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Association of Peripheral Pro-inflammatory Cytokines with Depressed Suicidal Patients: A Hospital-based, Cross-sectional Study

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Abstract

Background: Acute-phase reactants, such as C-reactive protein (CRP), along with pro-inflammatory cytokines like interleukin-6 (IL-6) and interleukin-1β (IL-1β), exhibit elevated levels in the peripheral bloodstream during acute mood episodes. In both major depressive disorder and bipolar disorder, instances of suicidal behavior, encompassing suicidal idealization and self-harm attempts, are linked to intensified depressive exacerbation. This study investigates the correlation between pro-inflammatory markers in patients experiencing major depressive episodes (MDE) with or without inclinations toward suicidal tendencies.

Methods: The study comprised a sample of 75 outpatients categorized into three equally sized groups. These groups consisted of patients experiencing major depressive episodes with accompanying suicidal behavior, individuals undergoing MDE without any indications of suicidal tendencies, and a control group of mentally healthy individuals. Pertinent demographic information was gathered, and levels of pro-inflammatory markers, namely CRP, IL-6, and IL-1β, were quantified in the serum. The data were analyzed using the Statistical Package for the Social Sciences version 22.

Results: Through bi variate analysis, it was observed that all three markers exhibited a noteworthy correlation with the subgroup experiencing MDE alongside suicidality (Pearson correlation coefficient ≤ 0.01). However, this correlation was not evident within the MDE subgroup lacking suicidal tendencies. Utilizing a one-sample t-test, it was established that both MDE groups displayed a substantial association (with a two-tailed significance level of ≤ 0.01) with the inflammatory biomarkers. Notably, the mean difference in biomarker levels was considerably more pronounced in the subgroup characterized by suicidality.

Conclusion: Suicidal behavior, encompassing both ideation and attempts, exhibits an independent association with a pro-inflammatory state, irrespective of the severity of the linked depression. This relationship warrants further investigation to identify reliable biomarkers to enhance prediction and facilitate more effective management of suicidal behavior.

Keywords: suicide, major depression, inflammation, C-reactive protein, cytokines.

Conflict of Interest: None **Funding Source**: None

Introduction

A major depressive episode (MDE), as defined by the Diagnostic and Statistical Manual – 5th Edition (DSM-5), is experiencing persistently low mood for a continuous period of 2 weeks. There is both subjective and objective evidence of depressed mood, and this is often accompanied by loss of pleasure in day-to-day activities or anhedonia[1]. Associated symptoms substantiating the MDE diagnosis are excessive feelings of guilt and self-blame, lack of energy, decreased appetite and weight loss, insomnia, recurrent suicidal

ideation and suicidal behavior. The latter symptoms signify that depression is severe and life-threatening, and immediate measures must be instituted to treat such cases[2]. MDEs occur in both unipolar major depression (MDD) and bipolar disorder (BD) and imply a severe disturbance in mood [3]. Research in the past couple of decades has consistently shown the relevance of inflammation, both in the periphery and center, as an essential factor in the pathogenesis of mood disorders[4]. Numerous original studies, systematic reviews and meta-analyses published in the recent past indicate an inflammatory imbalance in MDD and BD.

The principal findings are a rise in pro-inflammatory factors like C-reactive protein (CRP), pro-inflammatory cytokines and chemokines and a decrease in anti-inflammatory cytokines during acute episodes. With successful treatment, these abnormalities are significantly reversed, and during euthymic periods, inflammatory factors may return to normal[5]. Furthermore, there is evidence that severe depressive episodes associated with suicidal thinking/behavior are marked by more significant disturbance in the inflammatory milieu, with large rises in the inflammatory markers[6]. This finding implies that while affective episodes are associated with a pro-inflammatory state, this anomaly is markedly exaggerated in suicidal subjects[7].

With this background, our study intended to investigate key inflammatory markers in mood disorder subjects presenting as outpatients. We recruited consecutive patients with major depressive episodes and classified them as presenting either with or without suicidal behavior (suicidal ideation ± acts of self-harm). We measured levels of CRP, interleukins (IL) 6 and 1β in the peripheral blood of the study subjects and compared them with healthy controls.

The study aimed to examine whether there was any association between inflammatory factors and suicidality. We also examined the study subjects' demographic characteristics to further delineate the sample and clarify our findings.

Methodology

Study design and location: This observational, cross-sectional study was conducted from 01/10/2022 to 31/05/2023 at the Outpatients unit of the Department of Psychiatry, HBS General Hospital, a tertiary care teaching hospital of HBS Medical & dental College, Islamabad. The study protocol was approved by the Ethical Review Board of the medical college.

