Total Leucocyte Count and Platelet Count as Predictors for the Development of Acute Myocardial Infarction

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Total leucocyte count (TLC), platelet count (PC) and platelet aggregation (PA) were performed in 70 patients of acute Myocardial Infarction (MI) and the values were compared with 30 healthy control subjects. The patients were selected from casuality departments of Services Hospital, Mayo Hospital and Punjab Institute of Cardiology Lahore. Blood Samples were drawn before the institution of any antiplatelet therapy. Mean value of TLC in patients was 10.63 ± 1.65 x 10^9/L and it was significantly higher (P < 0.001) than the control value (7.46 ± 1.7 x 10^9/L). In 44 (62.8%) patients, TLC values were found to be higher than the normal value. Similarly the mean PC value was significantly higher in patients (309.4 ± 67.6 x 10^9/L) than the controls (236.5 ± 55.4 x 10^9/L) (P < 0.001). The aggregation response of platelets to ADP and collagen (primary and secondary agonists) was observed. In platelet aggregation studies, percentage aggregation, slope of aggregation, spontaneous aggregation (SPA) and threshold concentrations were measured in all the subjects. 66 (95%) of control subjects had enhanced values. Similar were the observations with SPA in both groups. The mean threshold concentration value with ADP was 1.1 umol in patients as compared with the control value of 4.9 umol. PC was in the upper normal range (350 - 450 x 10^9/L) in 41 (58.3%) patients and 3 (10%) controls. All the above values thus differed significantly between patients and controls (P > 0.001). We suggest that raised TLC and PC may be considered as independent risk factors for the development of acute MI.

Key words: Total leucocytes count in myocardial infarction, predictor for MI, platlet count in MI.

Various epidemiological studies[1,2] have found raised blood pressure (BP), elevated total cholesterol and smoking as major factors associated with an increased risk of Ischemic Heart Disease (IHD). Nevertheless on an individual basis, the prediction of the risk of IHD from levels of blood pressure, lipids and smoking is poor[3,4].

There is evidence[5] that occlusive thrombi are found in almost all the cases of acute MI and in majority with sudden cardiac deaths. Thus there may be a role for haemostatic and rheological factors in the etiology of the disease. In recent years there have been few reports[6,7] relating haemostatic factors to incidence of IHD showing white cell count to be associated with an increased risk of disease[8-10].

Paul M., (1991)[11] observed raised mean leucocyte count as a risk factor for the subsequent development of IHD. The raised leucocyte count was correlated positively with the smoking habits. Mechanisms that might explain the association of raised TLC with IHD include clumping of granulocytes and subsequent leucoembolism and the role of macrophages and monocytes in the development of fatty streaks[12].

It is also suggested that stimulated neutrophils and monocytes produce thromboxane A_2 ($T \times A_2$) and lipoxygenase pathway metabolites which enhance platelet aggregation[13,14].

Many agents cause platelet aggregation by direct effect on platelets (primary aggregating agents) or by releasing ADP and serotonin from platelet granules and the formation of prostaglandins and endoperoxides (PGG_2 and PGH_2) and $T \times A_2$ (secondary aggregation agents)[15].

Enhanced platelet aggregation has been involved in a number of disease states like MI, angina, strokes, peripheral vascular disease and venous thromboembolism[16].

Endothelial injury at sites of vascular stenosis, platelet attachment and the release of humoral mediators appear to be the major causes of reduced blood flow leading to the acute MI[17]. Thus in subjects with raised TLC there is release of platelet aggregating factors like $T \times A_2$ accompanied by leucoembolism and atherosclerosis, may play a major role in causing microvascular occlusion leading to ischemic necrosis of vital organs like heart.

Thus raised TLC along with increased platelet count and enhanced platelet aggregation are important risk factors for acute MI.

Subjects and methods

Subjects

The present study was conduced on seventy (70) patients of acute MI selected from casuality departments of Mayo Hospital, Services Hospital and Punjab Institute of Cardiology. They had the first episode of coronary ailment and had not taken any drug known to affect platelet aggregation during previous one week. The results were

compared with 30 healthy control subjects who were also age and sex matched with the patient group. Blood samples were taken in fasting state and special emphasis was placed on history of smoking habits. Subjects with history of diabetes mellitus and hypertension were excluded from the study.

