Soluble transferrin receptor, Ferritin index in Pakistani population

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Abstract: Inflammation affects the reliability of ferritin. The serum level of transferrin receptor protein (sTfR) represents true demand of iron in the body. This study attempts to identify levels of sTfR and correlate the trends of sTfR/ferritin index with BMI in the population of Karachi. 132 gender matched volunteers between the ages of 20-60 years were recruited for this cross-sectional study. BMI was calculated using the formula: (weight in kg / height in m^2). Following groups were made according to South Asian criteria of BMI; Group A: normal weight (18.0-22.9 kg/m^2), Group B: overweight (23.0-24.9 kg/m^2), Group C: obese (>25.0 kg/m^2). Serum ferritin, sTfR and CRP levels were determined using ELISA kits. Statistical comparisons were performed using Mann Whitney U and Spearman's rank correlation, where $p<0.05$ was considered significant. The results identified increased in TIBC, sTfR, ferritin and CRP in obese as compared to normal weight individuals ($p<0.001$). sTfR/ferritin ratio was 0.822 which signifies increased risk of acute myocardial infarction in group C. Serum iron ($r=-0.359, p=0.004$) showed negative correlation with BMI while serum ferritin ($r=0.237, p< 0.001$) and sTfR ($r=0.263, p= 0.036$) levels were positively associated to BMI. This study highlights a novel finding that sTfR is most likely a better clinical measure of iron status in inflammatory conditions as its expression is effected by erythropoiesis and not by inflammation. Risk of Acute myocardial infarction can also be predicted by increased sTfR/ferritin ratio.

Keywords: Obesity, ferritin, sTfR, inflammation, sTfR/ ferritin ratio.

INTRODUCTION

Presence of iron in hemoglobin makes its role extremely essential to health. Low iron levels results in iron deficiency anemia (IDA) and a decreased functional capacity. In cardio-myopathic patients, IDA has been commonly found to increase morbidity and mortality (Szachniewicz *et al.*, 2003). Iron deficiency can be detected by detecting low levels of ferritin or increased sTfR levels. Conventionally clinicians rely on ferritin levels for the assessment of iron stores present in the body.

Ferritin is a protein responsible for iron storage and a decreased level is associated with iron deficiency. However ferritin is an acute phase reactant that increases in inflammatory conditions thus becoming elevated despite normal or low iron content (Baynes *et al.*, 1986). Therefore, in inflammatory chronic conditions, ferritin is an unreliable marker (Fitzsimons and Brock, 2001). Similarly a new approach is required in diagnosing iron deficiency in the obese population. It has been shown that adipose tissue is responsible for secreting substances called adipokines that cause subclinical inflammation in obese individuals (Greenberg and Obin, 2006). In addition it is the obese population that is more predisposed to developing iron deficiency (Yanoff *et al.*, 2007). In such cases of inflammation, sTfR (soluble Transferrin Receptors) level has been hypothesized to be a more reliable marker for iron status (Baillie *et al.*, 2003). sTfR are cleavage products of the trans-membrane transferrin receptors expressed on the cell membrane of iron requiring cells. The levels of sTfR in the serum correlate with the level of receptors expressed on the cell surfaces of iron requiring tissue, and therefore serve as a marker for tissue iron requirement.

The debate still persists whether sTfR is a more reliable marker as compared to ferritin in diagnosing iron deficiency (Braga *et al.*, 2014). Researches have tried to observe trends of different iron parameters in their local populations and analyzed the results to produce better cut off ranges in order to increase specificity and sensitivity of the above tests. Discrepancies in normal values of iron parameters in different ethnic and geographical populations have been recorded (Braga *et al.*, 2014). In this study we attempt to identify and correlate the trends of different parameters of iron profile with the BMI variations in the population of Karachi.

