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Research Article

Role of inflammation as reflected by serum hsCRP and ferritin level in major depressive disorder

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ABSTRACT

Background: The relationship between depression and inflammation has been strengthened by different studies for which Immune Cytokine Mechanism has been proposed to explain pathogenesis of depression.
Methods: The role of inflammation in major depressive disorder (MDD) has been evaluated in this study among 101 patients suffering from MDD along with 106 age and sex matched control subjects. Two well known acute phase reactant proteins namely hsCRP and Ferritin were assayed in all study subjects by Enzyme Immuno Assay method.
Results: Concentration of hsCRP in serum was found to be significantly increased in all patients suffering from MDD whereas Ferritin level was found to be significantly decreased in male MDD subjects only (male: 70.6 ng/ml±53.54 ng/ml in MDD vs 100.16 ng/ml±39.93 in controls; female: 38.43 ng/ml±11.2 in MDD vs 39.93 ng/ml±15.0 in controls). There was no significant alteration of the parameters among different grades of MDD. The possibility of these two parameters to be used as biomarkers has also been evaluated by ROC curve.
Conclusions: The significant change in hsCRP & Ferritin which is not associated with grade wise alteration suggests that probably inflammation is the early event which causes depression. Therefore no gradual change is observed with increase in severity of the disease. hsCRP can be used as a biomarker of Inflammation in MDD cases.

Keywords: MDD, Inflammation, Acute phase reactant, Ferritin, hsCRP

INTRODUCTION

Various etiological factors have been postulated for depression which is the most common psychological disorder with a global prevalence varying from 8-20%).[1] The current established theory of depression is based on the beneficial role of selective serotonin reuptake inhibitors in depression which describes that synaptic depletion of serotonin is the key factor of depression.[2] However, this theory is not full proof. Recently, a theory called "*Immune cytokine mechanism*" has been proposed to establish the cause of depletion of serotonin in depression. The theory states that psychological stress can activate inflammation. The relationship between depression and inflammation was strengthened by the following observations:

1) Depression frequently is comorbid with many inflammatory illnesses like autoimmune disease, cardiovascular disease, diabetes, and cancer.[3]

2) Exposure to immunomodulating agents may increase the risk of developing depression. If animals are injected with IL-1α or TNF α, they show some sickness type of behaviour in a dose and time-related manner. As these inflammatory signalling proteins increase, sickness behaviours become more pronounced.[4]
3) Stress can activate pro inflammatory pathways and antidepressants can decrease inflammatory response. Moreover, inhibition of inflammatory pathways can improve mood.[5]

Increased inflammatory biomarkers have been found to be associated with major depressive disorder (MDD), as a supportive evidence of *Immune Cytokine Mechanism.* Numerous studies have established a probable association between depression and acute inflammatory biomarkers like hsCRP, various cytokines, ferritin and many others.

C-reactive protein (CRP) is one of the most sensitive acute-phase reactants. Although CRP levels are traditionally elevated only in severe inflammation, newer assays with improved sensitivity (high-sensitivity CRP, hsCRP) are able to measure CRP in healthy individuals, permitting an exploration of the association between subclinical systemic inflammation and risk of depression. In longitudinal studies, a positive association between CRP and depression has been reported by some but not all studies. Thus, researchers are unclear whether and to what extent elevated CRP levels are associated with psychological distress and depression in the general population.[6]

Another acute phase reactant protein is Ferritin, which is commonly used to measure body‘s iron status. Studies dealing with the possible relation between the iron metabolism and depression manifestation have been done with inconsistencies. Role of systemic, low-grade inflammation have been frequently used to explain the relationship.[7,8]

In the light of the above background, it seemed worthwhile-

a) To estimate serum hsCRP level and Ferritin in patients suffering from MDD.
b) To find out whether these parameters have any significant difference when the disease is classified according to grade and according to gender.
c) To evaluate serum hsCRP and Ferritin as biochemical marker in MDD.

METHODS

This Case control study was undertaken in Department of Biochemistry, College of Medicine & Sagore Dutta Hospital in collaboration with Department of Psychiatry of same Institute. The study period was from July, 2013 to Dec, 2015. The study was approved by Institutional Ethics Committee.

