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Study of the significance of platelet parameters in iron deficiency anemia cases

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Abstract

Anemia is a condition characterized by reduced concentrations of hemoglobin (Hb) in the blood below cut-off levels, and/or a diminished number of red blood cells (RBC or reticulocytes) according to age and gender. Iron deficiency anemia is the most common type of anemia and is diagnosed by serum iron panel studies. Platelet parameters usually altered in reactive mechanism. Reactive thrombocytosis is usually seen in iron deficiency anemia. Platelet indices like Mean Platelet volume (MPV) and Platelet Distribution Width (PDW) are raised during platelet activation. Plateletcrit (PCT) is an effective indicator for detecting platelet quantitative abnormalities. We aimed to study the significance of the platelet parameters in iron deficiency anemia cases. It is a prospective study conducted at Department of Pathology, Akshaya Health Care, Bangalore, India from January 2024 to April 2024. A total of 100 samples were studied, out of which, 50 were confirmed cases of iron deficiency anaemia and 50 were controls with normal haemogram (obtained from samples for general health check up requests from normal individual). Platelet parameters were analysed in both the groups. Data with continuous variables are presented as mean ± SD. Comparison of group means was done with student's t- test. P value <0.05 was considered statistically significant. The difference in mean platelet count, MPV, PDW and PCT between iron deficiency anemia cases and control was found to be statistically significant ($p < 0.05$). We concluded that there was a significant relationship between platelets parameters in microcytic hypochromic anemia.

Keywords: Iron deficiency anemia; Platelet parameters; Platelet Distribution Width (PDW); Mean Platelet volume (MPV) and plateletcrit (PCT)

1. Introduction

Anemia is defined as "a condition characterized by reduced concentrations of hemoglobin (Hb) in the blood below cut-off levels, and/or a diminished number of red blood cells (RBC or reticulocytes)". Hb is required to carry oxygen and if too few or abnormal red blood cells, or not enough haemoglobin is there, oxygen carrying capacity of Hb will be reduced to the body's tissues. It develops symptoms such as weakness, fatigue and dizziness. The optimal haemoglobin physiological concentration varies by age, gender, altitude, smoking and pregnancy status. Anemia is defined by World Health Organization (WHO) as Hb level less than 12g/dl and 13 g/dl in adult non-pregnant female and adult male respectively [1]. Iron deficiency anemia (IDA) is the most common type of anemia worldwide. WHO projects that almost 25% of the world's population are anemic, with roughly 50% of them having IDA [2].The causes of IDA include increase in red blood cell lysis, decreased red cell production, blood loss or due to increase in demand of the iron by the body [3]. IDA is commonly diagnosed by iron studies like serum iron, ferritin level, total iron binding capacity etc. [4]. Moderate IDA found to be associated with thrombocytosis while severe IDA mostly accompanied with thrombocytopenia [4]. Platelet indices like Mean Platelet volume (MPV) and Platelet Distribution Width (PDW) are increased during platelet activation. Plateletcrit (PCT) is an effective indicator for detecting platelet quantitative abnormalities [4]. Various studies suggested that platelet parameter change in IDA is due to platelet morphological features changes [4, 5]. The aim is to study the significance of the platelet parameters in iron deficiency anemia cases.

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2. Materials and methods

It is a prospective study conducted at Department of Pathology, Akshaya Health Care, Bangalore, India from January 2024 to April 2024. A total of 100 samples were studied, out of which, 50 were confirmed cases of iron deficiency anaemia (IDA) (group A) and 50 were controls with normal haemogram (obtained from samples came for general health check-up requests from normal individual) (Group B). Fresh venous blood samples were collected in ethylenediaminetetraacetic acid (EDTA) vacutainers tube as well as in tube without anticoagulants (Red top tube) and were stored at room temperature until they were analyzed within two hours. Clotted samples, inadequate samples as well haemolysed samples were excluded from the study.

EDTA blood samples were analyzed for red blood cell and platelet parameters estimation by processing in an automated hematology analyzer UNITRON BIO-MEDICALS (UBM) Fx-19T (Model : URIT – 3020, serial number: 3020ET03862) automated cell counter, manufactured by UBM. The quality control, calibration and maintenance of the analyzer were done as recommended by the manufacturer. Red blood cell related parameters and platelet count was confirmed on peripheral blood smear after staining with Leishman's stain, as per standard protocol [6, 7]. Serum were separated from samples collected in red top tube as per standard laboratory protocol, and were analyzed for serum iron parameters on Advia 1800 photometry analyser.

