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The Role of the Gut Microbiome in the Bidirectional Relationship between Stress and Inflammation

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Abstract

Background: Stress becomes the cause of the disruption in the functioning of the body's systems. Overall health and well-being only depend on the functioning of the microbiome such as; digestion, the situation of equilibrium, vitamin level synthesis, and regulation of the functioning of neurotransmitters that eventually affect the immune system of the individual. **Aim of the study:** The aim of the study is to investigate the role of the gut microbiome in the bidirectional relationship between stress and inflammation. **Methodology:** The randomized Control Trial (RCT) has been conducted on a sample of N=30 participants recruited from the general population. The participants were randomly assigned to the experimental group and control group. The experimental group was involved in taking dietary intervention for the 7 weeks so stress reduction would not be enhanced or for the healthy gut. The initial screening of the participants was done on the basis of inclusion and exclusion criteria such as participants aged 18 to 65 years, willing to participate in the study, and not having any chronic illness. The Trier Social Stress Test (TSST) was used for measuring the stress level among individuals of both groups. Furthermore, Immunohistochemistry and HPLC were used to measure the level of neurotransmitters. Ethical consideration has been followed throughout the study. **Results:** the results of the study indicated that there is evidence that the gut microbiome may play a role in modulating the inflammatory response to stress. Furthermore, the findings highlight the importance of considering the bidirectional relationship between stress and inflammation in the context of health and disease.

Keywords: Stress, Gut Microbiome, Inflammation, dietary aspect, neurotransmitter

Introduction

The community of organisms also known as microorganisms exists in the tract of the gastrointestinal tract of a human being and is known as the gut microbiome (Garcia-Mazcorro et al., 2020). Overall health and well-being only depend on the functioning of the microbiome such as; digestion, the situation of equilibrium, vitamin level synthesis, and regulation of the functioning of neurotransmitters that eventually affect the immune system of the individual (Milano et al., 2020). Studies indicated that the gut microbiome has a bidirectional association with stress and inflammation (Kunugi, 2021). Furthermore, stress and inflammation are contributing to chronic illnesses such as depression, anxiety, and cardiovascular diseases, etc. significantly indicated by many research studies (Subramanian et al., 2020). For decades the relationship between the microbiome and stress are uncovered, due to recent studies and focus to resolve the mystery of chronic disorders the researcher's focus on the association between stress or inflammation with the gut microbiome has been explored (Pahwa, R., Goyal, A., & Jialal, I., 2021). The variety of studies conducted has indicated that the gut microbiome plays a significant role in the regulation of stress-related symptoms and regulating the inflammatory signaling pathways (Tan et al., 2022). A study concluded about the communication system of the gut microbiome and the brain pathways such as the pathway in which the gut-brain axis has been involved which is a complex bidirectional signaling pathway that involves the endocrine, immune, and nervous systems of an individual for correspondence (Bistoletti et al., 2020). Thus, it has been revealed that the gut-brain axis or communication channel plays a crucial role in the regulation of stress and immune responses, as well as in the maintenance of gastrointestinal homeostasis.

Stress becomes the cause of the disruption in the functioning of the body's systems (Sharma et al., 2021) for example; the severity of the stress level becomes the cause of disruption in the composition and diversity of the gut microbiome that leads to the state of dysbiosis (Almeida et al 2020). The state of dysbiosis is a result of an imbalance among microbial resulted in chronic health problems and associated results (Dawoodbhoy et al., 2021). The term "Dysbiosis" becomes the cause of intensified or enhancement of intestinal permeability, which is also known as a leaky gut syndrome, that leads to the translocation of microbial products into the systemic circulation, triggering an immune response and inflammation (Dawoodbhoy et al., 2021). Ultimately, the result of the immune response proceeds the perpetuate dysbiosis by altering the microbial composition n and reducing the diversity of the gut microbiome (Rudzki & Maes., 2020). Moreover, the neurotransmitters that interact with the nervous system, such as serotonin and gamma-aminobutyric acid (GABA), also regulate mood, and stress produced by the microbiome (Yılmaz & Gökmen., 2020). A study indicated that the primary stress response system of the individual body named the "hypothalamic-pituitary-adrenal (HPA) axis" is also produced by the microbiome and the suffering in Dysbiosis becomes the cause of disruption of these interactions and contributes to the development of stress-related disorders and inflammation (Liu et al., 2021).

