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Original Research Article

"ROLE OF PLATELET INDICES IN TYPE2 DIABETES MELLITUS (DM) PATIENTS IN A TERTIARY CARE HOSPITAL"

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ABSTRACT

Background: Diabetes mellitus (DM) is the most common endocrine disease characterized by metabolic abnormalities, hyperglycemia, and by long term complications. There are two major subgroups of DM, type I insulin dependent (IDDM) and type II (NIDDM) [(1)]. Type 2 diabetes mellitus(T2DM) is a serious public health problem, considering its epidemic prevalence levels and high morbidity and mortality rate [(2)].

AIM: To study the platelet indices in patients with Type2 Diabetes Mellitus (DM) and in Non-diabetic group and to compare these platelet indices between DM and Non-diabetic patients.

MATERIALS & METHODS:**TYPE OF STUDY:**OBSERVATIONAL STUDY,**STUDY DESIGN :** CASE – CONTROL STUDY,**PLACE OF STUDY:** Mamata General Hospital, Khammam,Telangana.**SAMPLE SIZE:**100 Patients T2DM (Case group), 100 Non-diabetic individuals (Control group). **DURATION OF THE STUDY:**1 YEAR (October 2018 to October 2019). **Ethical consideration:** Institutional Ethical committee permission was taken prior to the commencement of the study. **Study tools and Data collection procedure:**Ethical clearance was obtained from institutional ethical committee. The data was collected from October 2018 to October 2019 by using pre-designed and pre-tested questionnaire which included socio demographic details, medical history and laboratory analysis. After explaining purpose of the study, written informed consent was taken from all the patients (cases) and control group. A detailed medical history was taken which included Age, gender, duration of Diabetes,

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medications in use, and previous diagnosis of micro vascular (Retinopathy, Neuropathy, Nephropathy) or macro vascular (Coronary artery disease, Stroke, and Peripheral artery disease) complications.

STATISTICAL ANALYSIS: The data was analyzed using SPSS (Statistical Package for Social Sciences) Version 20.0. We applied descriptive statistics and exploratory data analysis to obtain mean and standard deviations. For the qualitative variables, the Chi-square test was performed. Independent t test were used to test the difference between means. The statistical significance level was fixed at $P < 0.05$.

RESULTS: The mean and SD of MPV were 10.41 ± 0.95 and 8.89 ± 0.89 for DM group and control group respectively. The mean difference of MPV between DM and control group was found to be **statistically significant** ($p< 0.0001$) in our study. In other words, the mean and SD of MPV in DM group was significantly higher when compared to the MPV values of control group.

CONCLUSION: In conclusion, the present study showed significant differences in platelet parameters in patients with T2DM when compared to non-diabetic individuals. Once the platelet parameters analysis is a simple and cost-effective diagnostic tool, it could be a useful prognostic marker for chronic complications of diabetes. Therefore, it contributes to the early detection of these complications, as well as to a potential reduction in morbidity and mortality in this group of individuals.

Key words: Diabetes mellitus (DM), PLATELET INDICES, Mean platelet volume

INTRODUCTION:

Diabetes mellitus (DM) is the most common endocrine disease characterized by metabolic abnormalities, hyperglycemia, and by long term complications. There are two major subgroups of DM, type I insulin dependent (IDDM) and type II (NIDDM) [(1)]. Type 2 diabetes mellitus(T2DM) is a serious public health problem, considering its epidemic prevalence levels and high morbidity and mortality rate [(2)]. This type of diabetes that results from resistance to insulin action associated with a relative deficiency of this hormone, has an insidious development and is often diagnosed due to the presence of micro vascular or macro vascular complications[(3)]. During year 2014, the number of cases of diabetes worldwide is estimated to be around 422 million, of these more than 90% are type 2 diabetes. In 2015, an estimated 1.6 million people died from consequences of high blood sugar [(4)].

