THE ROLE OF INTERLEUKIN-1 AND INTERLEUKIN-15 IN AGGRAVATING THE EFFECTS OF ANOREXIA NERVOSA IN LOW BMI YOUNG ADULTS

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ABSTRACT

Objectives: This study exploring

1. To estimate the levels of IL-1 and IL-15 in the young adults suffering from anorexia nervosa (AN) with low BMI.
2. To evaluate and compare the levels of IL-1 and IL-15 with same age and same BMI group without AN.

Methods: The study design was a descriptive-analytic, conducted over a year i.e from March 2023 to April 2024 at RMI and HMC Medical and Psychiatry OPDs. We enrolled 50 young adults (both males and females) diagnosed with AN and compared them to 50 healthy controls matched by age and BMI. This study assessed levels of IL-1 and IL-15 in young adults (ages 15-30 years) with AN. A control group of the same age range and similar BMI was used for comparison. The BMI of all participants was calculated, and blood samples were collected for cytokine level analysis. Results were analyzed to compare cytokine levels between the two groups. After compiling the test results of these two groups, the results of both groups were analyzed. Data were analyzed using SPSS v25. Pearson correlation was applied to assess the relationship between cytokine levels, BMI, and psychiatric symptoms. A p-value of <0.05 was considered significant.

Result: Elevated levels of IL-1 and IL-15 were observed in AN patients compared to controls, suggesting these cytokines may disrupt hypothalamic function and neurotransmitter balance, contributing to the anorexic behaviors observed in these patients. IL-1 and IL-15 were also found to have a bidirectional relationship with stress, aggravating AN symptoms. Our findings suggest that targeting these cytokines could provide new therapeutic strategies**.**

Conclusion: IL-1 and IL-15 play a significant role in the biological mechanisms underlying AN, influencing appetite, mood, and cognitive function. Incorporating biological treatments into current therapeutic approaches could improve patient outcomes. Future research should focus on the immune system's role in AN to develop novel treatment interventions.

Keywords. Interleukine-1, Interleukin-15, Anorexia nervosa, BMI

INTRODUCTION

Anorexia nervosa (AN) is a life-threatening mental disorder characterized by self-imposed food restriction, an intense fear of weight gain, and a distorted body image.[1]. Worldwide the prevalence of AN was 0.9% of women and 0.3% of men [2].

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While AN was traditionally considered a disorder of Western cultures, globalization, and media influence has increased its incidence in non-Western countries, including Pakistan. Mostly adolescent girls and young women with age of onset between 15 and 24 years are diagnosed with it [3] [4]. Despite limited data in Pakistan on eating disorders, anecdotal evidence and smaller sample size studies reported an increasing incidence of AN, determined by cultural norms/ideals of thinness and social pressures.

Among the many factors contributing to its pathophysiology, the immune system has gained increasing attention. Many Research/studies have reported the immune system's responsible among the many factors contributing to its pathophysiology [5]. The cytokines (pro-inflammatory) like Interleukin-1 (IL-1) and Interleukin-15 (IL-15) are of particular

interest due to their effects on brain function, mood, and appetite regulation[6]. The function of IL-1 is known for its role in initiating inflammatory responses, while IL-15 functions as regulating immune cells and maintaining tissue homeostasis [7]. These cytokines have been implicated in the neuropsychiatric manifestations of AN, including cognitive dysfunction and altered mood states.[8].

The rationale of this study was to explore the biological underpinnings of AN, particularly the role of IL-1 and IL-15 in provoking its symptoms. By identifying these cytokines' contributions, we hope to uncover potential therapeutic targets that can lead to better patient outcomes.

METHODOLOGY

Study design: The study was descriptive-analytic, conducted over a year from March 2023 to April 2024 at RMI and HMC Medical and Psychiatry OPDs. Ethical approval was issued by IREB MTI HMC via NO. 2048. A total of 50 young adults diagnosed with AN were enrolled and compared with 50 healthy controls matched for age and BMI.

