associated biochemical, hematopoietic parameters to reflect the grade or diabetes disease burden as measurable index to evaluate diabetes control level along with cardiovascular and renal risks. It also gives evidence of platelet dysfunction to trigger the biochemical and hematological changes as measureable biomarkers to predict the diabetes disease burden and to distinguish non-diabetic factors in compounding risks of renal and cardiovascular disease. Platelet indices may be useful means of detect, identify the increased risk for developing vascular complications leading to increased morbidity and mortality. Main renal culprits were hyperglycaemia and systemic hypertension. We highlighted less known risk factors such as obesity, glomerular hyperfiltration, albuminuria, and dyslipidaemia to prevent diabetic kidney disease in patients with type 2 diabetes. Present study serves the purpose to explore multifactorial treatment strategies, substantial residual renal risk remains or therapeutic strategies. Several antihyperglycaemic pleiotropic actions are identified to reduce the renal risk factors, possibly useful in clinical care. Exploitation of these benefits may add clinical value in the reduction of renal and cardiovascular risk in the near future. However, large and long-term randomised trials will answer whether these off-target actions affect outcome in type 2 diabetes.

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DISCLOSURE OF CONFLICT
All authors have no conflict in this study and its contents.

References

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