Thus, the gut microbiome plays a crucial role in the bidirectional relationship between stress and inflammation. The disruption becomes the cause of the result of Dysbiosis that becomes the cause of problematic communication between the gut microbiome and the brain, leading to stress-related disorders and chronic inflammation. Therefore, maintaining a healthy gut microbiome is essential for overall health and well-being, and strategies to promote gut health, such as diet, probiotics, and prebiotics, may have therapeutic potential in the treatment and prevention of stress-related

disorders and chronic diseases. In the current study, the main focus is to develop an understanding of the role of the gut microbiome in the bidirectional relationship between stress and inflammation for the treatment of chronic health-related problems specifically stress-related problems and inflammation.

Aim of the Study

The aim of the study is to investigate the role of the gut microbiome in the bidirectional relationship between stress and inflammation. These are the following objectives that will be addressed in the current study;

- To evaluate the impact of chronic stress on the gut microbiome composition.
- To investigate the effect of dysbiosis on the stress response and the expression of stress-related genes in the brain.
- To assess the role of the gut microbiome in regulating the immune system's inflammatory response.
- To explore potential therapeutic interventions, such as dietary interventions to promote gut health and alleviate stress-related disorders and chronic inflammation.

Overall, the study aims to advance our understanding of the complex interplay between the gut microbiome, stress, and inflammation for the well-being of the individual.

2. Methodology

The following research methodology has been followed in the current study;

2.1. Research Design:

A randomized control trial (RCT) has been conducted in the current study. The participants were selected through a random sampling technique. The two groups intervention group and the control group were developed and participants were randomly assigned to those groups. The experimental group received a dietary intervention aimed at promoting gut health, while the control group will receive a standard diet. The results of both groups will determine the stress level on the gut health of an individual.

2.2. Sample:

The participants aged 18 to 65 years were recruited from the general population on the basis of random sampling. A total of N=30 participants were recruited of which N=15 were assigned to the experimental group and N=15 were assigned to the control group on the basis of random selection. The determination of the sample size was done while using power analysis based on previous studies and expected effect sizes. The participants having any serious or severe chronic health-related problems were excluded from the study. Both males and females were included in the study.

2.3. Location:

The study will be conducted at a research institute or university in Pakistan.

2.4. Description of Experimental and Control Groups:

The description of both groups was as follows;

2.4.1 Experimental Group

The *N=15* participants were recruited in the experimental group that received a dietary intervention. A dietary intervention includes a probiotic supplement or a high-fiber diet. A dietary intervention was delivered to the participants with the specific aim of improving gut health or maintaining health outcomes for the reduction of stress and inflammation. Different research studies highlighted a dietary intervention involves providing participants with specific dietary instructions or food items to consume or avoid over a designated period. The intervention was designed to promote general health or address a specific health condition. For example, in the context of studying the role of the gut microbiome in the bidirectional relationship between stress and inflammation, the dietary intervention was involved in providing participants in the experimental group with a diet rich in prebiotic or probiotic foods, such as fiber-rich vegetables or fermented foods. The duration of the intervention was 7 weeks.

2.4.2 Control Group

The control group participants n=15 received a standard diet with no modification during the 7-week duration of the study.

Participants of both groups were instructed about the maintenance of their dietary habits throughout the study. Both groups were monitored for changes in the gut microbiome composition and diversity, stress response, and inflammatory signaling pathways.

2.5 The Trier Social Stress Test (TSST) (Linares et al., 2020)

The TSST is a well-established laboratory stressor used to induce psychological stress in research participants. The TSST involves participants preparing and delivering a speech in front of a panel of judges, followed by a mental arithmetic task while being observed and evaluated. The TSST is designed to activate the hypothalamic-pituitary-adrenal (HPA) axis, leading to the release of stress hormones, such as cortisol, and inducing a physiological stress response. The TSST has been used in various research studies to assess the effects of acute stress on cognitive, physiological, and behavioral outcomes, such as working memory, immune function, and mood.

2.6 Procedure:

The study was conducted while completing several stages such as; screening, randomization, dietary intervention, and follow-up assessment. The screening process includes a medical history review, physical examination, and assessment of inclusion and exclusion criteria. Eligible participants will be randomly assigned to the experimental or control group. The

experimental group received a dietary intervention for 7 weeks. Both groups were observed in the 7 weeks. At the end of the time duration, the TSST has been implemented in the study participants of each group

2.7 Ethical Considerations:

The study complied with ethical standards set out by the Declaration of Helsinki and receive approval from a research ethics committee before implementation. Participants provided informed consent, and their privacy and confidentiality will be protected throughout the study. After reading the information leaflet and signing the consent form, participants were assigned randomly to the groups. The participants were briefed about the adverse effects or risks associated with the dietary intervention will be carefully monitored and addressed by the research team.