Hyperglycemia results in disturbances in cellular metabolism due increased production of reactive oxygen species (ROS) and nonenzymatic glycation of many macromolecules, which lead to changes in cellular structure and function, and formation of advanced glycation end products (AGEs). The formation of AGEs enhances metabolic disturbances and also increases reactive oxygen species production via interaction with the specific receptor for AGE (RAGE)

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[5,6]. This causes changes in structure and biophysical properties of the basement membrane which further causes changes in permeability and vasodilatation of blood vessels [7].

A study suggested that high platelet activity enhances vascular complications in DM patients [8]. Mean platelet volume (MPV) is a marker showing platelet function and activation.In the process of atherogenesis, the activity of the platelets and their potential aggregation actively participate in the development of thrombi. Furthermore, the function of these cells seems to be related to their sizes. Some studies have shown that large platelets are most reactive and aggregatable, have high amounts of dense granules, and present increased thrombotic potential when compared with smaller and less active platelets. Recent studies have shown significant increases in platelet parameters in diabetic subjects when compared with controls, particularly in Mean platelet volume (MPV) and Platelet distribution width (PDW).

Hence, the present study was undertaken to determine whether the platelets were activated and there was any changes in platelet indices in diabetic group when compared to the non diabetics by measuring various platelet parameters.

AIM: To study the platelet indices in patients with Type2 Diabetes Mellitus (DM) and in Non-diabetic group and to compare these platelet indices between DM and Non-diabetic patients.

MATERIALS & METHODS

TYPE OF STUDY : OBSERVATIONAL STUDY

STUDY DESIGN : CASE – CONTROL STUDY

PLACE OF STUDY : Mamata General Hospital, Khammam,Telangana.

SAMPLE SIZE : 100 Patients T2DM (Case group)

100 Non-diabetic individuals (Control group)

DURATION OF THE STUDY: 1 YEAR (October 2018 to October 2019)

INCLUSION CRITERIA:

1. Patients who already diagnosed as Type 2 Diabetes Mellitus with or without vascular complications.

2. Patients aged between 30 – 60 years.

3. Controls will be Non-diabetic of same age group (30-60 years).

EXCLUSION CRITERIA:

1) Patients who were not willing to participate in the study.

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2) Non-diabetic subjects with coronary artery disease (ECG changes)

3) Diabetics on antiplatelet drugs such as Aspirin and Clopidogrel.

4) Subjects with any diagnosed malignancy.

Ethical consideration: Institutional Ethical committee permission was taken prior to the commencement of the study.

Study tools and Data collection procedure:

Ethical clearance was obtained from institutional ethical committee. The data was collected from October 2018 to October 2019 by using pre-designed and pre-tested questionnaire which included socio demographic details, medical history and laboratory analysis.

After explaining purpose of the study, written informed consent was taken from all the patients (cases) and control group. A detailed medical history was taken which included Age, gender, duration of Diabetes, medications in use, and previous diagnosis of micro vascular (Retinopathy, Neuropathy, Nephropathy) or macro vascular (Coronary artery disease, Stroke, and Peripheral artery disease) complications.

Venous blood samples were collected in the morning time after a 12-hour fasting period. The following laboratory tests were performed: RBC count, Hematocrit, Hemoglobin, Total WBC count, platelet count, MPV, PCT, PDW, fasting blood glucose, creatinine, total cholesterol, high density lipoprotein cholesterol (HDL cholesterol), and triglycerides levels. In the DM group, glycated haemoglobin (HbA1C) levels were also measured.

Blood sample (approximate volume 4 ml) for complete blood count, platelet parameters, and HbA1C tests, was collected in vacuum tubes containing the anticoagulant ethylenediaminetetraacetic acid (K3 EDTA). The samples were kept at room temperature and processed within one hour after collection. For the biochemical tests, blood samples (5 ml) were collected in tubes without anticoagulant, and were centrifuged in LS-3 Plus (CELM) equipment for 5 minutes at 3,400 rpm for serum separation.

STATISTICAL ANALYSIS

The data was analyzed using SPSS (Statistical Package for Social Sciences) Version 20.0. We applied descriptive statistics and exploratory data analysis to obtain mean and standard deviations. For the qualitative variables, the Chi-square test was performed. Independent t test were used to test the difference between means. The statistical significance level was fixed at $P < 0.05$.

