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SERUM IRON, FERRITIN AND TRANSFERRED IN PATIENTS WITH ISCHEMIC HEART DISEASE

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Abstract

To shed *more light on the relationship between ischemic heart disease and iron, serum iron parameters (iron, ferritin and transferrin) were estimated in fifty ischemic heart patients (15 stable angina, 15 crescendo an gina and 20 acute Myocardiai Infarction, MI). Their ages ranged 43 – 58 years. Ten healthy subjects, matched with patients for age and sex. served as control group. Echocardiographic studies (ejection fraction (EF) and end systolic volume (ESV) together with peak serum creatine phosphokinase (CPK) were done for each case of infarction to correlate these variables with the corresponding serum iron parameters.*

Compared to controls, ischemic heart patients had highly signi/icon: *increase of serum iron (103.5 ± 25.6 vs 79.6 ±* 19.2, *p<0.0l) and serum ferritin (94.7 ± 54.2 vs 44.4 ±* 11.0, *p<0.01) whereas they showed insignificant increase of serum transferrin (273.8 ± 83.8 vs 244.1 ± 56.4, p>0.05). Subgroup analysis showed that there was statistically signif icant increase in serum iron and ferritin but not transferrin in patients with stable and unstable angina while in cases with acute MI, there was significant increase in* oil *serum iron parameters. Correlation studies showed that serum iron and ferritin but not transferrin were strongly correlated with peak CPK, EF and ESV. Patients with acute MI who had complicated in-hospital course showed significant higher value of serum iron, ferritin, transferrin, CPK and ESV but they had a significant lower EF than non-complicated cases.*

In conclusion, serum iron andferritin were significantly higher in ischemia *heart patients while serum transferrin was significantly higher only in patients with acute ML In the infarction group, the higher the ser - um iron and ferritin, the larger is the size of infarction, the worser is the in-hospital course and the more impaired is the systolic function. Thus, the present study might give a new meaning for the emerging role of iron in ischemic heart disease.*

Introduction

There are many risk factors for coronary artery disease. The most important are hypercholesterolemia, hypertension and cigarette smoking, (Shaper et al., 1985).

The association between coronary risk factors and clinical manifest disease have been established and the pathway of action has been hypothesized, as being through, building of coronary atherosclerotic raised lesions and stenosis finally resulting in occlusion, ischemia and myocardial infarction (Holmal et al., 1985).

In 1989 Sullivan suggested an iron paradigm of ischemic heart disease (IHD) as a complementary to that previously demonstrated for cholesterol. He constructed his hypothesis on many clinical and experimental evidences that high stored iron or iron depletion can affect the natural history of IHD regardless the serum cholesterol level. His hypothesis marked the beginning of a new era of investigations into the role of iron in IHD. To shed more light on the relationship between iron and IHD the present work aimed to assess serum iron parameters in patients with different clinical syndromes of IHD.

Patients and Methods

Fifty patients with IHD (stable angina, unstable angina and acute myocardial infarction, AMI, in addition to ten healthy subjects matched for age and sex as control group) were included in this study. The diagnosis of stable angina was based on the presence of recurrent typical ischemic chest pain; effort induced, stable in frequency, duration and severity for at least 60 days plus reproduction of typical chest pain with >2 mm

S-T segment depression in >2 contiguous leads during treadmil exercise ECG testing (Chaitman, 1986). Unstable angina was diagnosed when the anginal attacks showing changing in one or more of its characters: more prolonged, more severe, more frequent or occurring at rest plus finding of normal cardiac enzyme levels in the serum (Crescendo angina) (Abbate and Nicholas, 1996). Acute MI was diagnosed when the following criteria were present: typical prolonged chest pain, typical evolutionary changes in ECG characteristic of infarction and transient significant elevation of the serum cardiac enzymes (Higham et al., 1995). Patients with renal or hepatic failure were excluded from the study.

