https://doi.org/10.29309/TPMJ/2023.30.10.7675

The Professional Medical Journal
www.theprofesional.com
2023, Volume, 30 Issue, 10

ORIGINAL ARTICLE

Carbon Dioxide – Induced Anxiety; Effects of Carbon Dioxide on Behavior, Physiology, and Biochemistry in Patients with Panic Disorders and Healthy Subjects.

Fazeelat Hajra Karim[1], Muhammad Razaq[2], Fouzia Qadir[3], Ali Ahsan Mufti[4], Sikandar Ali Khan[5]

Article Citation: Karim FH, Razaq M, Qadir F, Mufti AA, Khan SA. Carbon Dioxide – Induced Anxiety; Effects of Carbon Dioxide on Behavior, Physiology, and Biochemistry in Patients with Panic Disorders and Healthy Subjects. Professional Med J 2023; 30(10):1339-1345. https://doi.org/10.29309/TPMJ/2023.30.10.7675

ABSTRACT… Objective: To investigate the processes that make people with panic disorder more vulnerable to the anxiety brought on by exposure to carbon monoxide. **Study Design:** Randomized, Cross-over Design. **Setting:** Hayatabad Medical Complex and Khyber Girls Medical College. **Period:** April 2022 to December 2022. **Material & Methods:** Patients with anxiety-related conditions and volunteers in good health who underwent CO2 testing were included in the research. Various gas mixtures, including air, 5% CO2, and 7.5% CO2, were administered to participants in a random order. Behavioral measures, physiological parameters, and biochemical markers were assessed during and after CO2 inhalation. **Results:** Panic episodes were elicited in both patients with panic disorder and healthy controls by inhaling 5% CO2. Panic disorder patients experienced significantly higher anxiety, nervousness, dread, and sadness levels during 5% CO2 inhalation compared to healthy subjects. Physiological effects, such as increased pulse rate, contrasted those with panic disorder with those without it. No significant changes were seen in either group as a result of inhaling CO2 with respect to bloodstream levels of "free 3-methoxy-4-hydroxyphenylglycol, cortisol, growth hormone, or prolactin". **Conclusion:** The inhaling of CO2 may trigger panic episodes and cause more intense behavioral and physicological reactions in those with panic disorder.

Key words: Anxiety, Behavior, Carbon Dioxide, Inhalation, Panic Disorders, Panic Attacks, Sensitivity.

INTRODUCTION

Anxiety can be brought on by carbon dioxide inhalation in both nervous individuals and healthy persons.[1] In healthy persons, the anxiogenic concentration range is between 6% and 13% for inhalation times ranging from two to twenty minutes.[2] Even when exposure to higher levels of CO2 is only restricted to a few inhalations, it is anxiogenic. The anxiogenic effects of C02 inhalation may be more sensitive in patients with panic anxiety disorders.[3] In two of three recent patient trials, it is shown that individuals with panic disorders are more likely than healthy participants to experience an increase in anxiety when exposed to CO2.[4] In Pakistan, the mean overall prevalence of anxiety and depression based on community samples is 33.62%, with a point prevalence of 45.5 % in women and 21.7% in men.[5]

Unknown are the processes by which CO2 causes anxiety as well as the reasons underpinning people with panic disorders' behavioral sensitivity to CO2.[6] These methods may entail C02 stimulating noradrenergic neural activity, according to a theory.[7] Carbon dioxide increases norepinephrine turnover and brain catecholamine synthesis in rats, as well as the rate at which neurons fire in the locus ceruleus noradrenergic nucleus of rats and "3-methoxy-4-hydroxyphenylglycol" levels in the plasma of rhesus monkeys, which is a metabolite of norepinephrine[8] Patients with PD always begin a description of their disorder by reporting symptoms related to breathing, the heart, the digestive tract and 'nerves.[9,10] Due to the somatic symptoms, 90% of patients with PD believe they have a physical condition instead of a mental disorder. Therefore, proper diagnosis and management of these patients is important

