https://doi.org/10.48047/AFJBS.6.12.2024.1303-1312

African Journal of Biological Sciences
Journal homepage: http://www.afjbs.com
Research Paper Open Access

# INVESTIGATING THE ROLE OF PLATELET INDICES AND ASPARTATE AMINOTRANSFERASE TO PLATELET RATIO INDEX (APRI) AS BIOMARKERS IN DIABETES MELLITUS PATIENTS ATTENDING A TERTIARY CARE HOSPITAL

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**Article History**
Volume 6 Issue 12, 2024
Received: 25 May 2024
Accepted : 25 June 2024
doi:
10.48047/AFJBS.6.12.2024.1303-1312

**ABSTRACT**

**INTRODUCTION:**

Diabetes Mellitus (DM) poses a global health challenge, representing a pandemic issue. It is recognized as a condition that increases the likelihood of blood clot formation, leading to heightened platelet activity. Elevated platelet activation can induce changes in platelet morphology and function, contributing to the onset of both microvascular and macrovascular complications in individuals with this metabolic condition. Despite being readily available, platelet indices such as Mean Platelet Volume (MPV), Platelet Distribution Width (PDW), and Plateletcrit (PCT) remain underused in clinical laboratory settings. Elevated levels of the AST to Platelet Ratio Index (APRI) show a significant correlation with Cardiovascular Risk (CVR), indicating a substantial increase in CVR, warranting attention and monitoring.

**MATERIALS AND METHOD:**

A cross-sectional investigation took place at the Department of Pathology, Chettinad Hospital and Research Institute, Chengalpattu, spanning three months from February 2023.The study examined 144 patients with Type 2 Diabetes Mellitus (DM) who were receiving care at the Outpatient Department (OPD).

**RESULTS:**

Among the 144 patients, 68% (98 individuals) suffered from diabetic complications, with 32% (46 out of 98) experiencing cardiovascular issues. Notably, patients with nephropathy demonstrated higher MPV and platelet levels, while those with retinopathy had elevated PDW. Furthermore, patients with cardiovascular complications exhibited increased Plateletcrit and HbA1c levels.A comparison between patients with and without diabetic complications revealed significant differences in age, duration of diabetes, Fasting Blood Sugar (FBS), HbA1c, MPV,

and PDW. Additionally, when platelet indices were compared with glycaemic control, patients with poorly managed diabetes displayed higher platelet indices compared to those with controlled diabetes, with statistical significance.Moreover, a positive and significant correlation was observed between MPV, PDW, APRI, duration of diabetes, FBS, HbA1c, and diabetic complications, whereas a negative correlation was found with platelet count, all of which were statistically significant.

**CONCLUSION:**

Platelet Indices and APRI offer a straightforward and accessible approach to assessing platelet hyperactivity in Diabetes Mellitus (DM).

**KEY WORDS:** Diabetes Mellitus, Platelet indices,AST to platelet ratio index (APRI), Cardiovascular risk.

## INTRODUCTION:

Diabetes mellitus (DM) presents as a persistent state of elevated blood sugar levels, marking it as a chronic metabolic disorder.(1) In 2015, the International Diabetes Federation (IDF) reported that about 415 million adults aged 20 to 79 years were afflicted with diabetes mellitus, a chronic metabolic condition marked by sustained hyperglycaemia resulting from compromised insulin secretion, insulin resistance, or both.(1) As per the 2012 statistics from the World Health Organization (WHO), diabetes affects 10% of the adult population.(2)

Diabetes, characterized by a gradual onset, arises from a complex interplay of environmental influences and polygenic inheritance. The majority of individuals with diabetes experience complications, primarily stemming from elevated blood sugar levels, which can contribute to heightened morbidity and mortality rates due to inadequate glycaemic management.(2)

Diabetes mellitus (DM) is a prevalent endocrine disorder known for its widespread metabolic disruptions affecting various organs such as the heart, nerves, eyes, CNS, kidneys, gastrointestinal system, and blood vessels. These metabolic irregularities lead to chronic complications, increasing both mortality and morbidity rates. In recent years, India has gained prominence as the epicentre of diabetes, boasting the highest population of affected individuals globally. With approximately one in every five people in the country living with DM, India has earned its reputation as the world's diabetes capital.(3)