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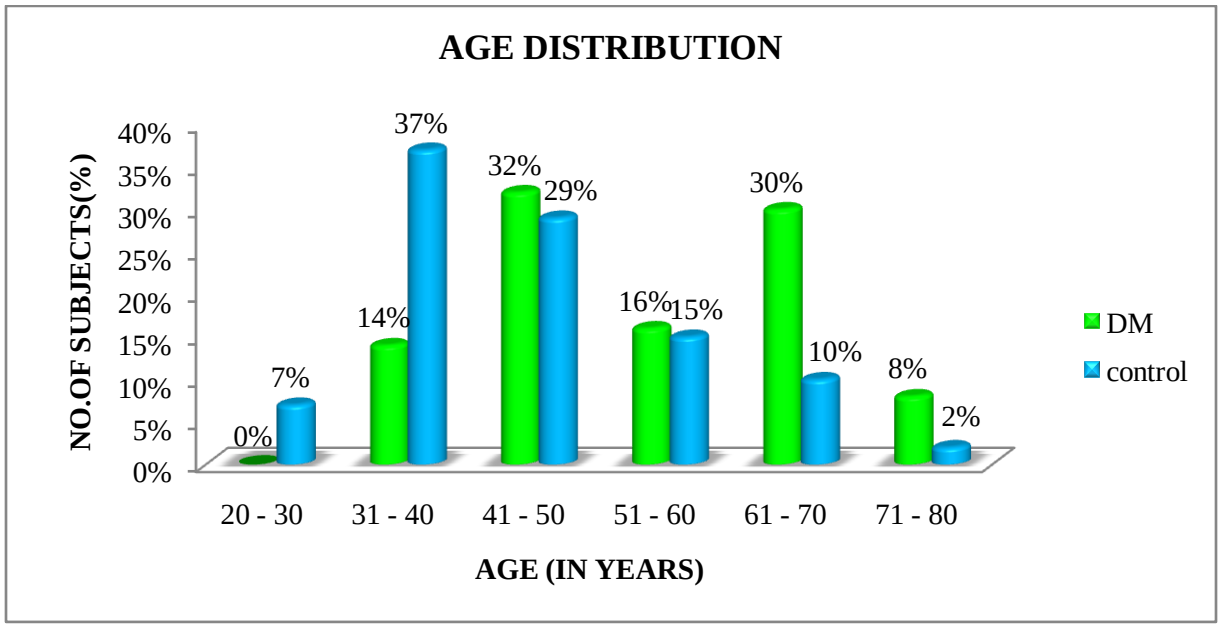
OBSERVATION & RESULTS:

FIGURE 1: AGE DISTRIBUTION in the study groups



Mean age of cases group was 54.96 ± 12.64 and for control group was 44.72 ± 11.88. Majority 32 (32%) of the cases group were between the groups of 41 – 50 years. Where as in the control group, majority 37 (37%) of the subjects were in between 31 – 40 years.

TABLE 1: ASSOCIATION BETWEEN AGE AND DISEASE

AGE	DM	CONTROL	TOTAL
≤ 40 YEARS	14	44	**58**
≥ 40 YEARS	86	56	**142**
TOTAL	**100**	**100**	**200**

X^2 = 21.8553 df = 1 p = 0.000003 (P < 0.05) (SIGNIFICANT)

Table 1show that the association between age and disease was found to be **statistically significant** ($p < 0.05$) in our study. Means, on increasing the age more than 40 years the chances of getting DM is high and it was significant as $p < 0.05$.