Sample collection: A non-probability, convenience sampling technique was utilized and consecutive patients presenting with a major depressive episode were enrolled after obtaining written consent. Patients, both males and females, aged between 18 to 65 years were included in the study if they were suffering from a DSM-5-defined MDE as determined by history and mental state examination. Hamilton Rating Scale for Depression (HRSD) was instituted to all cases to determine the severity of depression and those subjects admitting to suicidal ideation/behavior were also administered the Suicidal Risk Scale by Beck.

Patients were excluded if they concomitantly suffered from chronic inflammatory conditions like rheumatoid arthritis, systemic lupus erythematous, acute infections, and systemic disorders (diabetes mellitus, uncontrolled hypertension, and metabolic syndrome). Similarly, patients on long-term treatment with non-steroidal anti-inflammatory analgesics were excluded, and lastly, pregnant females were also not enrolled in the study. In this manner, 25 subjects of MDE with suicidal ideation/behavior and 25 MDE cases without this condition were selected to participate in the study. The control group consisted of 25 healthy adults with no previous psychiatric history. A semi-structured proforma with demographic details was administered to all participants.

Blood collection and laboratory techniques: After following the necessary precautions for phlebotomy, venipuncture was performed and 5 ml of blood was collected from each participant. Serum was separated at 4o C by centrifugation for 10 minutes, transferred to Eppendorf micro-centrifuge tubes, and stored at – 80o C until biochemical analysis. The latter was performed on the following biomarkers:

1. IL-1β
2. IL-6
3. CRP

Serum IL-1β and IL-6 were analyzed using human sandwich enzyme-linked immunosorbent assay (ELISA) kits provided by Elabscience and the detection range for both cytokines was from 7.81 to 500 pg/ml. briefly, the assay procedure was as following:

100µl of serum was added to each well of the micro ELISA plate, liquid was removed and 100µl of biotinylated detection antibody was added and the mixture incubated for 1 hour at 37o F. After the solution was aspirated and washed 3 times, 100µl of avidin horseradish peroxidase specific for human IL-1β and IL-6 was added and incubated for 30 minutes. It was then

aspirated and washed 5 times. This was followed by the addition of 90µl of the substrate reagent and the mixture was incubated for 15 minutes. Latter 50µl of the stop solution was added, optical density read at 450 nm spectrophotometrically, and results were immediately calculated.

CRP was analyzed by Avitex-CRP latex particles coated with antibodies to human CRP. When serum containing detectable levels of CRP was added, clear agglutination was observed within 120 seconds.

Statistical analysis; This was performed by Statistical Package for Social Sciences (SPSS) Version 22, copyright IBM Corporation, Armonk, NY, USA, 2013.

Results

Descriptive statistics were applied to demographic variables. For continuous variables like age, mean and standard deviation were calculated, while for categorical variables such as gender, marital status, etc., frequency and percentage were computed. The mean and standard deviation for HRSD scores for the 3 groups were also determined (Table I).

Table I: Demographic characteristics of study participants

Variable	Group	n	Mean ± SD
Age (years)	**Total**	75	33.32±8.93
	With suicidality	25	28.44±6.70
	Without suicidality	25	37.80±8.10
	Healthy controls	25	33.72±8.93
HRSD score	Total	75	19.36 ± 10.61
	With suicidality	25	26.96 ± 5.59
	Without suicidality	25	25 ± 6.15
	Healthy controls	25	6.12 ± 1.50
Gender	**Total**	75	Percentage
	Male	39	52.0%
	Female	36	48.0%
	With suicidality	25	
	Male	11	44.0%
	Female	14	56.0%
	Without suicidality		
	Male	13	52.0%
	Female	12	48.0%
	Healthy controls		
	Male	15	60%
	Female	10	40%
Marital status	**Total**	75	
	Married	58	77.3%
	single	17	22.7%
	With suicidality		
	Married	16	64.0%
	Single	09	36.0%
	Without suicidality		
	Married		
	Single	23	92.0%
	Healthy controls	02	8.0%
	Married	19	76.0%
	Single	06	24.0%
Occupational status	House wife	20	26.6%
	Student	20	26.6%
	Teacher	12	16.0%
	Own business	10	13.3%
	Miscellaneous	13	17.3%

With respect to the relationship between pro-inflammatory markers (CRP, IL-1β and IL-6) and the 3 groups studied (MDE cases with suicidality, MDE cases without suicidality and healthy controls) (Figure 1).