Diabetes creates a prothrombotic environment characterized by inflammation and expedited development of atherosclerosis.(4) Although the causes of diabetes mellitus (DM) are multifaceted, platelets, particularly large ones, assume a significant role in its pathogenesis and the ensuing complications. (5) The aberrant functions of platelets in DM primarily stem from their immaturity, larger size, and activation triggered by the metabolic environment characteristic of DM or vascular injury. These factors are intricately interconnected, contributing to the complex landscape of diabetic complications.(5)

The AST to Platelet Ratio Index (APRI score) is a convenient tool for predicting cardiovascular complications, offering a swift and cost-effective alternative to invasive tests. Considering the importance of systemic inflammation in Diabetes Mellitus, our study explored the potential of APRI scores in predicting cardiovascular risk among patients with Diabetes Mellitus.(6,7)

Platelet dysfunction in diabetic individuals leads to heightened platelet hyperaggregability and activation, resulting in the release of more granules from circulating platelets. This excessive release contributes to a shortened lifespan of platelets, prompting the bone marrow to produce larger platelets. This phenomenon arises due to increased ploidy and activation of megakaryocytes. These larger, more youthful platelets possess a larger volume and heightened functional activity, characterized by increased surface expression of key markers such as GPIIb/IIIa, GPIb-IX, GPIa/IIa, and CD62. Additionally, they exhibit enhanced capacity for thromboxane synthesis and express various platelet-specific proteins. (5)The heightened aggregation and activation of platelets have been linked to the onset and progression of both microvascular and macrovascular complications. (5)

Platelet indices (PI) are parameters obtained daily as part of an automatic blood count, serving as markers of platelet activation. These indices are associated with platelet morphology and proliferation kinetics. Among the commonly assessed PI are the mean platelet volume (MPV), platelet distribution width (PDW) and plateletcrit (PCT).(8)
Measuring platelet indices such as mean platelet volume (MPV), platelet distribution width (PDW), and plateletcrit allows for the assessment of larger platelets in diabetic patients. This monitoring of platelet function through such indices holds promise in mitigating morbidity and mortality associated with diabetes-related complications.(9)

**MATERIALS AND METHOD:**

A cross-sectional study was conducted at the Department of Pathology, Chettinad Hospital and Research Institute in Chengalpattu over a three-month period starting from February 2023. The study focused on 144 patients diagnosed with Type 2 Diabetes Mellitus (DM) who visited the Outpatient Department (OPD). The sample size was determined using the formula n= Z2pq/d2. Prior to participation, informed consent was obtained from all individual participants, and approval from the Institutional Ethics Committee was secured (IHEC-II/0316/23).
The study included patients with Type 2 DM who were receiving care at the Outpatient Department. Exclusion criteria encompassed individuals with anaemia (Hb <11 g/dL for females and Hb <12 g/dL for males), thrombocytopenia (platelet count <1,50,000/μL), thrombocytosis (platelet count >4,50,000/μL), and malignancy. This was done to eliminate potential confounding factors related to reactive platelets. Moreover, patients who initially had hypertension and later developed DM were excluded to mitigate the influence of hypertension as a confounding factor in the study.
A 2 mL blood sample was collected in a Dipotassium Ethylenediaminetetraacetic Acid (EDTA) tube for platelet indicesand promptly analysed using an automated haematology analyser (COULTER LH 780 Haematology Analyzer) within one hour of collection. This approach aimed to minimize potential variations arising from sample aging.
The samples for Fasting Blood Sugar (FBS), HbA1c, and Aspartate Aminotransferase (AST) were collected in sodium fluoride, EDTA, and plain vacutainer tubes, respectively. These samples were then kept at room temperature. FBS and AST were analysed using an automated clinical chemistry analyser, Beckman DXC 800. HbA1c was determined using the high-performance liquid chromatography method.
Reference ranges for various parameters were as follows: MPV: 7.4-10.4 fL, Platelet Distribution Width (PDW): 9-14 fL, Fasting Blood Sugar (FBS): <100 mg/dL, Haemoglobin A1c (HbA1c): <6.5%, Aspartate Aminotransferase (AST): 0-46 U/L.