MATERIAL AND METHODS

Total of 132 apparently healthy gender matched individuals between the ages of 20-60 year were recruited for this cross-sectional study after being questioned about their medical, surgical and personal history to match their compatibility with the inclusion of this study. Exclusion criteria included all conditions which led to change in the body iron stores or cause inflammation, such as pregnancy, smoking, alcoholism, hemoglobinopathies, diabetes mellitus, bleeding disorders, any acute illness during last 1 month, as well as iron deficiency as seen on complete blood picture (CP).

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Table 1: Biophysical & Biochemical Parameters of the Study Subjects

Variable	Group A Normal Mean ± SD (n=44)	Group B Overweight Mean ± SD (n=44)	Group C Obese Mean ± SD (n=44)	p value
Age (year)	36.59 ± 7.27	35.1±3.62	34.43 ± 8.16	>0.05
BMI (kg/m^2)	21.41 ± 1.93	24.3±0.55	29.99 ± 4.16	<0.001
Waist to Hip ratio	0.89 ± 0.11	0.85±0.11	0.88 ± 0.08	>0.05
Haemoglobin (g/dl)	13.11 ± 1.84	12.8±1.56	10 ± 1.53*	0.02
Ferritin (ng/dl)	11.25 ± 7.60	19.3±12.8*	22.8 ± 22.46*	<0.001
Iron (mg/dl)	2.18 ± 1.42	2.25±3.00	1.08 ± 1.28*	<0.001
TIBC (µg/dl)	1.94 ± 1.08	1.62±0.30*	3.31 ± 0.78*	<0.001
Transferrin Saturation (%)	50 ± 8.90	45.6±7.20	20.23 ± 2.10*	<0.001
sTfR (mg/l)	6.88 ± 0.68	8.07±4.58	18.76 ± 0.75$^{\#}$	>0.001
CRP (mg/dl)	3.48 ± 5.33	4.25±0.59	8.96 ± 5.33*	<0.001
sTfR/Ferritin ratio	0.61	0.42	0.822$^{\#}$	<0.001

Where: BMI (Body mass index), TIBC (total iron binding capacity), sTfR (serum transferrin receptor protein) & CRP (C-reactive protein). P value <0.05 considered statistically significant
*statistically significant when compared to group A
$^{\#}$statistically significant when compared to group A & B

The research protocol was permitted by the research ethics committee, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, (No. F.1-2/2013/BMSI-E.COMT/007/JPMC). All the participants were asked to complete a verbal and written informed consent.

BMI was calculated using the formula: (weight in kg / height in m^2) (Garrow and Webster, 1984) after measuring weight and height of all the subjects in kilograms and meters respectively, using Stadiometer (ZT -120 Health Scale, made in China). The Hip and waist circumference was measured by standard techniques (World Health, 2011).The subjects were divided into; Group A: normal weight (18.0-22.9 kg/m2), Group B: overweight (23.0-24.9 kg/m2), Group C: obese (>25.0 kg/m2) according to South Asian criteria of BMI (Sharma, 2013, Singh *et al.*, 2008).

Serum ferritin, sTfR and CRP levels in serum samples were determined using a commercially available sandwich ELISA kit (cat # YHB2785Hu; BioCheck (Foster city, CA, USA, cat #. BC- 1025) and Dia Source, (Immunoassay SA. Belgium, ref # KAPDB4360, respectively.

Statistical analysis of the data was achieved using the SPSS for Windows 19 software package (SPSS Inc., Chicago, IL). A descriptive statistical analysis of continuous variables was performed. Data on continuous variables i.e. biophysical and biochemical parameters were presented as Mean + Standard Deviation (SD). Statistical comparisons were performed by using Mann Whitney U test for non-parametric variables. Associations between circulating sTfR levels and BMI were determined using Spearman's rank correlation. Associations were considered significant if $P < 0.05$.

RESULTS

A total of 132 subjects were divided into Group A, B, C according to their BMI with reference to the South Asian Criteria (Sharma, 2013, Singh *et al*., 2008).

