

## Biochemical Biomarkers-Independent Predictors of Type 2 Diabetes Mellitus

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### Abstract

Biological markers or biomarkers are the indicators of a particular biological state before the appearance of manifestations in form of a disease. A variety of blood biomarkers represent pathology of diabetes. Analysis of blood is an important technique for determining physiological and pathological condition. People suffering from Type 2 diabetes mellitus (T2DM) were part of the study. The people with bleeding disorders and those suffering from other diseases were not included in the study. There were more female (F) diabetic patients than male (M) patients. This case control observational study is an attempt to interpret data obtained from biochemical investigation of diagnostic biochemical parameters of complete blood count and lipid profile for their pathological implication and in prediction risk of Type 2 diabetes mellitus and related complications among the healthy subjects. Normality test was done to check for normal distribution of the data among females (1) and males (2) and the correlation test showed no significant relation of the of biochemical parameters with PBSS.

**Keywords:** Biological markers; Type 2 diabetes mellitus

**Abbreviations:** FBS: Fasting Blood Sugar; PPBS: Post Prandial Blood Sugar; TC: Total Cholesterol; HDL: High Density Lipoproteins; LDL: Low Density Lipoproteins and TG: Triglycerides; CHD: Coronary Heart Disease

### Introduction

#### Biomarkers

“Biomarker or biological marker origin is the characteristic that is objectively measured and evaluated as an indicator of the normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention” (NIH Definition 2001).

Biomarkers provide a dynamic and powerful approach to understand the spectrum of diseases. Candidate biomarkers of health and disease discovery is the result of genome or proteome-wide search. From antibodies which are the simplest biomarkers to the widespread accurate genomics-based biomarkers has shown notable success. Biomarkers for T2 DM are able to answer the questions that move in our minds

- Am I going to get diabetes?
- Have I got diabetes?
- What is the effect of diabetes?

The biomarkers are risk factors that could be of either genetic or non-genetic origin. Among the non-genetic biomarkers the biochemical biomarkers are the independent predictors of Type 2 diabetes.

#### Type II diabetes mellitus

Complex interaction between biochemical, environmental and genetic factors can unmask T2DM [1] by predicting its outcome. It is known from ancient times and is designated as disease of affluence [2] and overfed, middle aged and elderly people but now the prevalence of diabetes for all age-groups is worldwide [3,4]. The overall burden on family and economy of nation is increasing worldwide as the prevalence of diabetes is increasing continuously at an alarming rate with T2DM accounting for >90% of all the cases of diabetes [5] There

is an increased incidence and prevalence in both the western world and in the developing countries. About 382 million people worldwide are affected by diabetes and the count will be raised to 529 million by 2035 (International Diabetes Federation estimation). By the end of the century it is estimated to reach indefinite proportions in every country and subsequently it has turned out to be a pandemic.

Diabetes mellitus is a multifaceted group of metabolic diseases characterized by the accumulation of sugar in the blood (hyperglycemia). This condition is due to non utilization of glucose by body cells due to insulin resistance shown by the cells [6,7] or due to insulin deficiency [8]. This results in abnormalities in carbohydrates, protein and fat metabolism [9]. The associated pathogenic processes are involved in the development of diabetes with a long-term damage dysfunction, and failure of various organs especially affecting the eyes, kidneys, nerves, heart, and blood vessels (American Diabetes Association). On entry of nutrients into the blood stream insulin brings down the blood glucose levels by decreasing the exogenous glucose production and stimulation of glucose transport [10]. Patients with long duration of type II diabetes and poor glycemic control have a significantly increased risk of developing related complications making it the most significant global health problems faced today [11-14]. The highest burden is borne by the South East Asian countries [15]. The Indians are more prone to diabetes whether they may be residing in India or outside due to the peculiar characteristics [16,17]. Prevalence of more among the urban population, now the prevalence is increasing in rural population due to change in socio economic life styles. The economic burden of treating

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diabetes and its complications is increasing at a fast pace with increase in number of upcoming cases of diabetes.

Complex diseases like diabetes do not have a single cause to occur. Several modifiable risk factors, non-modifiable risk factors of non-genetic origin and of genetic [18] origin have been studied for T2DM and related complications. Previously nationwide [19] and worldwide [20] studies have been carried to find out the importance of investigating the biochemical parameters in blood and lipid profile in advancement and progression of T2DM. These biochemical parameters are the risk factors for the development of T2DM and related complications when tested in controls [21-23]. These risk factors confer the risk validated by the presence of inheritable subset of SNP which do not change throughout one's life and the person could expect their effects in the later stages of life.