- **Inclusion criteria:-** Hemoglobin less than 12 gm/dl in adult non pregnant females and 13 gm/dl in male, serum iron < 10µmol/l, serum ferritin < 15 µg/l and transferrin saturation (Tfsat) < 16% were considered along with peripheral blood smear examination [8]. These reference values are used at the Department of Pathology, Akshaya Health Care.
- **Exclusion criteria:-** Cases with leucocytosis, leukemoid reaction, leukemia, parasite, platelet disorders or other causes of reactive thrombocytosis (infection, acute hemorrhage, chronic inflammatory disease, malignancy) were excluded from the study.
- **Statistical analysis:-** Data analysed for continuous variables are presented as mean ± standard deviation (SD). Comparison of group means was done with student's t- test. Platelet parameters were also correlated with different Hb range and Chi square test was applied as test of significance. P value <0.05 was considered statistically significant.

3. Results and discussion

We studied two groups with group A (50 cases of iron deficiency anemia) and group B (50 controls) and compared the platelet indices between the two groups. Most of the subjects in group A were females (90%) when compared to the group B (40% females) with significantly higher proportion of females in iron deficiency anemia group **(p < 0.00001). (Figure 1 and 2) (Table1)**

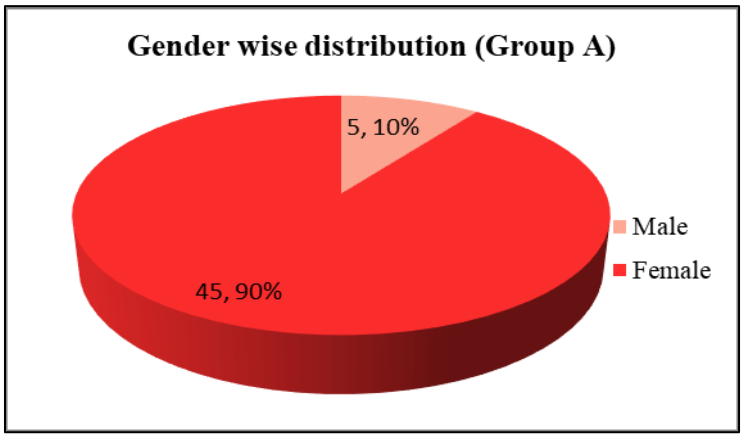

Figure 1 Gender wise distribution of the subjects in Group A (IDA cases).

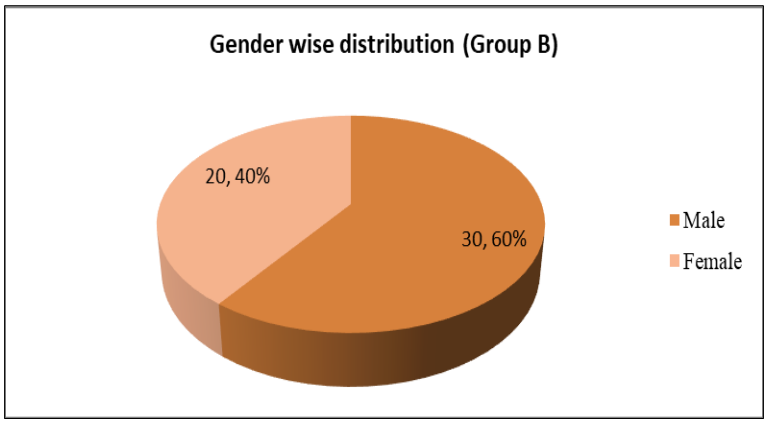

Figure 2 Gender wise distribution of the subjects in Group B (Controls)

Table 1 Comparison of gender wise distribution of the subjects in both the groups.

	Group A (cases) Frequency	**Group B (controls) Frequency**	**(Chi square test) p value**
Male	5 (10%)	30 (60%)	**< 0.00001**
Female	45 (90%)	20 (40%)	
Total	**50 (100%)**	**50 (100%)**	

The most common age group among group A subjects was 20-30 years (38%) followed by 40-50 years (22%) with the mean age of 34.2 ± 13.7 years. Similarly, the most common age group among group A subjects was also 20-30 years (26%) followed by 40-50 years and > 60 years (18% each) with the mean age of 40.24 ± 16.25 years. Control group was observed to be significantly older than the case group **(p = 0.0472). (Figure 3and 4) (Table 2).**