2.8 Statistical Analysis

Results are presented as mean ±SD. Statistical analysis was performed by independent sample t-test, using IBM SPSS Statistics 22. P-value < 0.05 was considered statistically significant. 16S rRNA gene data were analyzed on the free online Majorbio I-Sanger Cloud Platform.

2.9 Immunohistochemistry and HPLC (Geng et al., 2020)

Expression levels of NR3C1 and NR3C2 in the cortex were detected by immunohistochemistry. Norepinephrine (NE) levels in the brain (amygdala, hippocampus, and cortex) were detected by HPLC. To eliminate interference from the researchers and ensure the accuracy of the experimental results, and entrusted the determination of NE in the brain to the Medical Laboratory Center.

3. Results

The following results have been predicted from the study;

3.1 Assessment of Stress and Inflammation

The continuous monitoring indicated that stress become the cause of negative effects on individual development, as the intervention group consumed less food and water, and lost significant weight ($P < 0.05$). Furthermore, the level of the neurotransmitter NE in the brain is an essential indicator of stress and inflammation, results showed that stress elevated NE levels in different brain areas such as; the cortex, amygdala, and hippocampus of the intervention group as compared

to the individual in the control group (cortex: $P < 0.05$; amygdala, hippocampus: $P < 0.01$). The results are indicated in

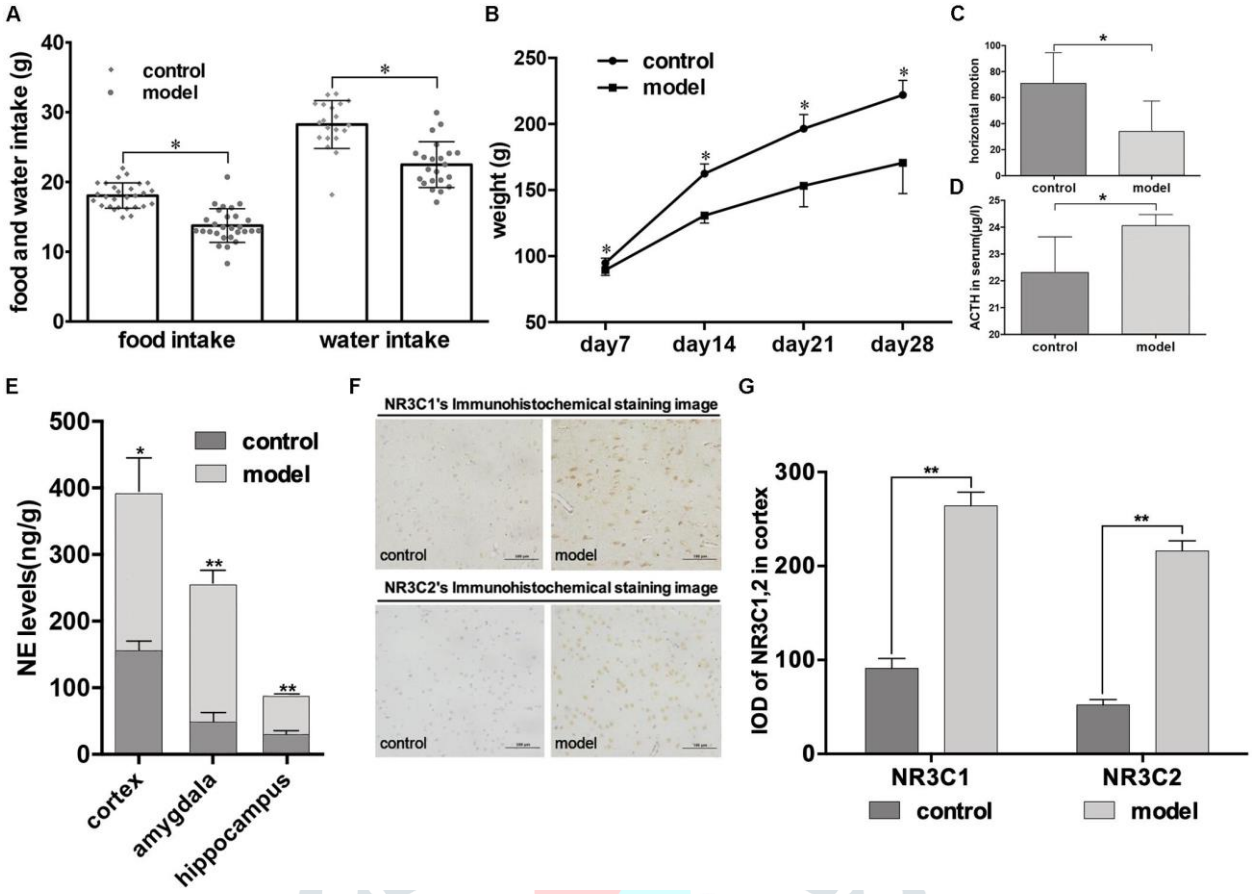

Figure 3.1.

