Platelets Of Precancerous Lesions As A Diagnostic Value: A Phase Contrast Microscopy Study

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ABSTRACT Platelets are cytoplasmic fragments of megakaryocytes, formed in the marrow, round oval in shape, approximately 2 µm in greatest diameter. They are multifunctionally involved in many patho-physiological processes including homeostasis, tissue repair, thrombosis, clot retraction, vessel constriction, repair host defense and even tumor growth/metastasis. Platelets may contribute to cancer progression by stabilizing tumor cell arrest in the vasculature, stimulating tumor cell proliferation & promoting tumor cells extravasation by potentiating tumor cell induced endothelial cell retraction which enhances tumor cell interaction with the extracellular matrix. Till date very few studies on platelets as a biomarker in the research of malignancy are conducted. Hence aim is to study and correlate the morphology of human blood platelets & degree of dysplasia in normal and oral precancer by using phase contrast microscopy.

KEYWORDS: Platelets, morphology, Precancerous lesions, homeostasis, metastasis

I. INTRODUCTION

Platelets are cytoplasmic fragments of megakaryocytes, formed in the marrow, round to oval in shape, approximately 2 µm in greatest diameter. They are multifunctionally involved in many patho-physiological processes including homeostasis, tissue repair, thrombosis, clot retraction, vessel constriction, repair host defense and even tumor growth/metastasis. Platelets may contribute to cancer progression by stabilizing tumor cell arrest in the vasculature, stimulating tumor cell proliferation & promoting tumor cells extravasation by potentiating tumor cell induced endothelial cell retraction which enhances tumor cell interaction with the extracellular matrix.[1] Till date very few studies on platelets as a biomarker in the research of malignancy are published.

II. AIM AND OBJECTIVES

The Aim and objectives of this study are

• To Study and correlate the morphology of human blood platelets
• To Compare the morphological changes of platelets in healthy and precancerous individuals by using phase contrast microscope

III Material and Method

10 healthy and 10 histopathological confirmed subjects of oral precancer were selected. Prior to study verbal, written consent were taken. 2 ml blood was retrieved from patient and 2-3 drops of EDTA were added. Centrifuged at 3000 rpm for 15 min. plasma was separated from the whole blood cells. To the separated plasma, 1 ml of solution of (1% disodium seguestrrane, 1% triton x100 solution prepare in 0.7% sodium chloride) was added. And again
centrifuged at 3000 rpm for 20 min. incubated at 6°C for 15 min. Then left at room temperature and gently shaken for every 10 minutes to obtain homogenous concentrate of platelets. One drop prepared solution was added on glass slide and immediately examined under phase contrast microscope.
Material and methods

One drop prepared solution added on glass slide and immediately examined under phase contrast microscope.
Platelet morphology under phase contrast microscope

Figure 4 Normal

Figure 5 Osmf patient
Fig 7 - Under 100x oil immersion, microphotograph showing slightly regular round to oval in space cells with regular size in normal patient.
Fig 8 - Under 100x oil immersion, microphotograph showing pleomorphic size of cell with increased granularity of osmf stage 11 patient.
Fig 9 - Under 100x oil immersion microphotograph Platelets observed in leukoplakia and slow variation in size, shape, increased granularity.
Fig 10 - Under 100x oil immersion cells are showing pleomorphic size and shape of cells in lichen planus patients.

IV. Results

Out of ten patients, three cases were OSMF, Five cases of Leukoplakia and cases of lichen planus.
80% cases of leukoplakia showed platelets with variation in size, shape and granularity and 20%, showed formation of aggregates.

While among lichen planus, 50% showed variation in size and granularity and no aggregates were seen.
All the OSMF cases showed platelates with variation in size, shape and granularity and 25% showed formation of aggregates.

Therefore, among the lichen planus variation in size of platelates were more profoudly seen in OSMF and leukoplakia when compared to lichen planus. Increased granularity was seen in OSMF when compared to Leukolpakia. Formation of aggregates was seen in both OSMF and leukoplakia while it was not observed in lichen planus.
However, even among the normal subjects few morphological change of platelets were observed. 40% showed variation in shape, 30% showed variation in size, 25% showed aggregates formation. There was no observation of granularity.

So comparing the morphological changes of platelets, between normal subjects and precancerous subjects; increased variation in size and shape: increased tendency to form aggregates and increased granularity are seen in the patients with precancerous disorders.

V.DISCUSSION

Platelets were described by Addison in 1841 as “extremely minute granules” in clotting blood and were termed platelets by bizzozero, who also observed their adhesive qualities as “increased stickiness when a vascular wall is damaged.” Platelets patrol in the blood stream to recognize structures representing damaged blood vessel walls, the incident which prompts them to aggregate and form a thrombus[1]
Nailin Li 2015, summarized that Cancer progress is accompanied by platelet activation and thrombotic complications. Platelets are a dangerous alliance of cancer cells, and are a close engager in multiple processes of cancer metastasis. Platelet adhesion to cancer cells forms a protective cloak that helps cancer cells to escape immune surveillance and natural killer cell-mediated cytolysis.[4]

Platelets have a pro-angiogenic effect in the setting of early injury, progressive tumour growth, atherogenesis or chronic inflammation, and an anti-angiogenic effect in the setting of a healing wound, dormant tumours, or receding inflammation (Almog NK,2010)[2]

Platelets are contain three types of granules Dense granules - serotonin ,ADP that amplify platelet activation. Alpha granules - proteins which help in activation and coagulation Lysosomal granules - glycosidases and proteases. Zhuge Y et al 2009 found ultrastructural changes in platelets from patients with lung cancer, included increase in number of alpha granules.In our study also there was increase in granularity of platelets in subjects with precancerous lesions.[4]

Erpenbeck L, 2010 reported that platelet adhesion receptors play a critical role in tumor-platelet cross talk and in the process of hematogenous spread. Tumor cells have the ability to aggregate platelets, first reported in 1968 as tumor cell induced platelet aggregation.[2]

Bambace NM 2011, Platelets can protect tumor cells by coating them and thereby shielding them from physical stressors within the vasculature and by permitting evasion from the immune systems effector cells. In the present study aggregates of platelets were observed in subjects who had precancer lesions.[2]

Pereira et al,2015, evaluated the MORPHOLOGY OF PLATELETS IN NORMAL AND PREMALIGNANT LESIONS USING PHASE CONTRAST MICROSCOPY. The study group included 15 healthy individuals and 15 patients with oral precancer lesions and they observed variation in the size of platelets, increase in number and no spider like process or spicules in the platelets in the precancerous patient. In few cases of premalignant lesions also showed an increase of aggregation of platelets with increase in the degree of dysplasia.[1]

VI. CONCLUSION

In the present study we observed variation in shape, size, formation of aggregates and increased granularity in platelets in the subjects having precancerous lesions. It is likely that the study of platelets in oral precancer will have wider clinical implications in the near future. Therefore additional studies with a larger population have to be conducted to ascertain these findings.

Even few studies conducted and published in platelet and precancer where platelets show variation in size, an increase of aggregation of platelets. We also observed changes of platelets in form of shape, size increased granularity and in few cases formation of aggregates in precancer.

Some differentiating observations was noticed in present study which may be valuable for the confirmation of morphological changes in precancer. So for the confirmation of platelet morphology needed to study in larger population. One of the important investigation to confirm the precancer lesion. This study concluded that the morphology of changes of platelets and degree of dysplasia; without any invasive investigation & by routine ,noninvasive procedure we can also confirm the early detection of precancer lesion.

REFERENCES