Methods

* Thorough history taking.
* Thorough clinical examina- - tion.
* 12-lead surface resting ECG.
* Symptom- limited treadmil exercise test (only for pa tients with stable angina).
* Sampling.

Venous blood sample was drawn while the patient is fasting and divided into 2"parts. The first part was taken on the EDTA for complete blood picture. The other part is left to be clotted, then centrifuged. The sera were separated for determination of the following routine investigations, serum glucose (Trinder, 1969), creatinine(Heniy, 1974), GOT, GPT (Reitman & Frankel, 1957), total cholesterol(Stein, 1986), triglycerides (Wahlefeld, 1974), creatine phospho-kinase(Nealon & Hlenderson. 1977).

Serum iron was determined according to the principle of Thompsen, (1984). In an acid medium and in the presence of *guani*-dine, ferric iron are released from their protein bonds, mainly from the iron-transferrin complex using hydroxylamine, the iron is reduced from ferric to the ferrous state which forms a colored complex with ferrozine acetate buffer.

Serum ferritin was determined by ELISA based on monoclonal antibody sandwich to ensure an optimal sensitivity and specificity (Ellis, 1979).

Determination of serum transferrin was done by immunoprecipitating analysis (Deverill, 1978).

*Echo-Doppler study (only for patients with acute MI before discharge from hospital): The study was performed using Hwelt Pak-ard sons 1000 + electronic phased transducer of frequency 3.5 MHz. The following echo-Doppler parameters were estimated accord - ing to Nishimura et al., (1984): -

* Endsystolic and enddiastolic volumes (ESV & EDS) using area length method.

* Ejection fraction (stroke volume divided by enddiastolic volume).

Patients with acute MI were classified into complicated and non-complicated based on the occurrence of one or more of the following during hospital stay: Death, pulmonary edema, shock, major arrythmias and/or infarct - tion extension.

The results were analyzed by the suitable statistical methods student t-test and correlation coefficient (r).

Results

Patients demographics and characteristics :

Among the studied fifty cases, there were fifteen patients with stable angina, fifteen with crescendo angina and twenty with acute ML Their ages ranged 43 - 58 year with a mean of 49.5 ± 4.4 years. Thirty-two patients were males (64%) and eighteen were females (36%), five pa tients (10%) were hypertensive, eighteen (36%) were cigarette smokers, seven (14%) were diabetics and thirteen (26%) had family history of IHD. Among the studied cases with acute MI, there were fifteen patients with anterior and five had inferior infarction.

Laboratory and echocardiographic data :

Ischemic heart group as well as subgroups (stable angina, crescendo angina and infarction) had highly significant higher serum levels of iron and ferritin than controls ($p<0.01$) (Table 1) and (Fig. 1&2). Serum transferrin was only significantly higher in infarction subgroup ($p<0.05$) (Table 1) and (Fig. 3).

Correlation studies showed that serum iron and ferritin but not serum transferrin were strongly correlated with peak serum creatine phosphokinase (r = + 0.33 and +0.44), ejection fraction (r = -0.61 and -0.69) and end-systolic volume (r = -1-0.58 and +0.56).

The infarction group was divided into complicated and non-complicated group. The first group included eleven patients who suffered complicated in-hospital course (two died of cardiogenic shock, one died of ventricular fibrillation, two had heart failure, three had infarction extension, three had complex.ventricular arrhythmias). Complicated group found to have statistically significant higher value of peak serum CPK ($p<0.05$), serum iron ($p<0.05$), serum ferritin ($p<0.05$), serum transferrin ($p<0.05$), end-systolic volume ($p<0.05$) than non-complicated group. Ejection fraction was significantly lower in complicated than in non-complicated cases ($p <0.01$) (Table 3) and (Fig. 4).

Table 1 : Mean ± *SO* and P values of serum iron parameters in ischemic groups compared with the control group.