I. Selection of study subjects

All patients who were suspected to suffer from Major depressive disorder (MDD) were selected from the Psychiatry outdoor of College of Medicine & Sagore Dutta Hospital. These patients were first evaluated by detailed history taking and clinical examination through a structured proforma designed for this study. Then they were screened with WHO Five well-being index. The raw score was calculated. When raw score was below 13 or if the patient had answered 0 to 1 to any of the 5 items, they were further tested. Patients were diagnosed as having major depressive disorder according to the Structured Clinical Interview for DSM-IV, and who scored at least 14 points on Major Depression Inventory (MDI). This inventory was also used to classify the patients according to ICD 10 criteria for depression.[9]

The exclusion criteria were significant psychiatric co-morbidity, organic mental disorder, mental retardation, bipolar disorder, intake of any psychotropic drugs during and at least 1 week before the study, substance abuse, history of endocrine disorders, pregnancy, postpartum depression and lactation.

Apparently healthy age and sex matched individuals were assessed using General Health Questionnaire (GHQ 12). A score of less than or equal to 15 were considered as not to suffer from major psychiatric illness.[10] Such individuals were selected as control group.

Informed consents were taken from the patients or legal guardians and from the control subjects.

II. Gradation of MDD cases

MDI score of 20 – 24 was considered as mild grade, 25 – 29 as moderate grade and > 30 was considered as severe grade.

III. Sample Collection, Separation & analysis of serum

An amount of 5 ml of fasting blood samples was drawn aseptically from the superficial veins of each of the study subjects (Both cases & controls) in plain vials and allowed to clot. Serum was separated at room temperature and later by centrifugation at 800 g for 10 minutes. Separated serum was kept in refrigerator in aliquots for maximum 1 week. Serum of all patients and controls included were investigated for hsCRP & Ferritin by immuno enzymometric assay (EIA).[11,12]

IV. Statistical analysis

The study groups were classified into male & female and concentration of hsCRP and Ferritin were expressed in mean±SD in each group separately. The mean values were compared for significance by student’s t test. A p value of <0.05 was considered to be significant.

The patients were further subdivided in mild, moderate and severe grade. One-way analysis of variance (ANOVA) with post hoc test is used to test the difference between the means of several subgroups.

Then ROC curve was used for determining the validity of the two parameters for diagnosis of Depression. Area under the ROC curve was found out with standard error and 95% CI. The optimum cut off value was found out from associated criterion calculated from Youden Index J, which is the measure of maximum potential effectiveness of a biomarker. Considering the cutoff point, sensitivity and specificity of the test was calculated.

The analysis was done using MedCalc Statistical Software version 16.4.3.

RESULTS

Table 1 shows level of Ferritin & hsCRP in study groups. Among 101 MDD cases 77 were female and 24 were male, whereas among 106 control subjects 76 were female and 30 were female. Ferritin level was found to be decreased significantly in male (70.6 ng/ml + 53.54 ng/ml vs 100.16 ng/ml + 39.93) but not in female (38.43 ng/ml + 11.2 vs 39.93 ng/ml + 15.0). Level of hsCRP was found to be decreased both in male and female subjects (male: 2.7 mg/L + 2.5 in cases vs 1.5 ng/ml + 0.7 in controls, female: 3.5 mg/L + 2.3 in cases vs 1.7 ng/ml + 1.0 in controls).

Table 1: Level of ferritin and hsCRP in study groups.

Biochemical parameter	Statistical parameter	Case (n = 101)		Control (n = 106)	
		Male (n=24)	Female (n=77)	Male (n=30)	Female (n=76)
Ferritin	Arithmetic mean (ng/ml)	70.60[1]	38.43[2]	100.16	39.93
	Standard deviation	53.53	11.20	18.02	15.01
	Standard error of the mean	10.92	1.27	3.29	1.72
hsCRP	Arithmetic mean (mg/L)	2.69[3]	3.40[4]	1.47	1.69
	Standard deviation	2.47	2.27	0.70	1.02
	Standard error of the mean	0.50	0.25	0.12	0.11

Test statistic t: 2.836, Two-tailed probability: P = 0.0065[1]; Test statistic t: 0.702, Two-tailed probability: P = 0.48[2]; Test statistic t: -2.572, Two-tailed probability: P = 0.0130[3]; Test statistic t: -5.963, Two-tailed probability: P < 0.0001[4].