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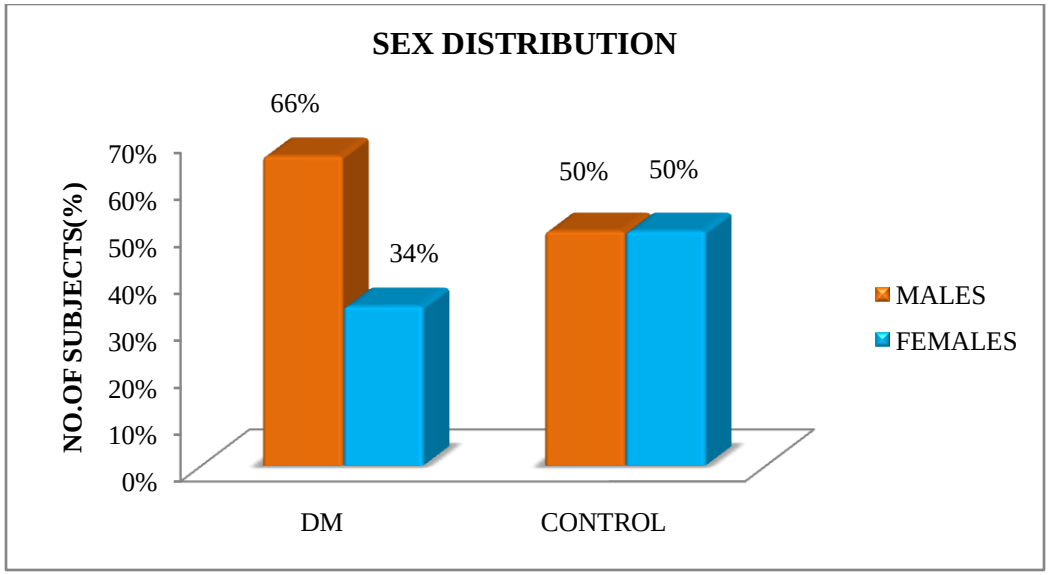
FIGURE 2: SEX DISTRIBUTION in the study groups



It was evident that there was a male predominance 66 (66%) in the DM group, while in the control group both the sex were equal (50 each).

TABLE 2: ASSOCIATION BETWEEN SEX AND DISEASE

AGE	DM	CONTROL	TOTAL
MALES	66	50	**116**
FEMALES	34	50	**84**
TOTAL	**100**	**100**	**200**

χ^2 = 5.2545 df = 1 p = 0.021 (P < 0.05) (SIGNIFICANT)

From table 2, we can say that the association between sex and disease was found to be **statistically significant** in our study ($p < 0.05$), means males were having higher chances of getting the disease than the females.

TABLE 3: FASTING BLOOD SUGAR & POST PRANDIAL BLOOD SUGAR VALUES

BLOODSUGAR	DM (Mean ± sd) (mg/dl)	CONTROL (Mean ± sd) (mg/dl)	Un-paired t-test
FBS	181.56 ± 68.40	116.42 ± 13.31	**P < 0.0001(SIG.)**

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PPBS	271.66 ± 88.61	175.12 ± 20.49	**P < 0.0001(SIG.)**

From Table 3, it is clearly evident that the mean difference of FBS & PPBS between DM group and control group was found to be **statistically significant** ($p < 0.05$). That means, DM group were having significant higher levels of FBS and PPBS values when compared to the control group in our study.

TABLE 4: HbA1c VALUES OF THE STUDY POPULATION

HbA1c	DM (Mean ± sd) (mg/dl)	CONTROL (Mean ± sd) (mg/dl)	Un-paired t-test
	7.28 ± 0.88	5.33 ± 0.59	**P < 0.0001(SIG.)**

From table 4, the mean value of HbA1c levels of DM was quiet higher than the controls. The mean difference of HbA1c levels between DM and controls was found to be **statistically significant** ($p< 0.05$) in our study.

