Manual Immature Reticulocyte Fraction: a reliable marker to assess post traumatic blood loss.

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Abstract. Hemorrhage is a common stress condition following acute trauma that needs prompt medical attention as it can be fatal. Traumatic haemorrhage be classified into three categories; mild, moderate and severe based on clinical manifestations and outcomes expected with each. However, some instances, clinical features may not reflect exact degree of blood loss due to other comorbid factors etc. Rapid marrow response to haemorrhage includes release of more immature red cells; reticulocytes and normoblasts depending on haemopoietic stimulus. Therefore, the presence of red cell precursors in peripheral blood is an expected marker following acute hemorrhage. Among reticulocyte parameters, Immature Reticulocyte Fraction (IRF) is widely used to indicate the erythropoietic activity of the bone marrow in stress conditions. Even though the manual reticulocyte count is performed in laboratories, calculation of manual IRF is not routinely practiced. Based on morphology, reticulocytes can be classified in to immature and mature sub types. Although automated method is available, it is costly to use. Therefore this study was performed to evaluate the relationship of manual IRF with degree of hemorrhage in acute trauma. In this analytical cross-sectional study, 38 blood samples of acute trauma patients admitted to emergency trauma care at a tertiary care hospital were analyzed. The IRF values were significantly higher in study subjects with severe hemorrhage than mild and moderate. When the time duration from trauma to admission was considered, subjects with clinically severe hemorrhage showed high IRF values within one hour. Appearance of the most immature (stage I) reticulocytes were noted after two hours of trauma in study subjects. Therefore, this study supports us ability of manual IRF in objective assessment of early marrow response to hemorrhage thus assessment of severity of acute trauma. Thus the manual IRF in peripheral blood can be considered an important, reliable and cheap laboratory indicator in acute trauma care in the diagnosis and management acute blood loss.

Keywords: Immature Reticulocyte Fraction, Degree of hemorrhage, Acute trauma

Introduction:

Haemorrhage is a common major complication in acute trauma that needs prompt medical attention as it can cause significant morbidity and mortality due to acute hypovolemia and shock. Clinically, blood loss in trauma care is classified into three categories according to the severity as mild, moderate and severe. This clinical classification aid patient management thus outcomes. Following acute hemorrhage, red cell production is activated in response to impaired tissue perfusion through increased erythropoietin secreted by kidneys. More immature stages of red cells are released into circulation and remain longer in circulation in the presence of erythropoietic drive together with massive production of red cells predominantly. This is well documented in both haemorrhage and haemolysis.

Immature red cells are detected using a special supra-vital stain and the test is called reticulocyte count. This test specifically stain

ribosomes and RNA present in the cytoplasm (and nuclear material). Due to the absence of nuclei in reticulocytes, only cytoplasmic stain of reticulum differentiates immature red cells from other cells with cytoplasmic RNA and ribosomes. According to the Heilmeyer classification, reticulocytes in peripheral blood smears can be classified into four stages (Piva et al., 2015).Those stages of maturation can be identified and counted manually by their morphological features ranging from the most immature reticulocytes with large clump of reticulum (stage I), to the most mature with few granules of reticulum (stage IV). Automated Immature Reticulocyte Fraction (IRF) is a new diagnostic parameter available in novel automated full blood count analyzers, which is widely used to indicate the erythropoietic activity of the bone marrow in stress conditions. However, automated method is prohibitively costly to use in routine care.Therefore, this study was carried out to evaluate the relationship of manual IRF with the degree of hemorrhage in acute trauma.

Methodology:

Ethical approval was obtained from ethical review committee of Faculty of Allied Health Sciences and the permission to collect data from Director, Teaching Hospital, Karapitiya and the relevant Consultants of the units. A total of 38 study subjects including 14 mild, 19 moderate and 5 severe trauma with acute haemorrhage who were admitted to Emergency Treatment unit of Teaching Hospital Karapitiya,Galle were recruited in this study. Individuals with known clinical conditions that could directly affect hematological results were excluded. Venous blood samples collected in to dipotassium ethylenediaminetetraacetic acid (K_2EDTA) containing tubes during routine standard care were used to perform reticulocyte counting. The reticulocyte staining was performed by the researcher according to the standard procedures previously described

within one hour of collection of samples. The quality of the stain was verified with the presence of stained platelets and white blood cells as the positive control. The reticulocyte counting was performed using oil immersion bright field microscopy (Olympus CX 32) fitted with an ocular graticule. Immature reticulocytes were enumerated as per the previously defined morphology, Heilmeyer classification (Figure 1).All the reticulocyte counts & IRF counts were performed in duplicates by the researcher and were verified by a Consultant Haematologist. A difference of 10% or less in the duplicate counts was considered as acceptable.
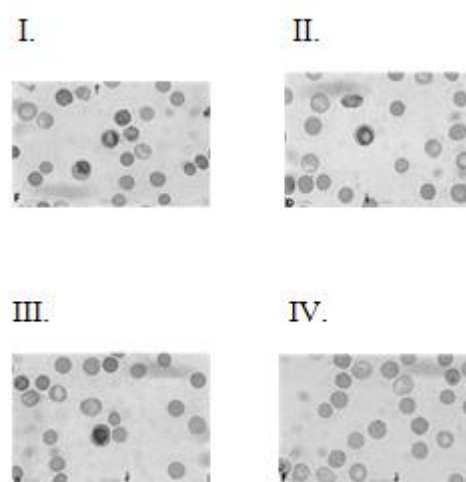

