



ANAEMIA AND ELEVATED LEUCOCYTE COUNT AS A RISK FACTOR FOR ISCHAEMIC HEART DISEASE

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Abstract :

Anaemia is the most common nutritional disorder in Indian females. It is well-known that children and women in the reproductive age group vulnerable to iron deficiency anaemia. Decreased Hb level is associated with increased work load and hypoxia (ischaemic damage) results in elevated leucocyte count. Many epidemiological studies have shown that anaemia and elevated leucocyte count as a risk factor for cardiovascular and ischaemic diseases. The present study was done to estimate the Hb(gm percent), RBC count, Total and Differential leucocyte count (TLC,DLC) in anaemic and non- anaemic females. Study group comprised of forty anaemic women (Hb less than 7 gm - Grade 3 severe anaemia with atleast 3 months of duration as it is associated with hypoxia and ischaemia that results in increased morbidity and mortality) in the age group of 20 -40yrs, forty age and sex matched healthy subjects (Hb more than 12 gm percent) were selected as controls. The results were analysed using Unpaired Students t test.

Our study result shows that Total leucocyte count was significantly higher (p less than 0.05) in severe anaemia Vs controls. On DLC, Neutrophil and Basophil were significantly higher (p less than 0.001), Eosinophil, Monocyte count were significantly less in anaemic Vs controls with no alteration in the lymphocyte count. There was a significant increase in Neutrophil to Lymphocyte ratio (NLR) in severe anaemia (p less than 0.001). In conclusion, leucocyte counts are altered in severe anaemia which plays an important role in predicting the future risk of ischaemic diseases. Further study is needed to find out whether leucocytosis is associated with mild and moderate cases of anaemia so that various intervention strategies can be implemented at an early stage to decrease the risk of CAD in developing countries.

Keyword : Anaemia , Leucocytes, Ischaemia, Neutrophil to Lymphocyte ratio

INTRODUCTION:

Anaemia is an important global public health problem affecting the greatest number of females of reproductive age group⁽¹⁾. There are two billion people worldwide who are anaemic. In 2001, WHO estimated that the prevalence of anaemia among women was 10% in developed countries and 42% in developing countries. An estimated 41% of pregnant women and 27% of preschool children worldwide have anaemia caused by iron deficiency⁽²⁾. Also deficiency of zinc, selenium, copper, folic acid and vitamins are documented to impair immune responses. Severe anaemia is associated with hypoxia and ischaemia that results in increased morbidity and mortality. Hypoxia induced by anaemia is a kind of stress, which increases vascular reactivity to catecholamines that results in elevated leucocyte count⁽³⁾. Hb levels defined by WHO^(4,5): Grade 1 (Mild Anemia): 10.0 – 11.9 g/dl; Grade 2 (Moderate Anemia): 7.0 – 9.9 g/dl; Grade 3 (Severe Anemia): below 7.0 g/dl. Tissue oxygen delivery is dependent on haemoglobin concentration, oxygen saturation and the capacity for the cardiovascular & pulmonary system to compensate. Reduced haemoglobin is responsible for inadequate oxygenation of myocardium, increased stroke volume, decreased peripheral resistance & ventricular remodeling⁽⁶⁾. A higher WBC count, particularly polymorphonuclear leucocytes (PMNs) is considered to be an independent risk factor for IHD & Stroke in middle aged & younger populations⁽⁷⁾. The Global status on Noncommunicable Diseases Report (2011) has reported that there were more than 2.5 million deaths from CVD in India in 2008, two-third due to CHD & one-third due to Stroke. A multi-variate analysis identified elevated Neutrophil to lymphocyte ratio (NLR) as a useful inflammatory index in predicting the future risk of cardiovascular events⁽⁸⁾.

AIM AND OBJECTIVES:

The present study was undertaken to estimate the Hb (gm%), RBC count, WBC count, DLC(%) and Neutrophil lymphocyte ratio (NLR) in anaemic women (Grade 3 anaemia) and non-anaemic healthy females. This is a low cost, good reproducibility test routinely measured by clinicians and may be helpful to predict the future risk of ischaemic events in high risk population so that considerable changes in the lifestyle & behavior, dietary habits, regular exercise & other strategic interventions can be planned at an early stage in anaemic cases.