**STATISTICAL ANALYSIS:**

Data collected were entered into an MS Excel spreadsheet and analysed using SPSS version 23 (IBM Corp.). Quantitative data were presented as counts and percentages, while qualitative data were summarized using the range (maximum and minimum values), mean, and standard deviation. Student’s t-test was utilized to compare categorical variables among different groups. Pearson's correlation test was employed to determine the correlation coefficient (r value) between variables. Statistical significance was set at a probability (p-value) of less than 0.05.

**RESULTS:**

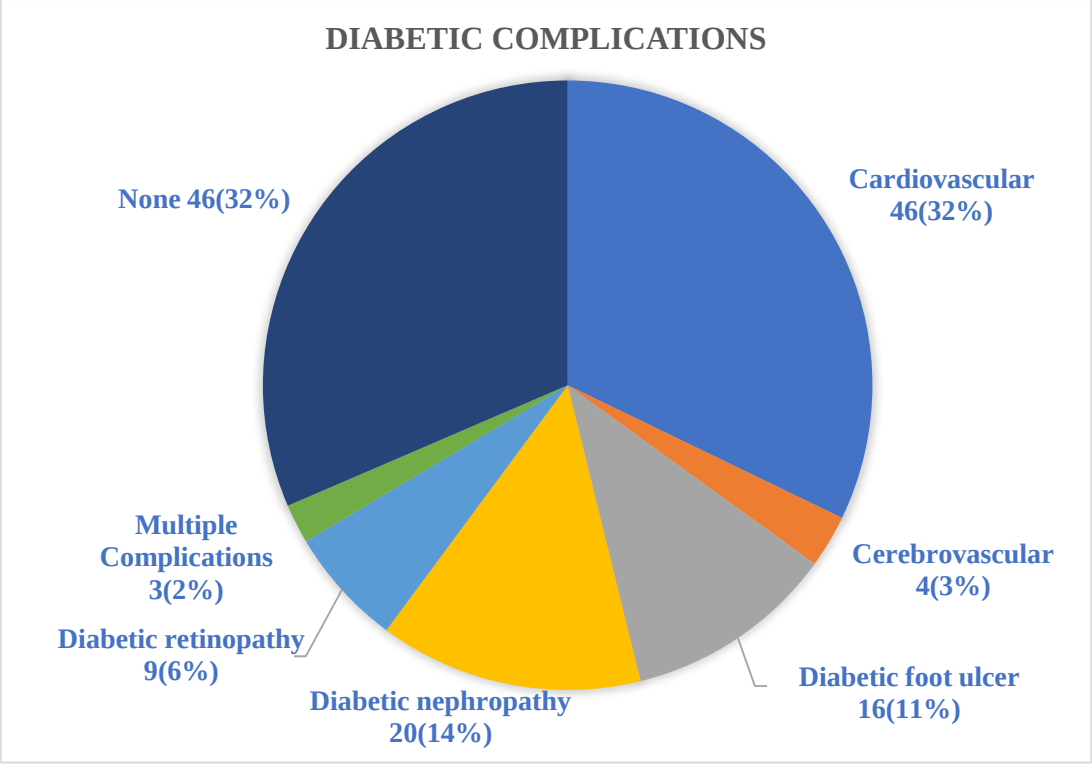
The present study was conducted over a period of three months. As shown in Table.1 Out of 144 patients, 79 (54.9%) were males and 65 (45.1%) were females. The majority of patients were in age group of 51- 60 years (mean age of the study group was 51.66 ± 11.82 years and

ranged between (26-80 years). Majority 49 (34.2%) of females were home makers and 46 (31.9%) of the study participants were employed. Majority 104 (72.2%) of the patients had diabetes for a duration of 1- 5 years. The average duration of diabetes 4.98 ± 2.93 years. Among 144 patients 120 (83%) were on regular treatment, 24 (16.7%) were on irregular treatment.