Figure1:*Maturation stages of reticulocytes according to Heilmeyer classification: Stage I: non nucleated red cells appearing with a dense clumped reticulum; Stage II: extended network of loose reticulum; Stage III: scattered granules with residual reticulum network; Stage IV: scattered granules*

Source: KDU IRC 2020

Clinical assessment of on admission severity of blood loss was extracted from patient record at ETU. Time taken for admission since traumatic event as well recorded. Results of IRF was tabulated for each patient with their blood loss severity. The results of IRF were expressed as mean along with the standard deviation (SD). Each IRF value fraction was counted & presented as a percentage (%). Data were analyzed by using Excel 2010 and R-Studio statistical software. The differences between

groups (mild, moderate, severe) were assessed by t-Test. Level of p <0.05 was considered as significant. Correlation was analyzed using the Spearman correlation coefficient.

Results:

A total of 38 patients with acute trauma including 14 mild, 19 moderate and 5 severe blood loss were included in this study. The mean age of the patients was 36.55±16.95 years. There were 24 males and 14 females reflecting male predominance for trauma.

The mean immature reticulocyte fraction of mild, moderate and severe hemorrhage were 1.86± 1.03%, 3.16 ± 2.32%and 10.4 ± 3.85% respectively. The IRF value of severe haemorrhage was significantly different from those of mild and moderate haemorrhage (P < 0.05) and the value of moderate haemorrhage was also significantly different from that of mild haemorrhage.

It showed a moderate positive relationship between manual IRF count and degree of haemorrhage according to the correlation coefficient (r) of IRF value with the degree of hemorrhage.

Table1: t-Test results of IRF values comparison between mild, moderate & severe haemorrhage conditions

Source: KDU IRC 2020

Severity	P(T<=t) two-tail
Mild – Moderate	0.021947
Mild – Severe	0.007165
Moderate - Severe	0.008740

When compare the time duration for reticulocyte / IRF increment, patients with severe haemorrhage showed higher IRF within 1st hour compared to mild and moderate blood loss. With the progress of the time, there was an increase of percentage of immature reticulocytes (stage I & II)while Stage III & IV fractions were decreased out of

total reticulocytes present in peripheral blood. There was a progressive increase of percentage of immature stages after two hours of trauma in patients with severe haemorrhage.

Percentage of maturation stages obtained within different time durations in severe haemorrhage and their trend lines are shown in following graph.
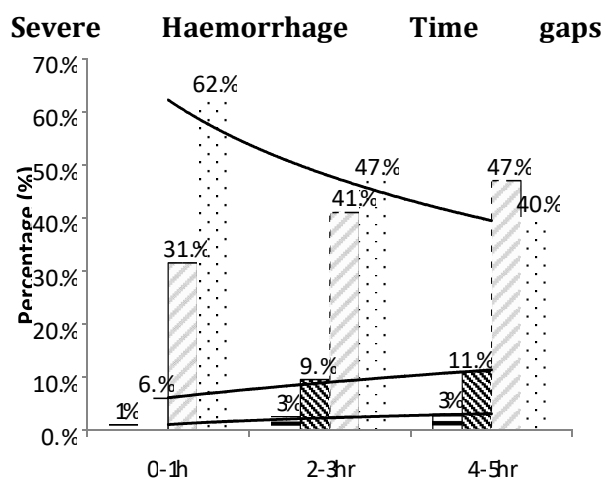
Severe Haemorrhage Time gaps

Figure 2 Stage I ☰ Stage II ⬎ Stage III ⬚ Stage IV ⋯

Source: KDU IRC 2020

Discussion and conclusion –

According to the literature, IRF is considered as one of the best parameter of marrow response (Buttarello et al., 2002). However, use of IRF in routine trauma care is limited to ascertain or to support the degree of haemorrhage. According to this study, the patients with severe haemorrhage showed higher IRF values compared to mild and moderate haemorrhage. Therefore, higher manual IRF results can be considered as an objective marker of severe haemorrhage. High IRF reflects prompt marrow response to blood loss thus it is useful even in acute concealed haemorrhage such as retroperitoneal, intra-abdominal or intra muscular bleeding following trauma or due to other reasons.

The reticulocyte count in the peripheral blood of a healthy individual is 1-2% and all of them belong to Heilmeyer group III and IV reticulocytes (considered mature forms).

The group I and II reticulocytes are not normally found in peripheral circulation (Crouch et al., 1985) in healthy subjects. In this study, stage I reticulocytes appeared in higher percentages (>3%) in the blood samples which were collected two hours after the trauma. In compliance with literature available, this study too shows that the time taken for marrow response to occur in severe haemorrhage is over two hours.

Findings of this study show the importance of properly performed manual IRF in differentiating the severity of haemorrhage in patients with trauma. Therefore, we conclude that, the manual IRF can be considered as a reliable, cheaper alternative marker of post traumatic blood loss.

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