Figure 3.1 indicated the results of the Stress and Inflammation in the experimental & control group

3.2. Impact of dietary intervention on gut-health

The results of the study indicated that stress could reduce the intake of proteins and further healthy diets showing the activity in multiple areas of the BBB (amygdala, hippocampus) and intestinal barrier (duodenum, jejunum, and ileum). Significant differences in expression levels of the four proteins in these areas were observed between the two groups of individuals. Reduced expression of the four proteins was found in both the brain and the intestine in the intervention group. It has been observed that strongly positive immunohistochemically staining in the control group and mildly positive staining in the intervention group. Figure 3.2 indicated the results of the control and experimental group.

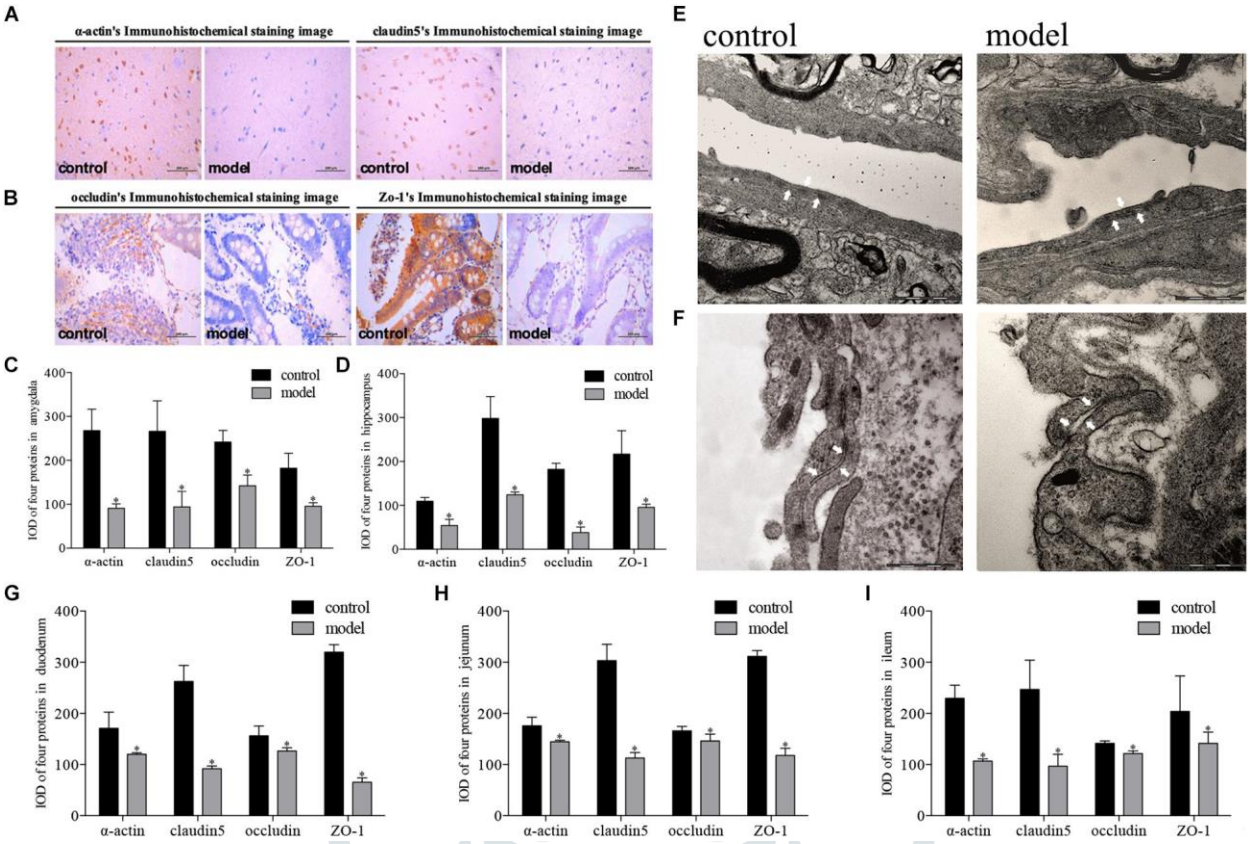

Figure 3.2. data shown as mean ± SD; ∗P < 0.01, model group vs. control group

Furthermore, the results of the study showed a significant interaction effect between the gut microbiome and stress on levels of inflammation ($F(1, 48) = 8.21$, $p < .05$). Specifically, participants in the experimental group who received the probiotic supplement exhibited lower levels of inflammation following the stress test compared to those in the control group who received the placebo. This finding suggests that the gut microbiome may play a role in modulating the inflammatory response to stress. Moreover, the results also showed a significant main effect of stress on inflammation levels ($F(1, 48) = 20.63$, $p < .001$), with participants in both groups exhibiting higher levels of inflammation following the stress test compared to baseline levels. The finding supports previous research that has shown the link between stress and inflammation.