***Table.1- Demographic characteristics of Study participants***

| **VARIABLE** | | **NO** | **%** |
|---|---|---|---|
| Age (Years) | 21-30 Years | 3 | 2.1 |
| | 31-40 Years | 28 | 19.4 |
| | 41-50 Years | 38 | 26.4 |
| | 51-60 Years | 41 | 28.4 |
| | 61-70 Years | 27 | 18.8 |
| | 71-80 Years | 7 | 4.9 |
| Gender | Male | 79 | 54.9 |
| | Female | 65 | 45.1 |
| Occupation | Employed | 46 | 31.9 |
| | Home Maker | 49 | 34.2 |
| | Retired | 4 | 2.7 |
| | Self Employed | 45 | 31.2 |
| Duration of DM(Years) | 1 to 5 | 104 | 72.2 |
| | 6 to 10 | 33 | 22.9 |
| | 11 to 15 | 7 | 4.9 |
| Treatment History | Regular | 120 | 83.3 |
| | Irregular | 24 | 16.7 |

***Figure.1- Diabetic Complications***




As shown in Figure.1 Among the study participants, majority of the patients 46(32%) had cardiovascular complications, 20 (14%) had diabetic nephropathy, 16(11%) had diabetic foot ulcer, 9(6%) had diabetic retinopathy, 4(3%) had cerebrovascular complications and 3(2%) had multiple complications.

***Table.2- Association between Demographic details and APRI***

| **VARIABLE** | | **APRI** | | **P-VALUE** |
|---|---|---|---|---|
| | | **< 0.5 n(%)** | **> 0.5 n(%)** | |
| Age | 21-30 Years | 3(3) | 0(0) | 0.424 |
| | 31-40 Years | 18(18.2) | 10(22.2) | |
| | 41-50 Years | 29(29.3) | 9(20.5) | |
| | 51-60 Years | 29(28.3) | 12(27.3) | |
| | 61-70 Years | 18(18.2) | 9(20.5) | |
| | 71-80 Years | 3(3) | 4(9.1) | |
| Gender | Male | 56(55.6) | 23(52.3) | 0.716 |
| | Female | 44(44.4) | 21(47.7) | |
| Occupation | Employed | 33(32.3) | 13(29.6) | 0.442 |

| | | | | |
|---|---|---|---|---|
| | Home Maker | 31(31.3) | 18(40.9) | |
| | Retired | 3(3) | 1(2.3) | |
| | Self Employed | 33(33.3) | 12(27.3) | |
| Duration of DM(Years) | 1 to 5 | 73(72.7) | 31(70.5) | 0.095 |
| | 6 to 10 | 26(26.3) | 7(15.9) | |
| | 11 to 15 | 1(1) | 6(13.6) | |
| Treatment History | Regular | 98(98) | 22(50) | 0.001* |
| | Irregular | 2(2) | 22(50) | |