### Methodology

This is a part of case control study to find how non-genetic and genetic risk factors together are responsible for outcome of T2DM and related complications in discrete population of Andhra Pradesh and Telangana States of India. This study is an attempt to interpret data obtained from biochemical investigation of diagnostic biochemical parameters of complete blood count and lipid profile along FBS and PPBS levels. Pathological implications of these biochemical parameters and the prediction of T2DM and the related complications are the risk factors among the healthy subjects.

The study is carried out with 180 subjects of which 96 were females (1) and 84 were males (2) (90 subjects (females 54; males 36) are diabetic cases and 90 subjects (females 42; males 48) are controls. Their left over blood sample was collected after taking their consent. The case subjects of the study were established diabetic patients based on their biochemical reports which showed FBS value of more than 126 mg/dl or  $\geq 7.0$  mmol/L and PPBS value of more than 200 mg/dl or  $\geq 11.1$  mmol/L (WHO diagnostic criteria for diabetes). The healthy subjects with no family history of diabetes served as controls. Subjects suffering from other infectious diseases were excluded from the study. They were either motivated or prescribed by the doctor for the screening of diabetes.

### Experimental Details

The Fasting and Postprandial whole blood samples were collected from diabetic patients aged between 15-85 years and normal subjects from Andhra Pradesh and Telangana States of India. The samples were collected in both EDTA [24] and non-EDTA vacutainers in accordance to the protocol of some of the investigations and not for the purpose of comparison. Blood samples were collected from them after obtaining information related to age, sex, life style and history. Blood samples were collected in sitting position by tying a band to make the vein more prominent. The data of lipid profiles and complete blood count along with the FBS and PPBS was obtained by investigation of the biochemical parameters. Blood sugar levels (FBS, PPBS) estimated by glucose oxidase and peroxidase (GOD-POD) method [25].

Hemoglobin was determined by cyanmethemoglobin method and lipid profiles (TC, HDL, LDL, VLDL, TG) were analyzed using Star 21 plus auto-analyzer were measured by Kellys end point method, the end point method is so called as the analytes are completely consumed in the reaction. The coloured complex thus formed at the end of reaction period is read for its absorbance using a spectro-photometer. All the chemicals used in this study were of analytical grade.

### Biochemical Evaluation

The Biochemical data and characteristics of the study participants was compared along with the normal reference range for the different Biochemical parameters as shown in the Tables 1 and 2 below. The data for different parameters are presented in the Tables as mean  $\pm$  standard deviation. The units of measurement for all the parameters is mg/dl and that of different types of WBC and total WBC are expressed as percent (%) and/cmm and Hb in grams.

### Discussion and Results

Glucose gets into the cells with the help of a hormone called insulin. T2DM is a condition characterized by hyperglycemic condition due to either insulin resistance and absolute or partial deficiency of insulin secretion by the pancreatic  $\beta$ -cell. Diabetes is a chronic disorder resulting from imbalance between the insulin sensitivity and the insulin secretion. There is no single cause for diabetes as it is a multifactorial disease.

The study showed that there is an increase in the values of some biochemical parameters investigated in the T2DM cases when compared to the control values but within the normal range. The blood sugar levels were found to be high in both male and female patients as per the WHO diagnostic criteria for diabetes. Poorly controlled PPBS levels have adverse affect on arteries that leads to either microvascular (small vessels) or macrovascular vessels (large) or both complications [26-29]. It independently show association with disability and death caused by cardiovascular disease irrespective of FBS. Occurrence of anaemia in patients with T2DM is a frequent condition [30,31]. Gender specific of investigation of anemia is done in females when the hemoglobin levels are less than 12 g/dL and in men when it is less than 13 g/dL [32] (WHO recommended criteria). Anaemia is an important predictor for microvascular disease [33] or progression of kidney diseases [34]. Anaemia contributes to tiredness in 74% of the diabetic people than those without anaemia [35]. The abnormalities of lipid profile or dyslipidemia are associated with the T2DM and it is one of the major risk factors for cardiovascular diseases [36,37]. The elevated levels of triglycerides with low HDL levels when compared to normal ones showed an increased risk of coronary heart disease (CHD) [38].