1. MBBS, M.Phil (Physiology), Lecture Physiology, Khyber Girls Medical College, Peshawar.
2. MBBS, M.Phil (Biochemistry), Assistant Professor Biochemistry, Jinnah Medical College, Peshawar.
3. MBBS, Master of Science and Molecular Medicine, Associate Professor Biochemistry, North West School of Medicine, Peshawar.
4. MBBS, FCPS (Psychiatric) HOD and Associate Professor, Jinnah Medical College, Peshawar.
5. MBBS, M.Phil (Biochemistry), Lecturer Biochemistry, Khyber Girls Medical College, Peshawar.

Correspondence Address:
Dr. Ali Ahsan Mufti
Jinnah Medical College, Peshawar.
Consultant Psychiatrist Ibadat Hospital &
Horizon NGO for Mental Health
aliahsanmufti@gmail.com

Article received on: 07/06/2023
Accepted for publication: 11/08/2023

for mental health professionals and general physicians alike.[11]

The underlying causes of anxiety disorders may be illuminated by investigating the mechanisms behind C02's differently anxiogenic and anxiogenic effects in people with panic disorders.[11] The goals of the current study were twofold: first, to confirm that patients with panic disorders are more vulnerable to the anxiogenic effects of CO2 compared to sound subjects; and second, to ascertain whether the consequences seen in these individuals at fewer carbon dioxide proportions are also present in healthy people at higher levels. Another goal was to discover how C02's noradrenergic actions contribute to its anxiety-inducing properties. Plasma methylhydroxyphenylglycine will employed as a surrogate marker for central and peripheral noradrenergic turnover. we suggest that the reported variability in CO2-induced panic rates across studies might, in part, be due to differences on important methodological parameters, including the panic provocation method, the approach taken for panic assessment, and the criteria used to define panic attacks. We argue that the lack of methodological standardization in studies using CO2 panic challenge reduces substantially the advances in our understanding of PD that these studies might provide.

MATERIAL & METHODS

The study objective was to investigate the processes that make people with panic disorder more vulnerable to the anxiety brought on by exposure to carbon monoxide. The study design was randomized, cross-over design in Hayatabad Medical Complex and Khyber Girls Medical College from April 2022 to December 2022 after approval from ethical committee 78-N(10-4-22). All participants in this study provided written informed permission before beginning. Patients themselves or were referred by experts who had heard about the therapy program via word-of-mouth or had read about it in the local press.

Diagnosis of severe depression and obsessive-compulsive disorder. In none of the individuals with comorbid conditions were the panic attacks limited to depressive episodes or primarily triggered by obsessive-compulsive symptoms. All of the patients had good overall health. Physical exam, electrocardiogram, and lab tests for haematology, hepatic, renal, and thyroid function all returned normal results in each patient. The mean age of patient was 35.8 1.5 years.

All participants in the research started weekly group psychotherapy after they were enrolled. The C02 testing was done while receiving a placebo for the first time. The inclusion criteria were prior to their first test day, all patients but two had maintained a drug-free lifestyle for a minimum of five weeks in a row. Both patients had gone three and two weeks without using any drugs. Throughout the trial, patients were asked not to take any additional psychotropic medicines. Exclusion criteria was patients who had chronic illness, and are on medication.

Advertisements and recommendations from other healthy individuals were used to find healthy volunteers. Each participant provided written informed permission to participate in the study. Based on a psychiatric interview, inclusion criteria were determined that the healthy participants had no mental disorders, and no one in their immediate family has ever been diagnosed with a mental disorder. The healthy volunteers had similar health screening results to the patients and reported no use of psychoactive drugs in the four weeks prior to screening.