Biophysical parameters

Mean age of the study subjects was 24.7 ± 6.34 years and mean BMI was 17.9 ± 2.21 kg/m^2. A trend of raised BMI was seen from group A–C (p=<0.001), whereas no significant change was noted in WHR in the study groups (p=>0.5) (table 1).

Biochemical parameters

There was a significant decrement in the hemoglobin levels (p= 0.02); serum iron levels (p < 0.001) and Transferrin saturation (p<0.001) from group A-C. TIBC and serum transferrin receptor protein on the other hand demonstrate a rise in group C when compared to Group A (p<0.001). This depicts a true picture of iron deficiency in Group C. Unexpectedly, serum ferritin levels were elevated in both group B and C as compared to the group A (p<0.001), respectively. In group C, sTfR/ferritin ratio was also calculated to be 0.822 which is suggestive of increased risk of acute myocardial infarction in obese individuals. CRP also showed a great surge in group C individuals as compared to group A individuals (p<0.001) (table 1). Spearman rank correlation portrayed a negative correlation of serum iron (r=-0.359, p=0.004) with BMI while serum ferritin (r=0.237, p< 0.001) and sTfR (r=0.263, p= 0.036) levels were positively associated to BMI (fig I A-C).

DISCUSSION

The study was focused on assessment of serum transferrin receptor (sTfR) levels in specific BMI groups with

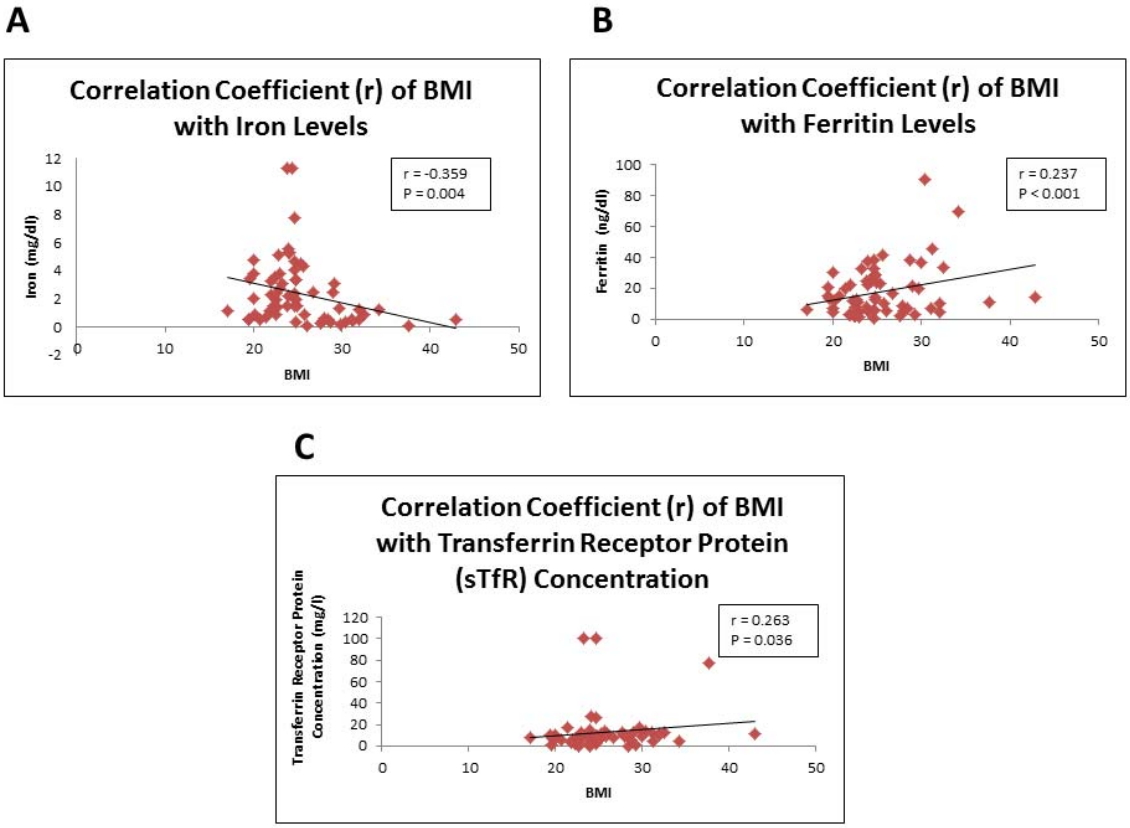

Fig. 1: Correlation of BMI with Iron Parameters

reference to South Asian criteria and finding out whether the serum transferrin receptor level is a better marker of iron status compared to ferritin levels.