T2DM has an impact on total and differential counts of WBC [39]. After controlling conventional risk factors WBC count worsens the insulin sensitivity [40]. Increased WBC count is independent

Parameter	T2DM Cases Mean $\pm$ SD	Controls Mean $\pm$ SD	Normal Range	*P=value
FBS	152 $\pm$ 92.8	92 $\pm$ 11.7	70-110	0.8
PPBS	229.3 $\pm$ 67.6	131.2 $\pm$ 18.8	170-200	0.7

**Table 1:** Blood sugar levels.

Parameter	T2DM Cases Mean $\pm$ SD	Controls Mean $\pm$ SD	Normal Range	*P=value
Total Cholesterol	176.8 $\pm$ 50.3	163 $\pm$ 51.7	130-250	0.09
Triglycerides	198.1 $\pm$ 87.7	141 $\pm$ 56.9	50-150	0.1
HDL	42.5 $\pm$ 4.7	43.2 $\pm$ 5.9	35-70	0.05
LDL	86.4 $\pm$ 25.8	44.0 $\pm$ 28.6	Upto140	0.09
VLDL	41.8 $\pm$ 20.7	28.6 $\pm$ 13.6	10-40	0.09
Total WBC Count	8116.6 $\pm$ 1196.3	7785 $\pm$ 1759	4000-11000	0.1
Neutophils	64.8 $\pm$ 7.4	63.88 $\pm$ 7.2	40-75%	0.8
Haemoglobin	11.9 $\pm$ 1.8	12 $\pm$ 1.2	M=13.5-18 g F=12-15 g	0.2

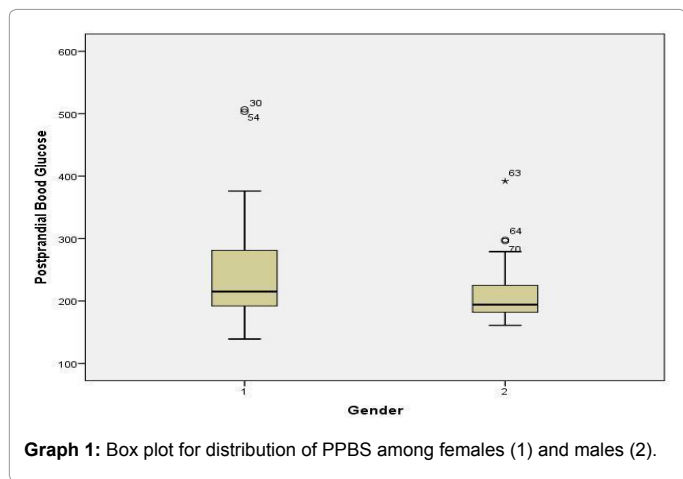
**Table 2:** Biochemical characteristics of study participants.

Parameter	Skewness	Kurtosis	Standard Error(SE)	Skewness	Kurtosis	Standard Error (SE)
PPBS	1.763	3.72	± 0.32 ± 0.63	1.8	4.33	± 0.39 ± 0.77
TG	1.298	1.288	± 0.325 ± 0.398	1.288	0.808	± 0.398 ± 0.778
HDL	418	-1.448	± 0.325 ± 0.639	0.073	-1.839	± 0.398 ± 0.778
LDL	0.466	-0.181	± 0.325 ± .639	-0.451	0.653	± 0.398 ± 0.778
N/L	0.878	1.927	± 0.512 ± 0.992	0.026	-1.359	± 0.398 ± 0.778

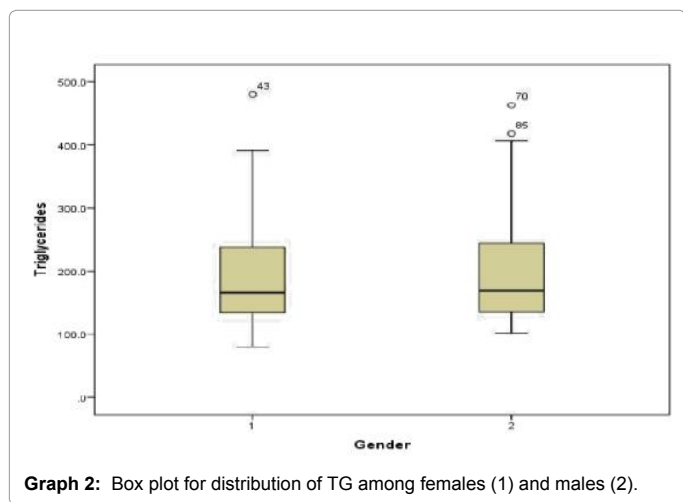
Table 3: Skewness and Kurtosis z- values.