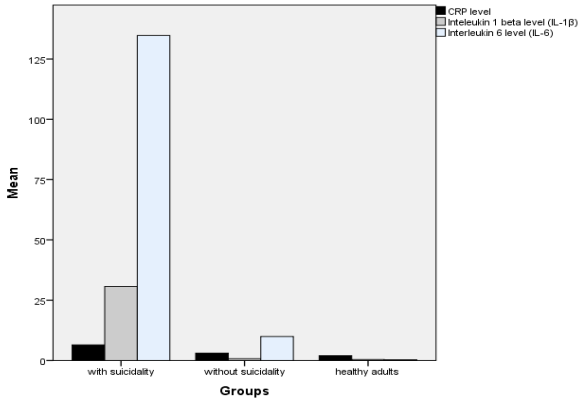

Figure 1. Graphical representation of correlation.

Classical bivariate analysis was performed on pro-inflammatory markers and the study groups using Pearson correlation coefficient. With p value significant at ≤ 0.01 (2-tailed) it was found that the biomarkers were significantly correlated to the MDE cases with suicidality but not to the other group i.e. MDE subjects without suicidality (Table II).

Table II: Bivariate analysis of the pro-inflammatory markers and the study groups

Study group	CRP	IL-1β	IL-6
MDE cases with suicidality Pearson correlation coefficient	$p < 0.01$	$p < 0.01$	$p < 0.01$
MDE cases without suicidality Pearson correlation coefficient	$P > 0.01$	$P > 0.01$	$P > 0.01$

Note: CRP – C-reactive protein; IL-1β – interleukin 1 beta; IL-6 – interleukin 6; MDE – major depressive episode.

The means of the inflammatory markers were analyzed using the one sample T- test (2-tailed significance ≤ 0.01) and the results are given in Table III.

Table III: Pro-inflammatory markers – comparison of means with one sample *t*-test.

	t	df	Sig. (2-tailed)	95% CI of the difference	Mean Difference	
					Lower	Upper
MDE cases with suicidality						
CRP level	6.024	24	0.000	6.48000	4.2663	8.6937
IL-1β level	3.823	24	0.001	30.710400	14.13161	47.28919
IL-6 level	4.289	24	0.000	134.761480	69.92025	199.60271
MDE cases without suicidality						
CRP level	6.363	24	0.000	3.04000	2.0539	4.0261
IL-1β level	2.818	24	0.001	0.730160	0.19547	1.26485
IL-6 level	8.651	24	0.000	9.957680	7.58213	12.33323
Healthy controls						
CRP levels	Standard deviation is 0 so results cannot be computed					
IL-1β	2.391	24	0.025	0.421200	0.05768	0.78472
IL-6	1.553	24	0.134	0.317280	- 0.10438	0.73894

Discussion

The mean age of the patients presenting with a major depressive episode and suicidality was 28.44 years, while this was 37.80 years in the MDE group without suicidality (Table 1). This finding that younger people were at greater risk for suicide was somewhat similar to that reported in an epidemiological study in Malaysia, another Asian country[8]. In that study participants between the ages of 16 and 24 had higher risk of suicidal behavior (OR: 2.6, 95% CI 1.08 – 6.2). The reasons for this association can be multifarious, including co-morbid anxiety disorders, melancholic depression or mixed features. Additionally, the social support network may not as robust as in the older, middle-aged cohort[9]. The mean HRSD score in the group with suicidal tendencies was 26.96, somewhat higher than the comparison group in which the mean HRSD score was equal to 25. Greater severity of depression may play a mediating role between hopelessness and suicidal ideation and this mechanism may be contingent on the level of impulsivity. This alludes to the fact that a higher degree of depression accompanied by impulsiveness precipitates attempts at self-harm[10]. Table 1 also shows that females predominate in the group with suicidal ideation or behavior (56% versus 44%). This is in line with reported findings from studies with large sample sizes in which it has been shown that the female gender is a risk factor for suicide attempts[11].

Figure 1 illustrates the relationship between the pro-inflammatory factors and the three study groups. It demonstrates that all three biomarkers are significantly raised in MDE cases with suicidal ideation ± behavior, while this increase is much less in MDE subjects without suicidality. In the case of healthy controls, it can be seen that the biomarkers barely rise above the baseline. Indeed, this is a well-replicated finding and a review of the extant literature supports the proposition that suicidal ideation and behavior are independently associated with a significant rise in inflammatory factors. A well-cited study showed that MDD patients with severe suicidal ideation or high lethality suicide attempts had a statistically significant increase in the inflammatory markers, and this rise was independent of the degree of depression[12]. Another noteworthy study is instrumental in understanding the role of inflammation in suicidal behavior. It measured serum IL-2, IL-6 and TNF- α in patients with recent suicide attempts, depressed subjects without suicidality and healthy controls. The study found increased levels of pro-inflammatory cytokines, namely IL-6 and TNF-α and decreased concentration of IL-2 (anti-inflammatory cytokine) in suicide attempters compared to the other two groups. The results remained statistically significant after adjusting for potential confounders like age, sex, body mass index, degree of depression, medication use, etc[13].