Table 2: ANOVA test of the study group.

Gender	Grade of MDD (n = 101)	Biochemical parameter			
		Ferritin		hsCRP	
		Mean (ng/ml)±SD	F ratio (significance)	Mean (mg/L)±SD	F ratio (significance)
Male (n = 24)	Mild (n = 04)	100.67±120.57	0.744 (P = 0.487)	2.55±1.85	0.0103 (P = 0.990)
	Moderate (n = 10)	65.60±39.64		2.67±2.21	
	Severe (n = 10)	63.57±20.55		2.76±3.08	
Female (n = 77)	Mild (n = 15)	37.99±9.72	0.863 (P = 0.426)	3.12±2.74	0.150 (P = 0.861)
	Moderate (n = 27)	40.65±9.51		3.33±2.16	
	Severe (n = 35)	36.91±12.87		3.42±2.20	

Table 2 shows ANOVA test done in different grade of MDD cases, followed by the F statistic and associated P value. Among 24 male patients suffering from MDD, 04 were of mild grade, 10 of moderate grade and 10 of severe grade. Among 77 female MDD cases, 15 were of mild grade, 27 of moderate grade and 35 were of severe grade. Neither for Ferritin, nor for hsCRP, is P value less than 0.05 in either gender. Hence, it cannot be said that the means of at least two of the subgroups differ significantly. As ANOVA test did not give significant value, post hoc test was not done.

Table 3 shows analysis of Ferritin and hsCRP as biomarker, which was done from ROC curve. Ferritin in male & hsCRP in female were found to be highly significant. The cut off value of Ferritin for male was 70.9, sensitivity of which is 70.83% and specificity was 100%. For hsCRP, cut off value was found to be 2 for male & 2.1 for female. Sensitivity & Specificity for male were found to be 50% & 90% respectively. For female the same were 90% and 77.63% respectively.

Table 3: Analysis of the biochemical parameters as biomarker from ROC curve.

Biochemical parameter	Statistical parameter	Male (n=24)	Female (n=77)
Ferritin	Area under the ROC curve (AUC)	0.810	0.529
	Significance level P (Area=0.5)	<0.0001	0.5384
	Youden index J	0.7083	0.1847
	Associated criterion (ng/ml)	≤70.9	≤52.8
	Sensitivity	70.83	96.10
	Specificity	100.00	22.37
hsCRP	Area under the ROC curve (AUC)	0.628	0.725
	Significance level P (Area=0.5)	0.1154	<0.0001
	Youden index J	0.4000	0.4516
	Associated criterion (mg/L)	>2	>2.1
	Sensitivity	50.00	67.53
	Specificity	90.00	77.63

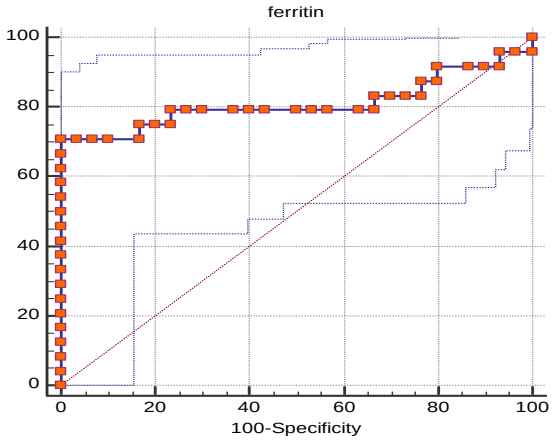

Figure 1: ROC curve of ferritin male.

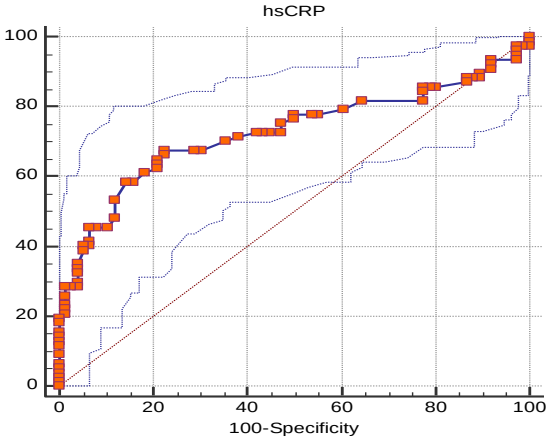

Figure 2: ROC curve of hsCRP of female.