MATERIALS AND METHODS:

The study was carried out in forty female subjects with anaemia (Hb < 7.0 gm% - Grade 3 anaemia with minimum of 3 months duration) in the age group of 20-40 yrs recruited from TMCH. Forty age & sex matched healthy subjects (Hb > 12 gm%) were selected as control group. Subjects with known general or systemic disease, acute or chronic infection, H/o drug intake/medication that affect blood cell count (Aspirin, statin, steroid therapy, haematinics etc), pregnant females were excluded from the study. The study was performed in accordance with Ethical Standards of the Institute. Experimental protocol was explained to the subject & informed written consent was obtained. A detailed history & clinical examination was noted for inclusion & exclusion criteria. Subject's Hb (gm%), RBC count, TLC & DLC were estimated. Blood investigations were done in forty anaemic and forty non-anaemic healthy women (20 - 40 yrs) having regular menstrual periods. Blood samples were taken

during proliferative phase of the intermenstrual period for both cases and control group. Research works have demonstrated that menstrual and secretory (luteal) phase were associated with significant increase in leucocyte count compared to proliferative phase^(9,10,11). Blood samples were taken between 11.00 am – 12.00 noon to avoid diurnal variations. Estimation of Haemoglobin concentration (gm%) was done by Haemoglobinometry (Sahli's method)⁽¹²⁾. RBC & WBC counts were done by using Haemocytometer⁽¹³⁾ & the diluting fluids -Hayem's fluid (for RBC count) & Turk's fluid (for TLC). The DLC (%) was carried out by staining the blood film with Leishman's stain. Neutrophil to lymphocyte ratio (NLR) was also determined. Statistical analysis was done using Unpaired Student's t test. The results were tabulated. *P <0.05 was considered as statistically significant.

RESULTS:

The results are expressed as Mean \pm Standard deviation (SD). The mean age of anaemic females was 30.6 \pm 4.76 yrs and the mean age of controls was 28.5 \pm 3.24 yrs. Our study result shows that Hb (gm%) in anaemic subjects was 6.6 \pm 0.6 gm% , significantly lower (p<0.001) as compared to normal subjects (13.1 \pm 0.46 gm%). RBC count in anaemic females was 3.0 \pm 0.38 million cells/cu.mm as compared to controls (4.2 \pm 0.28 million cells/cu.mm), which was statistically significant (p<0.001). Total leucocyte count was significantly higher in severe anaemic cases as compared to controls (Shown in Table 1 and Figure 1 & 2).

Table 1 : Comparison of basic parameters in Anaemic cases & Control

PARAMETER	CASES (n=40)	CONTROL (n=40)	P value
Age (yrs)	30.6 \pm 4.7	28.5 \pm 3.2	NS
Hb (gm%)	6.6 \pm 0.6	13.1 \pm 0.46	0.000 [*]
RBC (million cells/mm ³)	3.0 \pm 0.38	4.21 \pm 0.28	0.001 [*]
TLC (cells/mm ³ of blood)	7234 \pm 1494.8	6535 \pm 713.6	0.001 [*]

^{*} P <0.05 was considered as statistically significant. NS : Not Significant

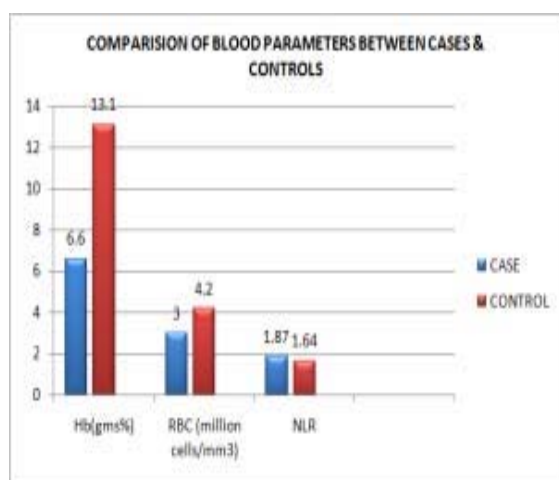
P <0.05 was considered as statistically significant. NS : Not Significant

On DLC, Neutrophil and Basophil were significantly higher (p<0.001), Eosinophil & Monocyte were significantly less in anaemic Vs controls with no alteration in the lymphocyte count. There was a significant increase in Neutrophil to Lymphocyte ratio (NLR) in cases of severe anaemia (p<0.05) as shown in Table 2 & Figure 3.