TABLE 5: PLATELET INDICES IN THE STUDY POPULATION

Platelet indices	DM (Mean ± sd) (In fL)	CONTROL (Mean ± sd) (in fL)	Un-paired t-test
Mean platelet volume(MPV)	10.41 ± 0.95	8.89 ± 0.89	**P < 0.0001 (SIGNIFICANT)**
PLATELET DISTRIBUTION WIDTH (PDW)	12.82 ± 1.91	12.23 ± 1.69	**P < 0.022 (SIGNIFICANT)**
PLATELET CRIT (PCT) (in %)	0.29 ± 0.06	0.23 ± 0.05	**P < 0.0001 (SIGNIFICANT)**
PLATELET LARGE CELL RATIO (PLCR) (in %)	25.422 ± 6.538	25.950 ± 6.181	P < 0.558 (NOT SIGNIFICANT)
PLATELET			

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LARGE CELL COUNT (PLCC) (in %)	65.20 ± 17.25	65.71 ± 20.13	P < 0.847 (NOT SIGNIFICANT)

The mean and SD of MPV were 10.41 ± 0.95 and 8.89 ± 0.89 for DM group and control group respectively. The mean difference of MPV between DM and control group was found to be **statistically significant** (p< 0.0001) in our study. In other words, the mean and SD of MPV in DM group was significantly higher when compared to the MPV values of control group.

The mean and SD of PDW were 12.82 ± 1.91 and 12.23 ± 1.69 for DM and control group respectively. The mean difference of PDW between DM and control group was found to be **statistically significant** (p< 0.05) in our study. In other words, the mean and SD of PDW in DM group was significantly higher when compared to the PDW values of control group.

The mean and SD of PCT were 0.29 ± 0.06 and 0.23 ± 0.05 for DM and control group respectively. The mean difference of PCT between DM and control group was found to be **statistically significant** (p< 0.05) in our study. In other words, the mean and SD of PCT in DM group was significantly higher when compared to the PCT values of control group.

The mean and SD of PLCR were 25.422 ± 6.538 and 25.950 ± 6.181 for DM and control group respectively. The mean difference of PLCR between DM and control group was found to be not statistically significant (p> 0.05) in our study.

The mean and SD of PLCC were 65.20 ± 17.25 and 65.71 ± 20.13 for DM and control group respectively. The difference of PLCC between DM and control group was found to be not statistically significant (p> 0.05) in our study.

DISCUSSION:

This case-control study was done in Mamata medical college, khammam, Telangana. A total of 200 members participated in this study, where 100 members were cases and 100 were controls. In the present study, there was a male predominance 66 (66%) in the DM group, while in the control group both the sex were equal (50 each). This was supported by studies done by Dayal et.al [(9)] and H. Pahim et.al [(10)] but was in contrast with Kamilla R. Alhadas et.al [(11)] and Kodiatte TA et.al [(12)] where there was female predominance in both the groups. The association between sex and disease was found to be **statistically significant** in our study (p < 0.05); means males were having higher chances of getting the disease than the females.

In our study, the mean age of cases group was 54.96 ± 12.64 and for control group was 44.72 ± 11.88. Majority 32 (32%) of the cases group were between the groups of 41 – 50 years, Where as in the control group, majority 37 (37%) of the subjects were in between 31 – 40 years. This was similar with studies done byDayal et.al [(9)], Kodiatte TA et.al [(12)] and H. Pahim et.al [(10)] but a

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study done byKamilla R. Alhadas et.al [11]show different result where majority of the case group belonged to the age 65 years. The association between age and disease was found to be **statistically significant** ($p < 0.05$) in our study. Means, on increasing the age more than 40 years the chances of getting DM was significantly high.

The mean ± sd (mg/dl) of FBS in DM group was 181.56 ± 68.40 and in control group was 116.42 ± 13.31 and the mean difference of FBS between DM group and control group was found to be **statistically significant** ($p < 0.05$) in our study. This was similar with studies done byDayal et.al [9], Kodiatte TA et.al [12] and Kamilla R. Alhadas et.al [11]. The mean ± sd (mg/dl) of PPBS in DM group was 271.66 ± 88.61 and in control group was 175.12 ± 20.49. The mean difference of PPBS between DM group and control group was found to be **statistically significant** ($p < 0.05$) in our study. This was also similar with studies done byDayal et.al [9], Kodiatte TA et.al [12].