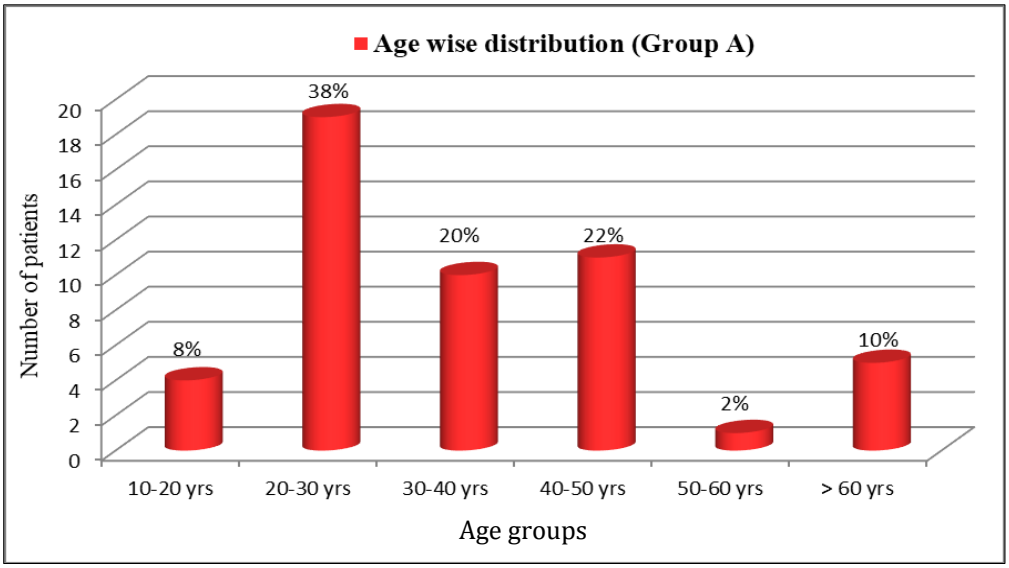

Figure 3 Age wise distribution of the subjects in Group A (Iron deficiency anemia cases)

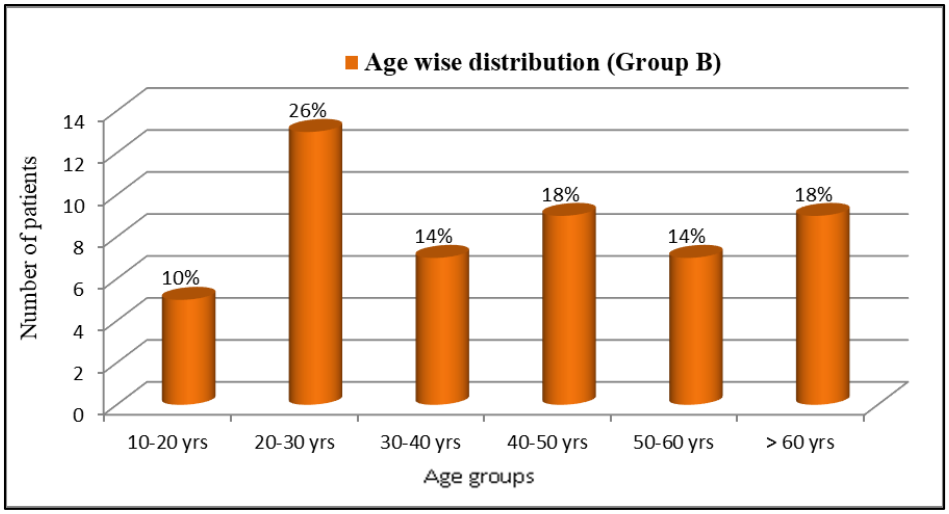

Figure 4 Age wise distribution of the subjects in Group B (Controls)

Table 2 Comparison of mean age of the subjects in both the groups.

Groups	Mean age ± SD	p value (Unpaired t-test)
A (cases)	34.2 ± 13.7 years	**0.0472**
B (controls)	40.24 ± 16.25 years	

Mean Hb of group A and group B was found to be 9.826 ± 1.874 g/dl and 14.184 ± 1.582 g/dl, respectively with significant difference between the mean Hb concentrations of the two groups ($p < 0.0001$). (Table 3)

Table 3 Comparison of mean Hb concentration of the subjects in both the groups.

Groups	Mean Hb ± SD	p value (Unpaired t-test)
A (cases)	9.826 ± 1.874 g/dl	**<0.0001**
B (controls)	14.184 ± 1.582 g/dl	

The difference in mean platelet count, MPV, PDW and PCT between iron deficiency anemia cases and control was found to be statistically significant ($p < 0.05$). (Table 4)

Table 4 Comparison of platelet parameters of the subjects in both the groups.

Platelet parameters	Group A (cases) (Mean ± SD)	Group B(controls) (Mean ± SD)	p value (Unpaired t-test)
Platelet count (x1000/ul)	274.1 ± 78.7	204.42 ± 32.28	<0.0001
MPV (femtolitre)	9.04 ± 1.309	8.194 ± 0.976	0.0004
PCT (%)	0.227 ± 0.0586	0.1626 ± 0.0314	<0.0001
PDW	12.728 ± 2.761	10.454 ± 1.494	<0.0001

Iron deficiency anemia (IDA) is the most common nutritional disorder globally, regardless of patient's age, gender as well as socioeconomic status. There are few previous studies on the relationship between IDA and altered platelet parameters, and has long been a subject of debate in the literature.