Table 2 : Comparison of Differential leucocyte count in Cases and Control

PARAMETER	CASES (n= 40)	CONTROL (n=40)	SIGNIFICANCE (P value)
DLC %			
Neutrophil	61.4 \pm 2.86 %	54.8 \pm 2.68%	0.001 [*]
Lymphocyte	33.4 \pm 2.61 %	33.2 \pm 2.6 %	0.701 (NS)
Eosinophil	1.63 \pm 0.54 %	4.28 \pm 0.75 %	0.000 [*]
Basophil	1.27 \pm 0.67 %	0.8 \pm 0.64 %	0.002 [*]
Monocyte	2.28 \pm 0.81 %	6.85 \pm 1.02%	0.001 [*]
NLR	1.87 \pm 0.26	1.64 \pm 0.19	0.000 [*]

*P <0.05 was considered as statistically significant. NS : Not Significant Data presented: Mean \pm SD; TLC: Total leucocyte count; DLC: Differential leucocyte count; NLR : Neutrophil-lymphocyte ratio



Thus, in conclusion our study result shows that leucocyte counts are altered in severe anaemia which plays an important role in predicting the future risk of CHD & ischaemic diseases.

Fig : 1 Comparison of Blood parameters in Anaemic cases & Control Fig :2 Comparison of Total leucocyte count in Anaemic cases & Control

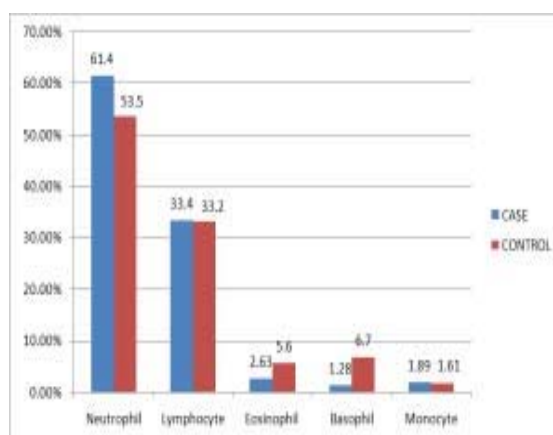
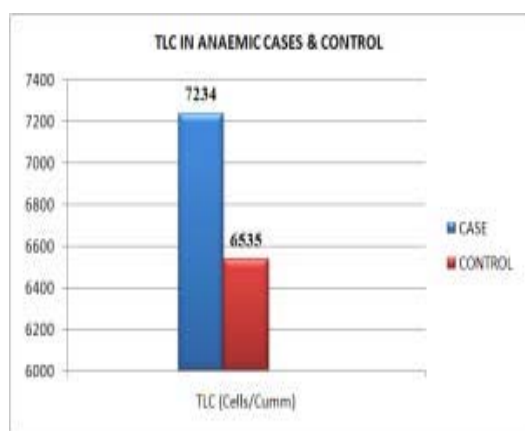


Figure 3 : Differential Count (DLC %) in Anaemic cases & Control

DISCUSSION:
Anaemia is said to be involved in the pathogenesis of Heart failure ⁽³⁾. In the study by Sanghvi et al., in India of 60 cases of severe anaemia, 20 were complicated by heart failure. In these cases, the factors of malnutrition and thiamine deficiency might be suggested as possible factors in the causation of heart failure -'Syndromes of Nutritional heart disease'. Decreased Hb level are known to be associated with an increased risk of coronary atherosclerosis due to an increase in blood flow and shear stress resulting in endothelial damage and vessel wall thickness ⁽⁶⁾. Elevated leucocyte count might be seen as a manifestation of 'Haematological stress syndrome' ^(3,14). Severe anaemia is associated with hypoxia & ischaemia which may alter the leucocyte count. Hypoxia induced by anaemia is a kind of stress, which increases vascular reactivity to catecholamines through glucocorticoids ⁽³⁾. There is a significant increase in leucocyte count in severe anaemia (p<0.05) in the present study. This elevated leucocyte count is associated with cardiovascular risk. Recent researches have shown that

leucocytes are involved in the pathogenesis of atherosclerosis due to proteolytic & oxidative vascular damage, abnormal leucocyte adhesion & aggregation, release of cytokines & chemokines ^(15,16). Activated leucocytes, most importantly neutrophils release substances like neutrophil elastase, cytotoxic material, hydrolytic enzymes that could lead to microvascular injury and ischaemic diseases ⁽¹⁷⁾. There are at least three mechanisms whereby leucocytes contribute to microvascular injury.