The mean ± sd (mg/dl) of HbA1c levels in DM group was 7.28 ± 0.88 in control group was 5.33 ± 0.59. The mean value of HbA1c levels of DM was quiet higher than the controls. The mean difference of HbA1c levels between DM and controls was found to be **statistically significant** ($p< 0.05$) in our study. This was in total agreement with studies done byDayal et.al [9], Kodiatte TA et.al [12], but disagreed with study done by Kamilla R. Alhadas et.al [11].

The mean and SD of MPV were 10.41 ± 0.95 and 8.89 ± 0.89 for DM group and control group respectively. The mean difference of MPV between DM and control group was found to be **statistically significant** ($p< 0.0001$) in our study. In other words, the mean and SD of MPV in DM group was higher when compared to the MPV values of control group. This was in agreement with the studies done by Hekimsoy et al [13], Zuberi et al [14], Dayal et.al [9], Kodiatte TA et.al [12], Demirtunc et al. [15], Khode V et.al [16] and Ulutas KT et.al [17].

Larger platelets are more active hemostatically and enzymatically, and they contain more prothrombotic molecules, such as platelet factor 4, serotonin, and platelet-derived growth factor, and possess greater aggregability in response to ADP. Mean platelet volume (MPV), which is used to measure platelet size, can reflect platelet activity. Increased MPV may lead to a prothrombotic condition with increased thromboxane A2 (TXA2) and B2 and adhesion molecule expression, such as P-selectin and glycoprotein IIb/IIIa, and β-thromboglobulin release. This suggests a relation between the platelet function especially MPV and DM vascular complications thus indicating changes in MPV reflect the state of Thrombogenesis. Thus, DM has been considered as a “prothrombotic state” with increased platelet reactivity. In diabetic patients, a high MPV is an important finding and could predict an increased risk for thrombosis and chronic complications.

The mean and SD of PDW were 12.82 ± 1.91 and 12.23 ± 1.69 for DM and control group respectively. The mean difference of PDW between DM and control group was found to be **statistically significant** ($p< 0.05$) in our study. In other words, the mean and SD of PDW in DM

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group was significantly higher when compared to the PDW values of control group. This was in consonance with study done by Khode V et.al [16] however the mean difference was not statistically significant in their study which was contrast to our study. Another study done by Dalamaga et.al [18] showed significant higher PDW values in DM group when compared to the control group PDW values.

The mean and SD of PCT were 0.29 ± 0.06 and 0.23 ± 0.05 for DM and control group respectively. The mean difference of PCT between DM and control group was found to be **statistically significant** ($p < 0.05$) in our study. In other words, the mean and SD of PCT in DM group was significantly higher when compared to the PCT values of control group. This was agreed with the study done by Kamilla R. Alhadas et al.[11].

The mean and SD of PLCR were 25.422 ± 6.538 and 25.950 ± 6.181 for DM and control group respectively. The mean difference of PLCR between DM and control group was found to be not statistically significant ($p > 0.05$) in our study. PLCR is another marker related to platelet volume, and it is an indicator of the largest platelet fraction. An increase in PLCR usually occurs together with an increase in the number of newly produced platelets, which are the largest platelet type. PLCR is usually correlated with MPV, but it is more sensitive to the increase in platelet size. Babuet.al [19]in their study showed that PLCR is inversely proportional to platelet count and directly related to MPV and PDW. The mean and SD of PLCC were 65.20 ± 17.25 and 65.71 ± 20.13 for DM and control group respectively. The mean difference of PLCC between DM and control group was found to be not statistically significant ($p > 0.05$) in our study.

CONCLUSION:

In conclusion, the present study showed significant differences in platelet parameters in patients with T2DM when compared to non-diabetic individuals. Once the platelet parameters analysis is a simple and cost-effective diagnostic tool, it could be a useful prognostic marker for chronic complications of diabetes. Therefore, it contributes to the early detection of these complications, as well as to a potential reduction in morbidity and mortality in this group of individuals.

This study revealed, significantly high MPV in diabetics as compared to controls thus establishing that MPV is strongly and independently associated with diabetes. Glycaemic control plays a role in the reactivity of platelets and thus MPV can be used as a simple cost effective tool for monitoring diabetic patients.

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