We observed that prevalence of IDA was significantly more in females as supported by Chaitra et al., (2023) [9] and Ai-alimi et al., (2018) [10]. Possible causes of the high prevalence rate of iron deficiency anemia among females population may include inadequate dietary iron intake, a concurrent inadequate intake of dietary micronutrients, poor bioavailability, nutritional status and lack of awareness of iron deficiency. Females are more prone to be anemic particularly at reproductive age because of menstruation bleeding, pregnancy related complications and because of various socioeconomic customs; getting a lower quality diet compared to males [11, 12]. Moreover, in our population, it could be due to the traditional cultural practices in some families to give more priority and rights to the males than females especially in food sharing.

The most common age group of IDA patients was 20-30 years (38%) with the mean age of 34.2 ± 13.7 years. Chaitra et al., (2023) [9] also stated that the most common age group among females was between 20 to 30 years in their study. Females in this age group are more prone to develop anemia secondary to increased physiological demands, menstrual losses, maternal blood volume expansion during pregnancy, and blood loss during and after childbirth, particularly in cases of postpartum haemorrhage. Due to all these etiological factors, females are more prone for developing IDA, as observed in the present study and also stated by previous scientific literatures [13].

It was evidenced significant difference between the mean Hb concentrations of the two groups ($p < 0.0001$). Apart from this, the present study results showed a statistical significant increase in platelet count ($p < 0.0001$) and plateletcrit ($p < 0.0001$) in IDA cases when compared with control as supported by various studies [4, 14, 15].

Munker M et al., (2007) [16] suggested that both erythropoietin (EPO) and thrombopoietin (TPO) belong to the same hematopoietic growth factor subfamily. Both of them are majorly produced in the kidney and act similarly through the JAK/STAT pathway activation and Ras signal transduction on their respective precursors. Some studies have suggested erythropoietin can affect platelet, but with controversial findings. The amino acid sequence homology of TPO and EPO may results in thrombocytosis in IDA [17]. However, in contrast, other previous studies have observed that the amino acid sequence homology of TPO and EPO as well as the cross-reactivity between TPO and EPO at the level of thrombopoetin receptor (Mpl) do not explain the relationship between iron deficiency and increased platelet count [18, 19]. Other study has suggested that increased EPO would stimulate megakaryopoiesis in moderate iron deficiency anemia, whereas high EPO response could cause thrombocytopenia in severe iron deficiency cases [20].

The duration as well as the degree of IDA may play an important role in determining the mechanism of thrombopoesis. In moderate IDA, shortening of megakaryocyte maturation; stem-cell shunt due to inhibition of erythropoiesis which results in increased production of other pluripotent cells (hemostatic compensatory mechanism); increased rate of influx of precursor cells into the megakaryocyte compartment with an increased rate of efflux; stimulator effect of transferrin on megakaryopoiesis and inhibition of iron on megakaryocyte maturation; may play role in increased platelet production. However, in severe IDA, megakaryocyte numbers decreased with increased size and platelet counts tended to normalize. This may be secondary to shortening of megakaryocyte maturation. This could, however, also be consistent with the previously described diphasic pattern of increased stimulation by endogenous Epoprecursors. It was also reported that an amino acid sequence homology between erythropoietin and thrombopoietin may explain thrombocytosis in children with IDA. [17]

In the present study, a statistical significant increase in platelet in MPV ($p = 0.0004$) and PDW ($p < 0.0001$) was observed in IDA cases when compared with controls, supported by Park MJ et al., (2013) [4] and Timuragaoglu et al., (2004) [21].

Various recent studies and meta-analyses have suggested that higher PDW and MPV values indicate enzymatically and metabolically more active platelets, with a great prothrombotic potential and can be used as an alternative marker for determining platelet activity. The megakaryocytic activation by EPO could be the one possible reason due to its homology with TPO. In IDA, increased EPO results from compensatory mechanism, to increase the red blood cell production and therefore, overall Hb for sufficient oxygen supply. The activated platelets differ in their size from non-activated form mainly due to alteration of its shape from a discoid to a spherical shape as well as pseudopodia formation which leads to a change in the PDW. The differences in platelet volume vividly correlates with differences in density, platelet aggregation to adenosine diphosphate (ADP) and serotonin uptake and release, indicating the relevance of the MPV estimation as a measure of platelet function. Thus, platelet size has become an important marker of platelet function and also a physiological variable of hemostatic importance in IDA cases. [9]

4. Conclusion

It was concluded that iron deficiency anemia is significantly associated with thrombocytosis, high MPV, PDW and PCT. Careful monitoring with proper treatment along with follow up is required for all IDA patients to prevent possible side effects such as thrombosis due to activated platelets. Further research workis warranted in a larger population for determining the relationship of platelet parameters and thromboembolic event. Also, these platelet parameters may contribute in diagnostic aids.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to declare.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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