(i) Pressure-dependent plugging of microvessels by leucocytes (PMNs)

ii) The rheological abnormalities of leucocytes (stimulating enhanced granulocyte aggregation and adhesion to endothelial cells) (iii) Endothelial cell injury (due to release of noxious chemicals) ^(14,18).

Neutrophil activation results in the release of Elastase, a serine protease, one of the most potent of the lysosomal enzymes is known to be implicated in endothelial damage. Release of leukotrienes (LTB₄), a potent chemoattractant initiates neutrophil aggregation and degranulation ^(17,18)

It is likely that the most important source of free radicals (FRs) are the neutrophils (PMNs). FRs such as superoxide (O₂⁻) & (OH⁻) are also produced during catecholamine metabolism and prostaglandin production. FR induced DNA damage will affect the cell function by interfering with protein synthesis. FRs damage cell membrane lipid and affects the cell membrane function. PMNs interact with platelets to produce platelet activation which plays an important role in thrombosis ^(16,18) The rheologic properties of blood cells (WBC's) are major determinants of microvascular perfusion. It has long been recognized that flow in the microcirculation is usually intermittent. This is because, the diameter of RBCs and leucocytes are greater than the internal diameter of most nutritive

When perfusion pressure is reduced, the WBCs become entrapped in the micro-circulation, causing prolonged disturbances of blood flow. In addition, the WBCs are less deformable (spherical in shape) than the biconcave disc shaped RBC. WBCs are 2000 times stiffer than erythrocytes with an average cytoplasmic viscosity of 1000 fold that of RBCs ⁽¹⁸⁾. Recent research works have shown that Neutrophil-lymphocyte ratio (NLR) as a significant inflammatory index in IHD ⁽⁸⁾. Regarding increased neutrophil count, several studies have shown that iron deficiency induces changes in apoptotic response (Paino et al., 2009), lower oxidative burst and oxidant product synthesis (Berrak et al., 2007) resulting in increased neutrophil lifespan. Furthermore, anaemic patients have reduced neutrophil-phagocytic activity (Banerjee et al., 1991). Thus the increased granulocyte count in anaemic patients could compensate for the reduced phagocytic capacity ⁽¹⁹⁾. Several reports show that there is increased lymphocyte DNA damage (Aslan et al., 2006) and reduced anti-oxidant capacities in adult female anaemic patients. Also, there is decreased cell mediated immunity (CMI) due to decrease in CD4 count in cases of anaemia ⁽²⁰⁾. Studies not only correlate an elevated WBCs with ischaemic events, but also show that a decreased count is associated with decreased risk. An epidemiological study of the Yemenite Jews who frequently have a benign constitutional neutropenia and have been shown to experience vascular events only infrequently ⁽²¹⁾. Thus, a treatment which inhibits WBC activation in the early stages of ischaemia may have beneficial effects.

It has long been considered that hormonal factors like estrogen plays a protective role in women against IHD in the pre-menopausal age group. For women, risk increases gradually in the five to ten years after the female hormone estrogen begins to decrease, around age 50⁽²²⁾. Recent works have shown that the risk of ischaemic diseases is increasing in young females (35-55yrs) in the developing countries due to change in the life-style & behavior, inappropriate dietary habit, Stress at work places, lack of regular physical exercise etc⁽²³⁾. CHD was the largest cause of death in males (20.3%) as well as in females (16.9%) and led to about two million deaths⁽²¹⁾. The proportion of unrecognized MI is greater in women than in men, particularly in young women. This may be because chest pain is absent in upto 43% of cases⁽²⁴⁾. Therefore, it is essential to implement various intervention strategies to decrease the risk of CAD in developing countries.

CONCLUSION:

In conclusion, elevated leucocyte count in severe anaemic cases is considered as a risk factor for cardiovascular and ischaemic diseases. In the present study, there is a significant increase in WBC count ($p < 0.001$) & Neutrophil-lymphocyte ratio (NLR) in cases of severe anaemia. This in future could contribute to atherogenesis and organ ischemia. Further follow up study is needed in these cases to find out the risk of CHD in anaemic women and also to implement various preventive measures at an early stage, such as changes in dietary habits (intake of plenty of green leafy vegetables), Stress management (WHO, 2003), use of conventional anti-oxidants (Vit E & Vit C) that have anti-platelet and free radical scavenging effects can be administered in high risk population.

Further studies are needed to find out whether leucocytosis is associated with mild and moderate cases of anaemia so as to implement various preventive measures at an early stage to decrease the risk of ischaemic heart disease in developing countries

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