Parameters / Studied group	S. iron (ug/dl)	S. Ferritin (ng/ml)	S. transferrin (mg/dl)
Controls n = 10	79.7 ±19.7	44.4 ±11.0	244.1 ±56.4
Stable angina n = 15	102.3 ±22.9 P<0.01	82.1 ±37.1 P<0.01	247.8 ±92.8 NS
Crescendo angina n = 15	11 0.8 ±30.0 P<0.01	129.5 ±75.1 P<0.01	262.4 ±85.8 NS
Acute MI n = 20	98.9 ± 23.8 P<0.01	78.9 ±32.1 P<0.01	301 .7 ±69.7 P<0.05
Total cases n = 50	103.5 ±25.6 P<0.01	94.7 ± 54.2 P<0.01	273.8 ±83.8 NS

NS = Non significant versus controls.

Table 2 : Correlation coefficient V and probability value "p" between serum iron parameters and other variables studied in acute myocardia! infarction (AMI) group.

Parameters	Serun iron		Serun Ferritin		Serun transferrin	
Variables	r	P	r	P	r	P
S. cholesterol	+ 0.17	NS	+ 0.24	NS	+ 0.13	NS
S. triglycerides	+ 0.23	NS	+ 0.20	NS	+ 0.05	NS
Serum CPK	+ 0.33	<0.05	+ 0.44	<0.05	-0.03	NS
Ejection Fraction	-0.61	<0.01	-0.69	<0.01	-0.09	NS
Endsystolic volume	+ 0.58	<0.05	+ 0.56	<0.05	+ 0.17	NS

NS = Non significant (p>0.05).

Table 3 : Comparison between complicated and non-complicated cases with acute myocardial infarction as regard laboratory and echocardiographic data.

Studied cases / Parameter	Non complicated n = 9	Complicated * n = 11	P
Peak CPK (U/1)	858. 9 ±49.0	1457.1 ±85.0	<0.05
Serum iron (}ig/dl)	87.2 ±15.4	113.3 ±25.0	<0.05
Serum ferritin (ng/ml)	61.3±18.6	98.4 ±34.3	<0.05
Serum transferrin (mg/dl)	273.8 + 63.0	330.4 ±71. 7	<0.05
Ejection Fraction	51. 2 ±4.4	38.2 ±8.1	<0.05
Endsystolic volume (ml)	53.6+16.8	92.7 ±47.1	<0.05

* Cases who had infarction extension, early post-myocara'ial angina, cardiogenic shock, heart failure or death.