Figure 1 and 2 shows ROC curves of Ferritin male and hsCRP of female respectively.

DISCUSSION

Both physical and mental stressors are known to activate immune system and release cytokines which can cause alteration of mood and behaviour, typical to that of Depression. This concept, known as Immune cytokine mechanism is thought to be present in Depression. The underlying mechanism between inflammation and depression though not fully understood, two mechanisms have been proposed so far.

First, inflammation may lead to depression. Some studies indicate that proinflammatory cytokines might activate the enzyme indoleamine-2,3-dioxygenase, which leads to decreased production of serotonin. This is an important factor in the pathogenesis of depression. Simultaneously, there is increased production of kynurenic and quinolinic acids, which lead to increased release of glutamate and thereby to decreased production of trophic factors, including brain-derived neurotrophic factor, a factor associated with depression.[13]

Second, depression may lead to inflammation. Psychological stress activates the hypothalamic-pituitary-adrenocortical axis and sympathetic nervous system, which releases stress hormones. These hormones, together with cytokine release induced by stress, initiate the acute-phase response triggering inflammation. This in turn increases levels of the proinflammatory cytokine interleukin 6, stimulating the production of acute-phase proteins, including CRP.[14]

Moreover, one study group has reported that though plasma cytokine assays are highly specific and sensitive measures in defining immune profile, they are quite expensive. The use of hs-CRP proved to be sufficiently reliable and potentially applicable to routine clinical practice, so to identify those subjects with the highest levels of immune dysregulation.[15]

In this study, hsCRP level was found to be increased significantly. This is supported by other study groups, which have reported that serum hsCRP is an independent risk marker for de novo major depressive disorder in women.[15] They also suggested an aetiological role for inflammatory activity in the pathophysiology of depression. One study group has however reported that symptoms consistent with major depression were significantly associated with hs-CRP in men only, even after adjusting for age, obesity class, metabolic variables and medications known to affect inflammation. They suggested that there are biologic differences between men and women that may modify the relationship between hs-CRP and depression.[15]

So far ferritin is concerned, a decreased value was observed in MDD cases in this study, which was

significant in male patients only. In a study conducted on 4181 participants, male participants with MDD plus comorbid CHD or hypertension were found to have lower levels of ferritin compared to men without MDD, while in women, results were inconsistent. They concluded that MDD reduced levels of ferritin, transferrin and fibrinogen in CVD in a gender specific way.[17] Another two studies concluded that depression is associated with decreased levels of serum ferritin concentrations.[18] But there are studies which did not find any significant association.[19,20]

Due to the fact that mean serum ferritin level was lower in depressed students than in healthy ones, some researchers suggest that probably iron plays a role in brain function and the establishment of depressive mood. Iron plays an important role in the oxygenation of brain parenchyma and the synthesis of dopamine, a neurotransmitter of the nervous system.[21]

However, when gradation of MDD was done, analysis by ANOVA shows no significant change in any of the parameter for any gender. This suggest that probably inflammation is the early event which causes depression and that is why there is no gradual change is observed with increase in severity of the disease.

The diagnostic ability of these two parameters was assessed by ROC curve, which is the first such approach to best of our knowledge. Ferritin was found to be a significant marker in male with a cut off value of 70.9 ng/ml, but for female, it was not found to a good marker as AUC was found to be only 0.529. So far hsCRP is concerned, though significance was observed only in female, cut off value was almost similar among male (2 mg/L) and female (2.1 mg/L). Not much difference was also observed in ROC curve for male and female.

CONCLUSION

It has been hypothesized that major depression is accompanied by alterations in cell-mediated and innate immunity, which is reflected by change in “acute-phase” proteins. In this study, hsCRP was found to increase significantly in MDD cases when compared with controls. Ferritin, another acute phase reactant was found to be decreased significantly in male subjects but not in female. This change can be attributed to decrease in iron but as iron was not estimated, it can be considered as a limitation of this study. No grade wise change in either parameter was observed. The cut off value to use hsCRP as a biomarker for MDD was found as 2 mg/L for male and 2.1 mg/L for female.

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