To further clarify our findings, we performed bivariate analysis of the inflammatory biomarkers with the study groups (Table 2). Using Pearson correlation co-efficient, we were able to show that CRP, IL-1β and IL-6 were significantly associated ($p \leq 0.01$) with the MDE group with suicidality but this relationship did not achieve statistical significance in the case of MDE subjects without suicidality (Table 2). This is a noteworthy discovery and review of the extant literature revealed that it is in line with published research. A recent study measured high sensitivity C-reactive protein (hsCRP) levels in inpatients admitted after attempted suicide, patients with suicidal ideation, and non-suicidal psychiatric controls. The investigators were able to demonstrate an apparent inflammatory gradient assessed by CRP levels from recent suicide attempters, cases with suicidal ideation and psychiatric controls in the inpatients setting[14]. This showed a linkage between pro-inflammatory state and suicidal behavior in the background of the real world in severely ill psychiatric

patients. In an interesting study, inpatients aged 15-30 years were assessed for multiple measures including serum cortisol as determined by hair cortisol concentration, serum CRP and mRNAs for inflammatory factors including TNF-α, IL-1 β, glucocorticoid receptor, etc. In this young cohort, the researchers were successful in differentiating suicide attempters from ideators on the basis of the above biomarkers with a distinct biological profile in both the hypothalamic-pituitary- adrenal axis and the inflammatory pathways[15].

Finally, we analyzed our results by comparing the means of the inflammatory factors in each of the study groups and the conclusions are provided in Table 3. The mean values were very high in the group of MDE cases with suicidality as compared to the other group, however it remained statistically significant (2-tailed significance ≤ 0.01) in both sub-groups. It implies that while pro-inflammatory markers are associated with depressive episodes without suicidal ideation or behavior, this linkage is robust in the presence of suicidality. In a pioneering study Kim and colleagues were the first to show differences in the balance of cytokines in MDD patients with and without suicidal behavior. Their sample included MDD patients with recent suicide attempts, major depression cases without suicidal tendencies and normal controls. They used mitogen stimulated whole blood and measured the levels of several pro and anti-inflammatory cytokines. Their study revealed a

distinctive immune response, with the non-suicidal patients showing increased IL-6 production, a Th1/Th2 imbalance and a shift to Th1 lymphocytes, while suicidal MDD cases had decreased IL-2[16]. To conclude the discussion, a worthwhile study must be mentioned which measured plasma kynurenine (KYN) levels in three groups of participants, namely MDD cases with or

without recent suicide attempts and healthy volunteers. KYN levels varied across groups (F=4.03, df= (2,58), p=0.023) and this was higher in the suicide attempter subgroup compared with non-attempters (t=2.105, df=58, p=0.040), while the latter did not differ from healthy controls (t=0.418, df=58, p=0.677). In post hoc analysis, KYN but not tryptophan (TRP) was associated with attempt status, and only suicide attempters showed a positive correlation of the cytokine activation marker neopterin with the KYN:TRP ratio, signifying that KYN production may be influenced by inflammatory processes among suicide attempters[17].

Limitation

While interpreting the results of this study the following caveats must be considered:

(1) The sample size is small and studies with greater number of participants are required to replicate the findings.

(2) The design is cross-sectional; only prospective, cohort studies can confirm the results presented here.

(3) As serum cytokine levels are still not performed routinely, there are technical and financial barriers in conducting such studies, so that many investigators face severe difficulties in carrying out their research work.

Conclusion

In order to discover biomarkers for the better prediction and treatment of suicide, researchers have been trying for decades to elucidate its neurobiology; the fact is that only in the past decade has the focus shifted to inflammatory factors. In this respect, different teams of investigators have been successful in showing the connection between suicide and inflammation, and this has mainly been achieved by measuring the peripheral values of pro-inflammatory markers. A picture is emerging indicating the central role of inflammatory abnormalities in developing suicidal behavior. The present study demonstrates this fact emphatically and is instrumental in further extending the published findings. It is hoped that valid biomarkers will be discovered that would assist in the overall management of suicidal behavior in psychiatric patients.

List of Abbreviations

C-reactive protein (CRP), interleukin-6 (IL-6), interleukin-1β (IL-1β),major depressive episodes (MDE), Diagnostic and Statistical Manual – 5th Edition (DSM-5),bipolar disorder (BD),Hamilton Rating Scale for Depression (HRSD),Statistical Package for Social Sciences (SPSS),high sensitivity C-reactive protein (hsCRP), kynurenine (KYN),tryptophan (TRP)

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Authors Contribution:
[1,3]Substantial contributions to the conception or design of the work;
[4,5,6]the acquisition, analysis, or interpretation of data for the work & Final approval of the version to be published
[1,2,6] Drafting the work or revising it critically for important intellectual content.