Fig. (l): Comparison of serum iron between control and ischemic groups

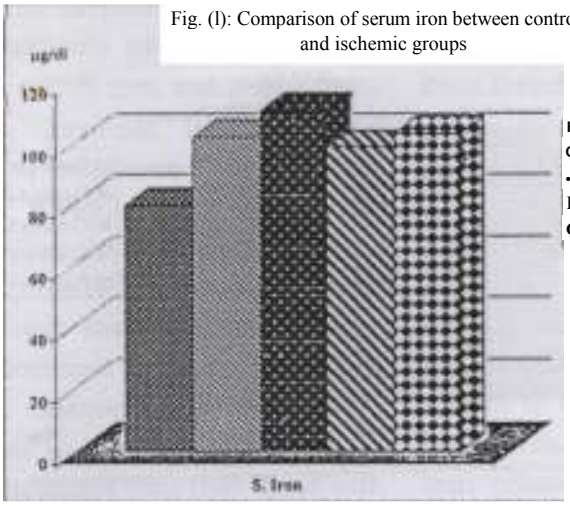


Fig. (2): Comparison of serum Ferritin between conirol and ischemic groups

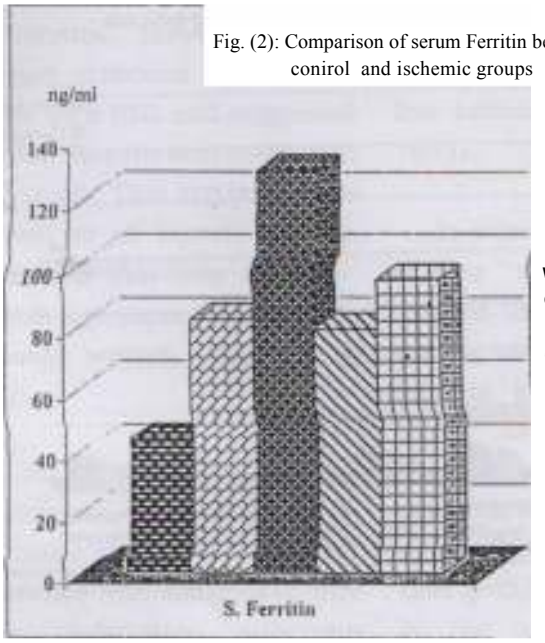


Fig. (3): Comparison of serum Transferrin between control and ischemic groups

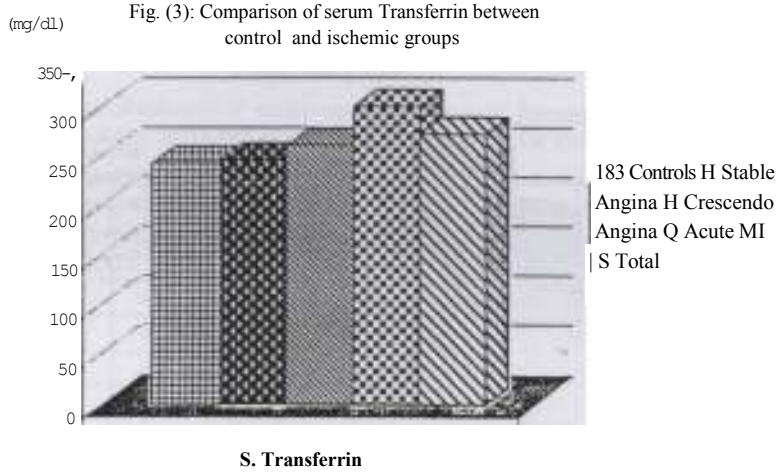
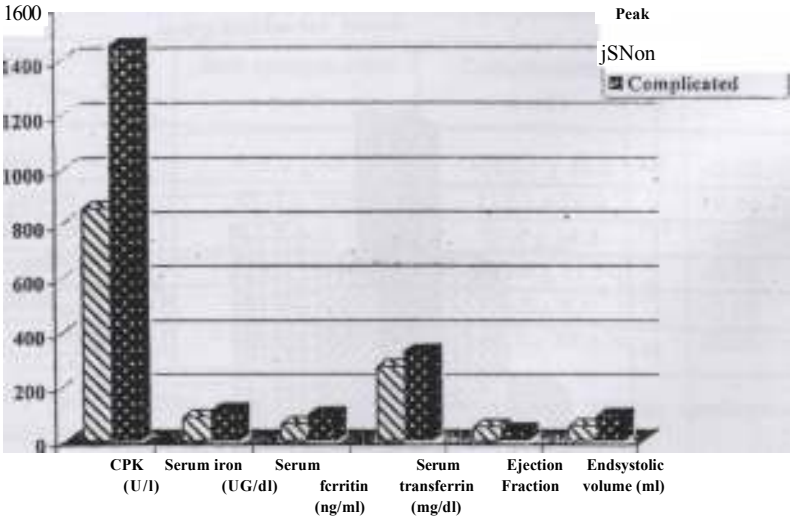


Fig. (-4): Comparison between complicated and non-compiicated groups as regard laboratory and cchocardiographic data



Discussion

In the present study, there was a statistically significant increase in the serum iron and serum ferritin in ischemic heart patients ($p<0.01$) compared to the control group (Table 1) and (Fig 1&2). These results are in agreement with those of Sullivan (1981) who suggested that high stored iron may increase the risk of IHD. In 1992, Salonen and Co-Workers showed in a prospective study of eastern finish men that those with high serum ferritin were more than twice as likely to have acute MI than men with a low or normal serum ferritin. However, Helen (1993) had criticized the role of iron store with IHD and suggested that serum ferritin decreases with increasing age. This argument was in contrast to all known studies, which showed that iron store increase with age especially in postmenopausal women (Salonen et al., 1992).