Sample Size Calculation: Based on previous studies that show a significant difference in cytokine levels between anorexia nervosa patients and controls. we calculated the sample size using a 95% confidence interval and 80% power. The effect size for IL-1 and IL-15 differences was assumed to be medium. To account for dropouts, 50 participants were enrolled in each group. Based on the formula, the calculated sample size was 45 participants per group. To account for possible dropouts, a final sample size of 50 patients with anorexia nervosa and 50 control participants was selected.

Ethical approval was obtained from the Institutional Review Board (IRB) of RMI and HMC. All participants provided written informed consent before participation. Confidentiality and anonymity were maintained throughout the study.

Inclusion Criteria were young adults aged 15-30 years, diagnosed with AN, with a BMI of less than 18.5. Control participants were required to have the same age range and BMI but no history of eating disorders.

Exclusion Criteria Participants with chronic illnesses, pregnancy, or other psychiatric disorders that might influence cytokine levels were excluded.

The data collection was obtained demographic data, clinical characteristics, and medical history were recorded for all participants.

Cytokine Analysis: Blood samples were collected for the analysis of IL-1 and IL-15 levels using ELISA. BMI was calculated as weight (kg) divided by height (m^2). The clinical characteristics recorded included hematological parameters, liver function tests, electrolyte levels, and psychiatric symptoms (anxiety, depression, and cognitive dysfunction). The steps for cytokine measurement were conducted using a sandwich ELISA technique, with specific antibodies for IL-1 and IL-15.

First all collection of blood samples from patients and then centrifuging to separate the serum from it.to quantify the IL-1 and IL 15 the sandwich technique was used. The plates were coated with the A 96-well microplate with specific antibodies against IL-1 and IL-15. After that, the blocking procedure was performed in which the plate was blocked with a protein-based buffer to avoid non-specific binding. The third step includes adding the serum of both samples of AN and control groups. Fourth step, a primary antibody was added for IL-1 and IL-15 to bind the cytokines present in the samples. The fifth step includes the secondary antibody an enzyme-linked was added after washing the plate to remove unbound primary antibodies.
Sixth step the to develop color proportional to the cytokine concentration, and an enzyme substrate was added. At the end, the optical density was measured using a spectrophotometer, and cytokine concentrations were intended based on a standard curve.

Statistical Analysis: Data were analyzed using SPSS v25. independent t-tests were performed to compare cytokine levels between groups. Pearson correlation assessed the relationship between cytokine levels, BMI, and psychiatric symptoms. A p-value <0.05 was considered significant.

RESULTS

Table 1 shows that there is no significant difference between the groups of age, gender distribution, and BMI.

Table 1: Demographic Characteristics of Participants

Characteristic	AN Group (n=50)	Control Group (n=50)	p-value
Mean Age (years)	23.5 ± 3.2	23.8 ± 3.4	0.56
Sex (Males/Females)	10/40	12/38	0.72
Mean BMI (kg/m²)	17.2 ± 0.5	17.6 ± 0.7	0.47

Table 2: Clinical Characteristics of Participants

Clinical Characteristic	AN Group (n=50)	Control Group (n=50)	p-value
Total Leukocyte Count (TLC) (x10³/µL)	11.5 ± 1.4	6.2 ± 0.8	<0.01
Hemoglobin (g/dL)	10.3 ± 0.9	13.5 ± 1.1	<0.01
Platelet Count (x10³/µL)	170 ± 15	240 ± 18	<0.01
Albumin (g/dL)	3.1 ± 0.4	4.2 ± 0.3	<0.01
Serum Sodium (Na+) (mmol/L)	133.5 ± 3.6	138.7 ± 2.1	<0.01
Serum Potassium (K+) (mmol/L)	3.2 ± 0.6	4.0 ± 0.4	<0.01
Aspartate Aminotransferase (AST) (IU/L)	42.6 ± 6.8	22.7 ± 5.3	<0.01
Alanine Aminotransferase (ALT) (IU/L)	38.1 ± 7.3	19.6 ± 4.9	<0.01
Frequency of Infections (episodes/year)	3.2 ± 0.7	0.8 ± 0.4	<0.01
Fatigue Severity Score (0-10 scale)	7.5 ± 1.2	2.1 ± 0.8	<0.01
Amenorrhea (%)	65%	5%	<0.01