Methods

Ten milliliter (10 ml) of venous blood was drawn aseptically from all the subjects in a fasting state using a 19 gauge butterfly needle avoiding stasis and frothing 8 ml was preserved for platelet aggregation studies and was mixed with 0.9 ml of 3.8% trisodium citrate thus making a dilution of blood with the anticoagulant 9 : 1. The remaining 2 ml of the sample was delivered to a test tube containing dried EDTA for routine blood examination and platelet count which were performed on Sysmex K - 1000. Two blood films were also made for differential leukocyte count (DLC).

Platelet aggregation studies were performed on platelet rich plasma (PRP) obtained by centrifuging the sample at 200 G for 15 minutes. Platelet count was adjusted between 200 - 400 x 10^9/L by diluting with platelet poor plasma (PPP) before performing platelet aggregation. PPP was also used as a reference in this study. PA was performed within 6 hours of sample collection on Chronolog aggregometer Model 540 VS in Pathology Department PGMI Lahore. The following agonists were used:

1. ADP (10 umol, 5 umol, 2.5 umol, 1 umol).
2. Collagen (4 ug, 2 ug, ug).

The following parameters were included in PA studies.

1. Percentage aggregation (%).
2. Slope of aggregation (% / min).
3. Spontaneous aggregation (%) aggregation of activated platelets without the addition of agonist.
4. Threshold Concentration (umol). (Minimum amount of agonist required to produce irreversible aggregation).

The statistical analysis was done by applying student "t" test.

Results

Routine Hematological Parameters (Table 1)

There was no significant difference between the mean haemoglobin values of patients (14.4 ± 1.57 gm/dl) and controls (13.57 ± 1.62 gm/dl) (P > 0.05).

Table 1:Comparison of mean Hb, TLC, ESR and platelet count values between patients and control.

Group	Hb (10^9.L)	TLC ((10^9/L)	ESR (mm/hr)	Platelet count (10^9/L)
Control	13.57 ±1.62	7.46 ±1.17	9.87 ±3.72	2.36 ±55.45
Patients	14.4 ±63 ±1.57	31.33 ±1.65	309.4 ±8.63	67.61
P Value	*>0.05	**<0.0	**<0.0	**<0.0

*Non Significant **Highly Significant

However the mean values of ESR were significantly higher in patients (31.33 ± 8.63 / mm/hr) than the controls (9.87 ± 3.72 mm/hr) (P < 0.001).

The mean value of TLC was 7.46 ± 1.17 x 10^9/L (6 - 10 x 10^9/L) in patients. Difference was highly significant between the two groups (P < 0.001). In 44 (62.8%) patients TLC values were found to be higher than normal (> 10.9 x 10^9/L), while in 19 (37.2%) patients the values were within normal range. None of the control subjects had higher values than the normal range.

The mean platelet count in controls was 236.5 ± 55.45 x 10^9/L.

Platelet Aggregation Studies

In 67 out of 70 acute MI patients enhanced platelet aggregation was clearly demonstrable and was pronounced in all the parameters.

Spontaneous Platelet Aggregation (SPA)

It was seen in 3 (10%) control subjects and 66 (95%) patients. Mean value thus differed significantly between control and the patient group (p < 0.001) Table 2.

Table 2 Comparison of mean values of threshold concentration and spontaneous aggregation between patients and controls.

Group	Threshold Concentration	Spontaneous Aggregation (%)
Control	μ (mol) 4.98 ± 3.29	0.620 ± 2.23
Patients	1.11 ± 0.67	16.73 ± 9.15
P Value	**<0.001	**<0.001

** *HighlySignificant*

Threshold Concentration

With ADP it was significantly reduced in patients than the control subjects (p < 0.001) Table 2.

Percentage Aggregation

The percentage aggregation was significantly higher in patients than the controls with all the concentrations of ADP and collagen used (p < 0.001) Table 3, 4.

Table 3: Comparison of mean aggregation (%) and slope of aggregation (% / min) of adp between patients and controls.

Group	Concentration (%)				% / min			
Control	10 μ mol	5 μ mol	2.5 μ mol	1 μ mol	10 μ mol	5 μ mol	2.5 μ mol	1 μ mol
	47.67 ± 13.41	33.23 ± 14.49	23.64 ± 14.85	11.48 ± 10.55	77.89 ± 17.67	69.91 ± 19.83	61.61 ± 26.32	42.79 ± 32.74
Patient	66.91 ± 1.82	59.88 ± 4.35	53.53 ± 15.71	43.31 ± 15.69	94.25 ± 12.19	94.62 ± 10.34	87.71 ± 19.59	78.92 ± 23.17
P-Value	*<0.001	*<0.001	*<0.001	*<0.001	*<0.001	*<0.001	*<0.001	*<0.001

**Highly Significant*

Table 4 Comparison of mean percentage aggregation (%) and slope of aggregation (% / min) of collagen between patients and controls.