* P value <0.05

As shown in Table.2 Out of 144 patients, among three patients belonging to 21 to 30 years of age, 3 patients had APRI less than 0.5, among 28 patients belonging to 31 to 40 years of age 18 patients had APRI less than 0.5, 10 patients had APRI more than 0.5. Among 38 patients belonging to 41 to 50 years of age 29 patients had APRI less than 0.5, 9 patients had APRI more than 0.5. Among 41 patients belonging to 51 to 60 years of age 29 patients had APRI less than 0.5, 12 patients had APRI more than 0.5. Among 27 patients belonging to 61 to 70 years of age 18 patients had APRI less than 0.5, 9 patients had APRI more than 0.5. Among 27 patients belonging to 61 to 70 years of age 18 patients had APRI less than 0.5, 9 patients had APRI more than 0.5. Among 7 patients belonging to 71 to 80 years of age 3 patients had APRI less than 0.5, 4 patients had APRI more than 0.5. Among 79 male patients 56 patients had APRI less than 0.5, 23 patients had APRI more than 0.5 and among 65 female patients 44 patients had APRI less than 0.5, 21 patients had APRI more than 0.5. Among 46 employed patients 33 patients had APRI less than 0.5, 13 patients had APRI more than 0.5 and among 49 home maker patients 31 patients had APRI less than 0.5, 18 patients had APRI more than 0.5. Among 4 retired patients 3 patients had APRI less than 0.5, 1 patient had APRI more than 0.5 and among 45 self-employed patients 33 patients had APRI less than 0.5, 12 patients had APRI more than 0.5. 104 patients had diabetes for a duration ranging from 1 to 5 years, among which 73 patients had APRI less than 0.5, 31 patients had APRI more than 0.5, 33 patients had diabetes for a duration ranging from 6 to 10 years, among which 26 patients had APRI less than 0.5, 7 patients had APRI more than 0.5 and 7 patients had diabetes for a duration ranging from 11 to 15 years, among which 1 patient had APRI less than 0.5, 6 patients had APRI more than 0.5. Among 120 patients on regular treatment, 98 patients had APRI less than 0.5, 22 patients had APRI more than 0.5 and among 44 patients on irregular treatment 2 patients had APRI less than 0.5, 22 patients had APRI more than 0.5.

***Table.3- Platelet indices and HbA1c in patients suffering from diabetes mellitus***

| **COMPLICATIONS** | **MPV** | **PDW** | **PLATLETCRIT** | **PLATELET** | **HbA1c** |
|---|---|---|---|---|---|
| Cardiovascular (46) | 14.47±1.28 | 22.03±1.31 | 0.24±0.05 | 203.76±30.68 | 6.81±1.27 |
| Cerebrovascular (4) | 14.70±1.77 | 21.07±1.34 | 0.23±0.04 | 286.25±68.96 | 5.87±0.43 |
| Diabetic foot (16) | 15.03±1.02 | 23.15±2.06 | 0.23±0.04 | 284.81±72.70 | 6.16±0.86 |

| | | | | | |
|---|---|---|---|---|---|
| Nephropathy (20) | 15.14±1.28 | 21.66±1.20 | 0.22±0.03 | 292.25±50.54 | 5.84±0.47 |
| Retinopathy (9) | 14.92±1.48 | 23.56±1.84 | 0.22±0.04 | 266.78±67.31 | 6.08±0.30 |
| Multiple complications (3) | 14.867±1.15 | 20.13±0.97 | 0.20±0.02 | 262.33±48.12 | 6.60±1.15 |
| Absent (46) | 8.14 ± 0.99 | 16.76±0.53 | 0.23±0.03 | 274.56±55.38 | 5.849±0.56 |

As indicated in Table 3, nephropathy patients exhibited elevated levels of Mean Platelet Volume (MPV) and platelets, while retinopathy patients demonstrated higher Platelet Distribution Width (PDW). Moreover, diabetic patients with cardiovascular complications displayed increased Plateletcrit and HbA1c levels.