Table 2 there were significant differences in hematological and biochemical markers between AN and control groups

- **Platelet Count**: Lower in the AN group, indicating possible bone marrow suppression or nutritional deficiencies.
- **Albumin**: Lower in the AN group, reflecting malnutrition and poor protein status.
- **Serum Sodium and Potassium**: Lower levels suggest electrolyte imbalances commonly associated with anorexia nervosa.
- **AST/ALT Levels**: Elevated in the AN group, which may indicate liver stress or damage due to malnutrition.
- **Fatigue Severity**: Higher in the AN group, likely due to nutritional deficiencies and overall poor health status.
- **Amenorrhea**: Higher percentage in the AN group, indicative of hormonal dysregulation commonly seen in anorexia nervosa.

Table 3: Cytokine Levels in AN and Control Groups

Cytokine	AN Group (pg/mL)	Control Group (pg/mL)	p-value
Interleukin-1 (IL-1)	45.3 ± 5.2	23.4 ± 4.1	<0.01
Interleukin-15 (IL-15)	37.8 ± 4.9	19.2 ± 3.7	<0.01

Table 3 demonstrates significantly higher levels of IL-1 and IL-15 in the AN group compared to controls. indicating a potential role in the pathophysiology of AN.

Table 4: Correlation of Cytokine Levels with Psychiatric Symptoms in AN Group

Variable	IL-1 Correlation Coefficient (r)	IL-15 Correlation Coefficient (r)	p-value
Anxiety Severity	0.56	0.49	<0.01
Depression Severity	0.52	0.47	<0.01
Cognitive Dysfunction	0.48	0.54	<0.05

Table 4 shows a significant positive correlation between cytokine levels (IL-1 and IL-15) and psychiatric symptoms such as anxiety, depression, and cognitive dysfunction in the AN group.

There was a significant difference between the AN group and the control group of cytokine levels and clinical parameters.

The levels of cytokines IL-1 and IL-15 in AN group had significantly increased in individuals than in healthy controls. Our study, the levels of cytokines suggest that an inflammatory response may be certain to the primary mechanisms of AN. The clinical parameters of AN participant's total leukocyte count TLC, had a significantly increased along with levels of hemoglobin and platelet were decreased. Furthermore, this group showed lower serum sodium, potassium, and albumin concentrations, demonstrating electrolyte disturbances and malnutrition. Liver enzymes AST and ALT were considerably high, signaling potential liver strain associated with poor nutritional status.

In the AN group, the health symptoms include frequent infections and reported higher levels of fatigue, linking these health issues to the increased inflammatory response suggested by increased levels of cytokines.

There were significant differences between both groups ($p < 0.01$) across all measured parameters. The higher cytokine levels were associated with more severe psychiatric and medical symptoms in the AN group. These findings suggest that IL-1 and IL-15 contribute to the aggravation of anorexia nervosa symptoms, highlighting the need for therapeutic approaches that integrate both physiological and psychological interventions.

DISCUSSION

In our research, the role of interleukin-1 (IL-1) and interleukin-15 (IL-15) in anorexia nervosa (AN) supports both international and national research, yet also provides exceptional insights, especially in terms of gender diversity and the interplay of inflammation in AN patient with low BMI.

Our research reported, increased levels of IL-1 in AN patients, following international studies like that of Nilsson et al. (2021), which reported, increased IL-1levels in patients with AN [9]. Both studies highlight IL-1 plays a significant role in inflammation and its role in altered neuroendocrine pathways disturbing appetite regulation. Chronic inflammation acts as a driver and consequence of AN, emphasizing a vicious cycle of inflammation and eating disorders.

In accordance with our finding of IL-15, Roczniak et al. (2020) report significantly higher levels of IL-15 in girls with AN than to healthy controls. These studies emphasize the IL-15 role in reducing adipose tissue while sustaining lean mass, indicating its importance in the catabolic processes in AN [10]. In our study, the increased IL-15 levels support the hypothesis that this cytokine plays a main role in modulating energy balance and contributing to anorexic behavior.