Parameter	Males	Females
PPBS	0.00	0.00
TG	0.00	0.00
HDL	0.00	0.000
LDL	0.198	0.590
N/L	0.015	0.121

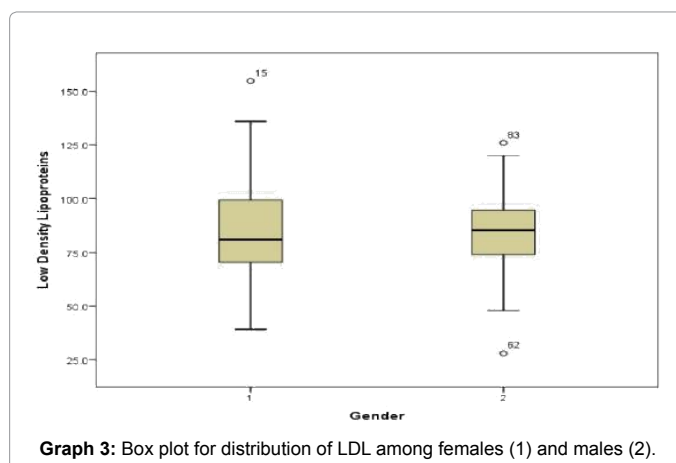
Table 4: Shapiro-Wilk test p-values.



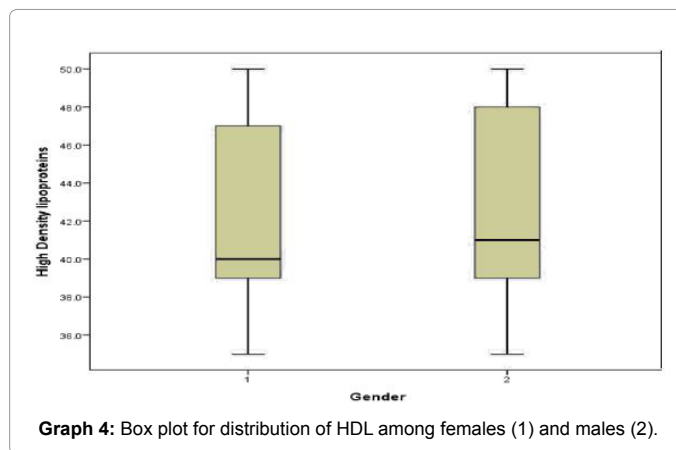
Graph 1: Box plot for distribution of PPBS among females (1) and males (2).



Graph 2: Box plot for distribution of TG among females (1) and males (2).



Graph 3: Box plot for distribution of LDL among females (1) and males (2).