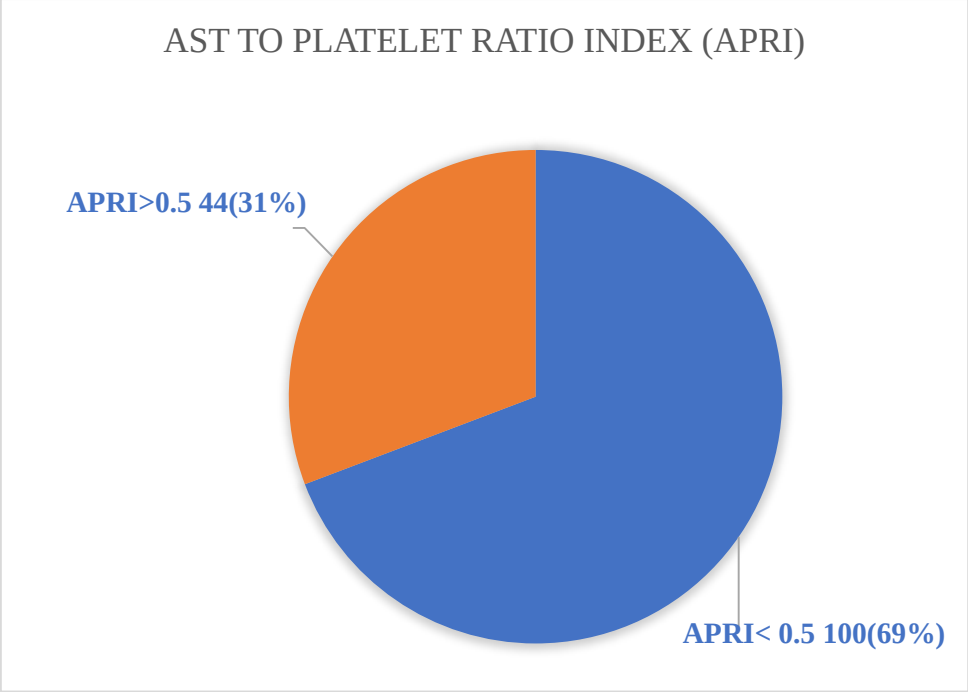
***Table.4- Association between Diabetic complications and APRI***

| **VARIABLE** | | **APRI** | | **P-VALUE** |
|---|---|---|---|---|
| | | **< 0.5 n(%)** | **> 0.5 n(%)** | |
| Diabetic Complications | Cardiovascular | 3(3) | 43(97.7) | 0.001* |
| | Cerebrovascular | 4(4) | 0(0) | |
| | Diabetic foot ulcer | 16(16.2) | 0(0) | |
| | Diabetic nephropathy | 20(20.2) | 0(0) | |
| | Diabetic retinopathy | 8(8.1) | 1(2.3) | |
| | multiple complication | 3(3) | 0(0) | |
| | None | 46(45.5) | 0(0) | |

* P value <0.05

As shown in Table.4 Among patients 46(32%) with cardiovascular complications, 3 had APRI less than 0.5, 43 with APRI more than 0.5. 4 patients with cerebrovascular complications had APRI less than 0.5, 16 patients with diabetic foot ulcer had APRI less than 0.5, 22 patients with diabetic nephropathy had APRI less than 0.5, 3 patients with multiple complications had APRI less than 0.5. Among 9(6%) patients with diabetic retinopathy, 8 had APRI less than 0.5, 1 with APRI more than 0.5 and were statistically significant.

***Figure.2- APRI among Diabetic patients***




As shown in Figure.2 Among diabetic patients with complications, 44 (31%) patients had APRI more than 0.5 and 100 (69%) patients had APRI less than 0.5.

***Table.5-Comparative Analysis of Variables in Patients with and without Diabetes Mellitus Complications: Mean ± Standard Deviation***

| VARIABLES | COMPLICATIONS PRESENT | COMPLICATIONS ABSENT | P VALUE |
|---|---|---|---|
| Age (Years) | 54.11 ± 11.33 | 46.13 ± 11.15 | 0.001* |
| MPV (fL) | 14.76± 1.28 | 8.14± 0.99 | 0.001* |
| PDW (%) | 22.18± 1.63 | 16.76± 0.53 | 0.001* |
| Plateletcrit (%) | 0.23 ± 0.04 | 0.23 ± 0.03 | 0.501 |
| Platelet count (x103/mm3) | 246.00 ± 63.03 | 274.56 ± 55.38 | 0.010* |
| AST | 38.37 ± 18.32 | 23.04 ± 7.49 | 0.001* |
| APRI | 0.37 ± 0.21 | 0.18 ± 0.07 | 0.001* |
| Blood Sugar (mg/dL) | 142.18 ± 52.69 | 119.7 ± 37.66 | 0.011* |
| HbA1c | 6.40 ± 1.06 | 5.849 ± 0.56 | 0.001* |

* P value <0.05

When various parameters were compared between diabetic patients with complications and those without complications, as presented in Table 4, it was noted that Mean Platelet Volume (MPV) and Platelet Distribution Width (PDW) were significantly higher in patients with complications compared to those without complications. Additionally, age, Aspartate Aminotransferase (AST), APRI (AST to Platelet Ratio Index), blood sugar, and HbA1c were

all significantly higher in patients with complications than in those without, with p-values <0.05.
However, there was no significant difference between the groups in relation to Plateletcrit. Notably, Platelet count was lower in patients with complications compared to those with uncomplicated diabetes, with statistical significance.