The difference between our and the above study was that Roczniak's research also investigated metabolic markers such as lipid profiles and insulin resistance IR while these studies reported increased IL-15. Levels, but Roczniak reported that IL-15 was related with abnormal lipid metabolism, a variable that our study did not explore. The future research in our study could be enhanced by including metabolic indicators to gain a broader understanding of IL-15's role in inflammation as well as in the metabolic values of AN [10]. Another study conducted by Himmerich et al (2021) showed that IL-1 and IL-15 were increased significantly in patients with AN than in healthy subjects [11]. In contrast to our study, Dalton et al., (2019) showed no significant difference in IL-15 levels between those with AN compared to healthy subjects. Their study also reported that the comparison of patients with AN before and after weight gain showed no significance in IL-15 before and after weight gain. Also the comparison between patients with AN after-weight gain and healthy subjects showed no significance in IL-15 in those with AN following weight gain compared to healthy subjects [12].

international studies mostly emphasize female populations [7], a study conducted by Specht et al. (2022), a total of 22 female adolescents with AN also at an early stage of their illness. The authors felt studying adolescents with shorter illness duration and fewer confounding factors may help to elucidate the role of inflammation in the pathophysiology of AN [13]. our study expands the scope by including both male and female participants, making our research more inclusive. This broader approach provides a more comprehensive understanding of IL-1 and IL-15's roles across genders, addressing a gap in much of the existing international literature.

Our findings resonate with national studies,

such as the one reported by Zahra et al. (2023), which similarly reported elevated levels of IL-1 and IL-15 in AN patients [14]. These studies confirm the involvement of inflammatory pathways in AN within the Pakistani population. Zainab et al reported in (2023) conducted in Karachi the link between body dissatisfaction and eating pathology and their fundamental mediators in the chain. Adolescents with disturbed eating, along with body image issues and the fear of negative evaluation and negative effects, should also be acknowledged by the mental health practitioner to design effective treatment plans [15].The consistency between our findings and this local study reinforces the relevance of cytokine dysregulation in AN globally, yet also emphasizes the importance of studying these mechanisms in specific cultural and healthcare contexts.

National research has tended to focus almost exclusively on female populations, reflecting the higher prevalence of AN among women [16]. However, by including males in our study, we provide new insights into how these cytokines behave across genders in AN patients. The inclusion of males broadens the understanding of how IL-1 and IL-15 contribute to AN in a more diverse population, offering valuable data that challenges the traditionally female-centric focus in AN research within Pakistan. The future national studies to implement more broad methodologies to better understand gender differences in cytokine response.

Our study supports international and national research by confirming the significant roles of IL-1 and IL-15 in the inflammatory processes underlying anorexia nervosa. However, by incorporating both genders and additional physiological markers, our research contributes new scopes to the understanding of AN, advocating for more general methods in future studies that consider metabolic, inflammatory, and gender-related variables. The elevation of these cytokines in AN proposes their potential as biomarkers and treatment targets, demanding further surveys in larger and more diverse populations.

CONCLUSION

This study demonstrates that IL-1 and IL-15 contribute to the aggravation of AN symptoms by disrupting hypothalamic function and neurotransmitter balance. The findings suggest complex interaction between the immune system and neuropsychiatric mechanisms in AN. Targeting these cytokines, alongside psychological treatments, could offer new therapeutic avenues. Further research is necessary to explore the immune system's role in AN, potentially leading to improved treatments for this debilitating disorder.

Author contributions: Author 1: Conceptualized the research idea, designed the study, and performed data analysis. Additionally, [Author 1] wrote the first draft of the manuscript and contributed to interpretation of results.
Author 2: Played a key role in data collection and lab work, including sample preparation and cytokine level measurements. Author 2 also contributed to the methodology and reviewed the manuscript for scientific accuracy. Supervised the project, provided critical. revisions, and contributed to editing the manuscript. was also responsible for overseeing the final version of the manuscript. Other co-authors also helped me in consent taking, counselling of the patients, BMI calculation, data collection and preserving the blood samples.

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