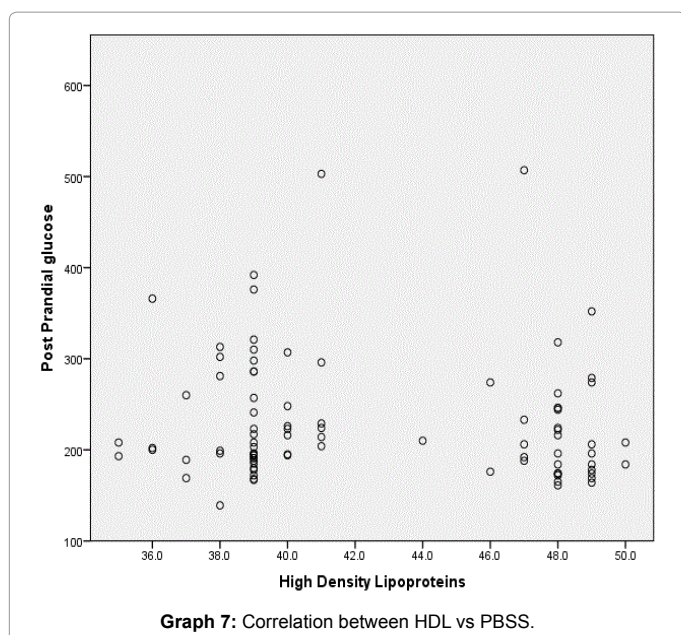
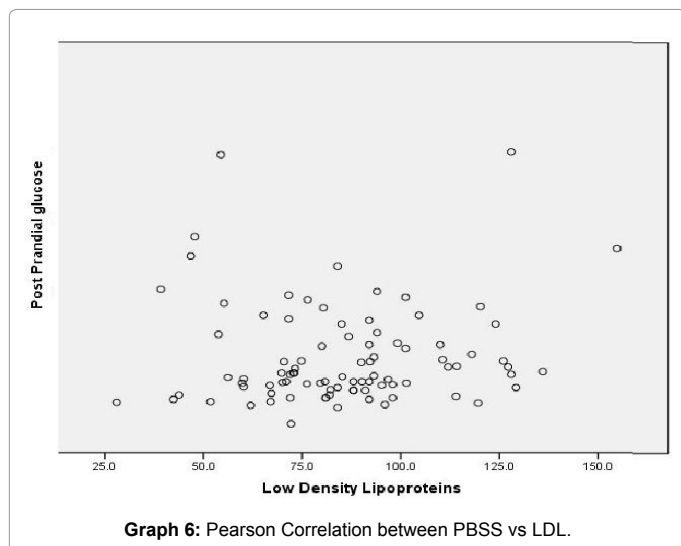
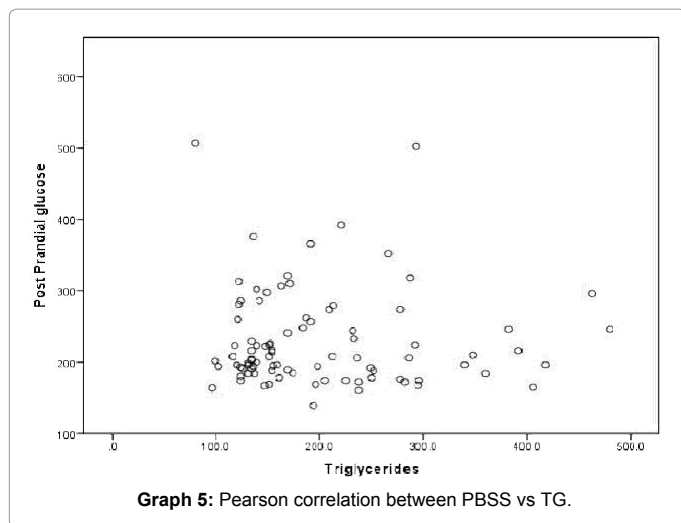
Graph 4: Box plot for distribution of HDL among females (1) and males (2).

### Statistical analysis

The following numerical and visual outputs are investigated with the help of IBM SPSS v.21 Software. Normality test was performed to check whether the post prandial blood sugar levels and other lipid profile values are normally distributed among female (1) and male (2) diabetic patients. The Graph 1 box plot shows that the PPBS were not normally distributed among the males and females. The Graphs 2,3 and 4 box plot shows that the TG, LDL and HDL were not normally distributed among the males and females.

Significant difference was observed between the PBSS scores for female (1) (M=24.31, SD=76.05) and male (2) (M=211.86, SD=48.81);  $t(87)=2.03$ ,  $p=0.045$  on conducting an independent T-test. The observed lipid profile variables were analyzed for correlation by Pearson's correlation test and showed no statistically significant correlation between PPBS vs TG, HDL vs PBSS, LDL vs PBSS. R square values are 0.001, 0.002 and 0.009 for TG, LDL and HDL respectively. The Pearson correlations are shown below in Graphs 5,6 and 7.

biomarkers for macro and microvascular complications [41] responsible for death and disability in T2DM patients. Lymphocyte count is decreased with the advancement of diabetic nephropathy even in the normal range [42]. The future incidence of CHD is predicted by the total [43,44] and the differential cell counts of WBC (eosinophils, neutrophils, and monocytes) [45]. NLR (Neutral Lymphocyte Ratio) is an essential marker of systemic inflammation and T2DM and are indicator of increased risk for cardiovascular events in patients [46].



## Conclusion and Future Directions

Diabetes has no cure but can be kept under control by making small modifications in the life style and it will certainly help to prolong the onset of diabetes and related complications in affected people. The higher levels of cholesterol is one of the risk factors for dyslipidemia in the subjects normal for T2DM. Our further studies had revealed the presence of SNPs for dyslipidemia in controls also. Increased levels of Lipids and decreased HDL along with high post prandial blood sugar will result in cardiovascular complications and will lead mortality and morbidity. Lower levels of hemoglobin cause tiredness and vascular complications. Increased WBC count but within range is an independent biomarker for onset of T2DM decreased of lymphocyte count indicates the advancement diabetic nephropathy. The difference in normality shows the difference in susceptibility of T2DM among female (1) and male (2). There is an urgent need for research to identify other risk factors for predisposition of diabetes. Further, the study is continued to find the genetic and other non genetic factors for predicting the out come of T2DM and related complications.

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