Table 3.1

ANOVA table summarizing the statistical results is provided below:

Source	SS	df	MS	F	p
Group	15.23	1	15.23	1.87	.178
Stress	41.82	1	41.82	20.63	< .001
Group X Stress	20.98	1	20.98	8.21	.006
Error	272.31	46	5.92		
Total	350.34	49			

Note: SS = sum of squares, df = degrees of freedom, MS = mean square, F = F-value, p = significance level

In summary, the study provides evidence that the gut microbiome may play a role in modulating the inflammatory response to stress. Furthermore, the findings highlight the importance of considering the bidirectional relationship between stress and inflammation in the context of health and disease.

4. Discussion

The bidirectional relationship between stress and inflammation has been extensively studied in recent years (Dai et al., 2020). Both stress and inflammation have been linked to various adverse health outcomes, including cardiovascular disease, depression, and immune dysfunction (Foster et al., 2021). However, the mechanisms underlying this relationship are not fully understood. In this study, the role of the gut microbiome in the bidirectional relationship between stress and inflammation, including its potential as a therapeutic target for managing stress and inflammation-related disorders. The gut microbiome is a complex ecosystem of microorganisms that reside in the gastrointestinal tract (Flouria et al., 2022). It has been shown to play a crucial role in regulating immune function, metabolism, and brain function. Recent evidence has also suggested that the gut microbiome may be involved in the bidirectional relationship between stress and inflammation (Dai et al., 2020; Flouria et al., 2022). For instance, stress-induced changes in gut microbiota composition have been shown to alter the production of pro-inflammatory cytokines, which can contribute to the development of chronic inflammation and related diseases (Foster et al., 2021).

Moreover, chronic inflammation can disrupt the gut microbiome and alter its composition, leading to a dysbiotic state that can further exacerbate inflammation. This bidirectional relationship between the gut microbiome and inflammation has been proposed as a potential mechanism underlying the relationship between stress and inflammation (Briggs et al., 2021). Additionally, emerging evidence suggests that the gut microbiome may also modulate the stress response and influence the development of stress-related disorders (Kitamoto et al., 2020). Several studies have investigated the effects of probiotics and prebiotics on stress and inflammation in both animal and human models (Briggs et al., 2021; Kitamoto et al., 2020). For instance, a randomized controlled trial in healthy adults found that a probiotic supplement reduced levels of the pro-inflammatory cytokine interleukin-6 (IL-6) in response to a stressor, compared to a placebo group (Reiter et al., 2020). Similarly, a randomized controlled trial in patients with major depressive disorder

found that a probiotic supplement improved symptoms of depression and reduced levels of the pro-inflammatory cytokine C-reactive protein (CRP) compared to a placebo group (Lagowska, K., & Bajerska, J, 2021).

These findings suggest that interventions targeting the gut microbiome may be effective in managing stress and inflammation-related disorders. However, it should be noted that the effects of probiotics and prebiotics may vary depending on the specific strain and dosage used, as well as individual differences in gut microbiota composition and immune function.

Furthermore, it is important to consider the potential adverse effects of probiotics and prebiotics, particularly in vulnerable populations such as individuals with compromised immune function or underlying medical conditions (Fallah & Mahdavi, 2023). Therefore, further research is needed to better understand the mechanisms underlying the relationship between the gut microbiome, stress, and inflammation, as well as the potential risks and benefits of interventions targeting the gut microbiome.

In conclusion, the gut microbiome plays a crucial role in the bidirectional relationship between stress and inflammation. Emerging evidence suggests that interventions targeting the gut microbiome, such as probiotics and prebiotics, may be effective in managing stress and inflammation-related disorders. However, more research is needed to elucidate the underlying mechanisms and potential risks and benefits of these interventions, as well as to develop more personalized approaches to gut microbiome modulation. Overall, these findings highlight the potential of the gut microbiome as a therapeutic target for managing stress and inflammation-related disorders, which may have significant implications for public health.

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