In this study the sTfR levels are elevated in obese group individuals although, the individuals did not have any apparent iron deficiency (table 1), which was initially confirmed by the microscopic picture. So far the sTfR cut off levels are not well documented in South Asian population, this study is highlighting the levels of sTfR in Pakistani population. The increased sTfR levels in this study indicate the functional picture of iron in the body. This suggests that the demand of iron is more in obese individuals probably because the iron is sequestered within the macrophages and as a result sTfR increases to fulfill the demand.

The results of this study show that the serum ferritin levels of obese were higher than those of overweight individuals. It was also seen that with an increase in BMI there is a corresponding increase in the C reactive protein (CRP) in the body (Table 1). CRP is an acute phase reactant that is produced by the liver under the influence of adipokine released by the adipocytes in the body (Alam *et al.*, 2014, Brooks *et al.*, 2010, Choi *et al.*, 2013). Since an excess of adipocytes means additional adipokine in circulation hence the body enters a state of sub-clinical inflammation. This leads to a decrease in iron levels of the body due to the inflammatory mediated sequestration of iron within the reticulo-endothelial system (Karl *et al.*, 2009). Although the increase in ferritin level trend may seem vague but as identified by anoother study of our group; its levels increase when the body is in a state of subclinical inflammation such as obesity (Alam *et al.*, 2014) . Given that it could be postulated that ferritin is responsible for giving false positive results for IDA in overweight and obese (fig. 1B) and thus, sTfR is likely to be the best clinical measure of iron status in these subjects as its expression is not effected by inflammation but demand of erythropoiesis by the body (Zimmermann, 2008) (fig. 1A and C).

Obesity is one of the causes of increased risk for acute myocardial infarction (AMI). Furthermore, some studies have established increased serum ferritin as a risk factor for AMI (Holay *et al.*, 2012). Recently attention has been given to evaluate whether sTfR/ferritin ratio can be used as a better marker in certain conditions including AMI. A ratio of sTfR and Ferritin provides highest sensitivity and specificity in this regards (Punnonen *et al.*, 1997). The

current study shows that sTfR/ferritin levels in obese group is almost near 1 (0.822), that is it is increasing from normal weight to obese individuals. sTfR and ferritin ratio has been studied in different patients of coronary artery diseases (CAD) elucidating an increase in sTfR and ferritin ratio as the disease progresses from stable angina to the potentially life taking AMI (Braun *et al.*, 2004). Hence it is logical to conclude that an increase in sTfR to ferritin ratio can be a good predictor of AMI in our obese subjects.

The limitation of this study has been its cross-sectional design; prospective studies would be a better evaluator. Relatively larger population studies are not available to define the cut off values of the iron parameters Pakistani population. However, this study proposes a causal connection between change in BMI, inflammation and low iron levels.

CONCLUSION

This study highlights a novel finding of increasing levels of sTfR in obese Pakistani population. On the contrary ferritin levels could not depict the true iron deficient status. It could be postulated that sTfR is likely to be the best clinical measure of iron status in obese subjects. sTfR is most likely a better clinical measure of iron status in inflammatory conditions as its expression is effected by erythropoiesis and not by inflammation. Furthermore; risk of acute myocardial infarction can also be predicted by increased sTfR/ferritin ratio.

ACKNOWLEDGMENT

The authors wish to thank the Department of Biological and Biomedical Sciences, Aga Khan University for funding this project.

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