Also, in the present study serum transferrin was higher in ischemic cases than in controls but the difference was significant only in the infarction subgroup ($p<0.05$) (Table 1) and (Fig 3). Magnus et al. (1993) detected strong correlation between transferrin and risk of acute ML In 1989, Sullivan found a circadian rhythm for serum transferrin similar to that of acute ML Transferrin has been known to be one of the acute phase reactants that released in response to inflammation or tissue necrosis (Salonen et al., 1992). Also, it has also been known to be a strong antioxidant due to its capacity to bind free iron released during ischemic or reperfusion myocardial injury (Halliwell and Guttenidge, 1990). So, the increased serum transferrin in acute MI may be a protective mechanism against oxygen free radical injury (Jonsson et al., 1991).

In infarction group; correlation studies of the present work showed that serum iron and serum ferritin had a -statistically significant positive correlation with peak creatine phosphokinase ($p<0.05$) and endsystolic volume ($p<0.05$) but it had a strong negative correlation with ejection fraction ($p<0.01$) (Table 2). Additionally, the present study showed a statistically significant rise of ser-

um iron parameters, peak creatine phosphokinase and endsystolic volume but it showed a significant decrease of ejection fraction in complicated compared to non complicated cases of acute MI ($p<0.01$) (Table 3) and (Fig 4). These results mean that the higher the serum iron or serum ferritin the worser is the systolic function, the larger is the size of the infarction and the worser is the in-hospital prognosis. Similar conclusion was reported by Lauffer (1990) who demonstrated a strong positive correlation between the median value of hepatic stored iron and mortality from IHD in different countries. Experimental studies showed that iron depletion (by deferoxamine) during ischemic myocardial injury (induced by coronary artery ligation) resulted in decrease of creatine phosphokinase release (Myers et al.$_f$ 1985), decrease in the incidence of arrhythmias (Bernier et al., 1986) and rapid recovery of contractile function (Farber et al., 1988).

The exact role of iron in the pathogenesis of IHD remain undecided, however substantial evidences have been put forth during the last few years that free iron catalyzes oxygen free radicals production which generate a wide range of potent oxidants that might be involved in the pathogenesis of atherosclerosis and in production of myocardial ischemic injuries (Steinberg, 1991 and Salonen et al. 1992). Also. Antonius et al., (1988) suggested that iron plays an important role in the occurrence of tissue damage and ventricular fibrillation during anoxia and reperfusion probably through the formation of hydroxyl radicals and / or perferryl oxide.

In (1993), Franscesco and Associates had shown that high levels of iron store could increase the risk of acute coronary syndromes by favoring platelets activation. Mackler et al. (1984) suggested that iron activates iron-dependent enzymes (such as xanthine oxidase, cyclooxygenase and lipooxygenase) with the subsequent platelets leucocytic activation as well as the production of oxygen free radicals, vasoconstrictive agents and many cytokines that implicated in the development of coronary artery disease and myocardial infarction.

In conclusion, serum iron and serum ferritin were significantly higher in the ischemic heart patients compared to the controls. Serum transferrin is significantly higher only in patients with acute MI. High serum iron parameters were associated with impaired systolic function, large infarct size and worse in-hospital prognosis the infarction group. These data provide an important indirect support for new paradigm, based on a central role for iron in the development of IHD. Also, this study recommends regular blood donation as a prophylactic measurement to reduce serum iron parameters and hence the risk of IHD. It explains why postmenopausal women are more highly susceptible to IHD than menstruating women.

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