**DISCUSSION:**
In a study conducted by Tanima Dwivedi and Reshma Davangeri et al., it was noted that all platelet indices (Mean Platelet Volume [MPV], Platelet Distribution Width [PDW], and Plateletcrit) exhibited higher levels in patients with complications compared to those without, and these discrepancies were statistically significant. Similarly, in our study, upon comparing various parameters between diabetic patients with and without complications, it was found that mean platelet volume, platelet distribution width, and Aspartate Aminotransferase to Platelet Ratio Index (APRI) were elevated in patients with complications in comparison to those without, with statistically significant differences.(2)
In patients with complications attributed to diabetes mellitus (DM), both Fasting Blood Sugar (FBS) and HbA1c levels were notably elevated compared to those without complications. Statistical significance was observed for both FBS (p-value < 0.011) and HbA1c (p-value <0.001). (2)
Furthermore, when examining platelet indices in relation to glycaemic control, patients with HbA1c levels exceeding 6.5% exhibited higher values in platelet indices (Mean Platelet Volume – MPV and Platelet Distribution Width - PDW) compared to those with HbA1c levels below 6.5%. These differences were statistically significant (p-value <0.001 for all two platelet indices), aligning with previous studies conducted by Kodiatte TA et al. and Demirtunc R et al. (9)
In diabetic patients, inadequate glycaemic control leads to heightened platelet activity directly and via the glycation of platelet membrane proteins. This process accelerates platelet destruction and prompts enhanced thrombopoiesis, culminating in elevated platelet indices in patients experiencing complications.Patients with complications exhibited a significantly lower platelet count compared to those with uncomplicated diabetes, indicating a notable difference between the two groups in this study.The authors also noted that while platelet count showed no association, it displayed an inverse relationship with all platelet indices, a conclusion consistent with findings from previous studies. In this study platelet count was lower in patients with complications compared to those with uncomplicated diabetes, with statistical significance.(9)
In a study conducted by Carlo De Matteis et al., the role of APRI score as a valuable predictor for CVR demonstrated that in a mixed cohort of non-metabolic and metabolic syndrome subjects, APRI significantly correlated with CVR and determined when > 0.5 a significant increase in CVR in both genders, especially females. The spike in CVR was relatively highin older patients but is significantly higher in younger and premenopausal women, approaching risk values thatare usually typical of men.Similarly in this study,Patients with complications exhibited notably higher levels of age, Aspartate Aminotransferase (AST), APRI (AST to Platelet Ratio Index), blood sugar, and HbA1c compared to those without complications, which was statistically significant.(7)

**LIMITATIONS:**
The study's limitations included the inability to conduct patient follow-ups to ascertain the prognostic implications of the observed findings to validate the current results. However, it's important to note that these factors comprise a minor aspect of the study.

**CONCLUSIONS:**

The recent research highlights that individuals with diabetes mellitus (DM) exhibit larger and more active platelets, along with heightened thrombogenic potential, leading to increased platelet indices. These larger platelets serve as significant risk factors for atherosclerosis development and are closely linked to vascular complications. Moreover, elevated platelet indices and APRI correlate directly with diabetic complications and glycaemic management. Monitoring platelet indices and APRI during routine haematological assessments can readily identify diabetic patients at higher risk and potentially facilitate timely intervention with antiplatelet therapy, thereby mitigating disease progression and reducing the likelihood of thromboembolic events. Thus, platelet indices and APRI emerge as a valuable tool for assessing DM control, disease advancement, and thrombotic risk.

**ACKNOWLEDGEMENTS:**Nil

**CONFLICT OF INTEREST:** There are no conflicts of interest to declare.

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