Group	%			% / min		
Control	4 μ gm	12 μ gm	1 μ gm	4 μ gm	2 μ gm	1 μ gm
	52.53 ±	43.32 ±	31.35 ±	67.32 ±	61.29 ±	49.69 ±
	14.27	18.09	18.54	24.66	23.74	29.45
Patient	74.24 ±	67.73 ±	59.06 ±	98.16 ±	94.94 ±	91.08 ±
	10.11	10.86	13.38	5.30	10.85	18.61
P-Value	*<0.001	*<0.001	*<0.001	*<0.001	*<0.001	*<0.001

Highly Significant

Slope of Aggregation

Mean values of SA were also significantly higher in patients than control subjects with ADP as well as collagen ($p < .001$) Table 3, 4.

Discussion

There are a number of studies[1,9,10] which show an association of white cell count with the incidence of coronary heat disease (CHD) but it is not certain whether the association arises solely as both are dependent on smoking habit. Laloker et al[9], concluded that WBC count is an excellent indicator of exposure to cigarette smoking. However, Frideman I[8] and Grimm et al[10], observed that part of the association is independent of smoking habit. The present study supports the latter conclusion as out of 70 patients of acute MI, 24 were non - smokers and 23 of them had raised TLC values (>10 x 10^9/L).

Ridker et al[18], (1991) studied the relationship of leucocyte count with CHD. They measured TLC as base line parameter among 464 patients who subsequently had first episode of acute MI, along with two control groups. The first control group consisted of patients matched for age, sex and race. The second was made up of patients also matched for a number of other coronary risk factors[8]. Over all the mean leucocyte count in the case group was significantly higher than either of the control groups ($P < 0.001$). The absolute difference in TLC was relatively smaller 0.5 x 10^3/mm^3. In a follow - up study of this population the total white cell count was also found to be a predictor of sudden cardiac death, particularly for the subgroups of men older than 40 years[8].

In another prospective study[9] a significant relationship of white cell count with acute MI was noted. Similarly multiple risk factor intervention trials[10] strongly supported the total WBC count a strong independent predictor of CHD.

Similar were the observation by Famell[12] et al., (1991) who observed the association of multiple hemostatic risk factors with IHD. They found that fibrinogen level and WBC count remained predictive even after controlling for smoking 'status variables. They concluded that the white cell count alone was an independent risk factor for ischemic events as are possibly both the fibrinogen and the viscosity levels.

Mechanisms that might explain this association include clumping of granulocytes and monocytes and subsequent leucoembolism and the role of macrophages and monocytes in the development of fatty streaks[12].

The important hemostatic risk factor is raised platelet count associated with platelet hyperaggregability as they also correlate with other coronary risk factors.

Initial thrombus formation seems to be an important factor in the development of atherosclerotic coronary artery and cerebrovascular disease[19]. This has been attributed to platelet interaction with damaged vessel wall in the process of platelet aggregation and adhesion. Enhancement of platelet aggregation may accelerate this process and initiate vascular thrombosis.

In the present study mean platelet count was found to be significantly higher in patients than controls ($P < 0.001$). Increased platelet count was found to be associated with SPA.

All these subjects had platelet count in the upper normal range (350 - 400 x 10^9/L). Increased platelet aggregation may thus be considered as a risk factor for development of thromboembolic phenomenon.

Mean values of percentage aggregation and SA with all the concentrations of ADP and collagen used were significantly higher in patients than controls ($P < 0.001$)[20,21].

Abbate et al.[22], also had similar observations. Hyperaggregability of platelets in patients with acute MI was indicated by a significantly reduced threshold concentration in patients than the control subjects ($P < 0.001$). Similar conclusions were by Zahavi (1977)[23] who observed an increased response by ADP of PRP in acute MI patients. Thus at an ADP concentration of 1 u mol, platelet aggregation curve of most patients was either biphasic or monophasic and the secondary wave was clearly apparent indicating irreversible platelet aggregation even at this lower concentration.

It is therefore suggested that hemostatic risk factors are closely linked with acute ischemic events. It seems appropriate for future epidemiological studies to continue assessing new hemostatic variables as CHD predictors.

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