The Clinical Neuroscience Research Unit was used for the C02 testing. Patients received one session of 5% C02 testing and one session of placebo testing.[1] Test sessions with air, 5% CO2, and 7.5% CO2 were performed on healthy volunteers. The testing sessions occurred on different days and in a jumbled schedule, and the administration instructions were similarly chaotic.

The used gas mixtures included air (0.03% CO2, 21.1% oxygen, and 79.1% nitrogen), 5% C02 (5% CO2, 21.1% oxygen, and 74% nitrogen), and 7.5% C02 (7% CO2, 21.1% oxygen, and 71.5% nitrogen)[12] The C02 mixtures were of a clinical blood gas grade manufactured specifically for

equipment calibration. The test gas mixtures were transported in a clear plastic canopy hood with a removable top and a sloppy neck seal.

The hood had a 70 L/min airflow rate, which entered via a hole behind the subject's head and exited through a port near the subject's neck. A C02 analyzer was utilized to continuously measure the percentage of CO2 in the hood, with its sample intake positioned around 13 cm in front of the subject's lips. On days with 5%, 7.5%, and air-grade 5% C02, as well as on days with 5% and 7.5% C02 with the gas that would be provided to the person, the C02 analyzer was calibrated.

Physiologic and Biochemical Methods

The individual was supine when a study nurse took their vital signs. 15 seconds were spent taking the wrist pulse. With the use of a mercury sphygmomanometer, the blood pressure (BP) was measured. The individual was allowed to lie still for one minute while breaths were softly counted.

Gas chromatography-mass spectrophotometry was used to measure the plasma free MHPG concentrations. Through the use of a double-antibody radioimmunoassay, plasma growth hormone was evaluated. 45 Plasma cortisol and prolactin concentrations were measured using radioimmunoassay kits. Duplicate plasma specimens were analyzed to lessen technique variation. The specific values provided are the means of the two specific values obtained from these samples.

Behavioral Measures

Self-report measures included visual analogue scales (VASs) and the Panic Attack Symptom Scale.[13] Thirteen different emotional states, including those in Table-III and others including sadness, sleepiness, activity, and anger, were rated using the participants' VASs. The subject marked his present emotional state perpendicular to a 100 mm line at the left edge of the scales, and the distance between the two points was measured in millimeters. As a result, the scores might be between 0 (not at all) and 100 (the highest ever). Additionally, participants took the PASS, a test intended to gauge the intensity of symptoms frequently connected with panic episodes or elevated states of functional autonomy.

RESULTS

C02-Induced Panic Attacks

At 5% carbon dioxide, both patients and healthy persons experienced anxiety that was diagnostic of a panic attack "Fisher's exact test, two-taile". Patients did not have any anxiety attacks after CP.01's air session. The anxiety caused by 7.5% CO2 was severe enough to cause a panic attack in 3 out of 8 healthy volunteers (Fischer's exact test, two-tailed; no significant difference between patients breathing 5% CO2 and healthy subjects experiencing 7.5% CO2). Whether a patient suffered a panic episode had no discernible relationship to the sequence in which the test gases were administered.

Patients who panicked when exposed to C02 had a higher frequency of spontaneously occurring panic attacks. Patients with more than 2.5 occurrences per week, and even those with fewer incidents, reported C02-induced panic attacks. The subset of those who experienced C02-induced panic reported more spontaneous panic attacks per week than the control group. Results on the Clinical Global Impairment Scale, the Patient Rated Anxiety Scale, and the Hamilton Anxiety Scale were similar across panic attack sufferers and those who did not experience these symptoms. There were no significant differences between the panicking and nonpanicking groups at baseline with respect to VAS or PASS ratings, systolic or diastolic blood pressure, pulse rate, respiration rate, or the blood level of free MHPG, cortisol, growth hormone, or prolactin.

At the end of the 15-minute C02 inhalation phase, all sick and healthy subject groups showed the maximal behavioral effects of CO2, and at the second measurement, taken fifteen minutes after resuming to breathing room air, they had returned to baseline. In what follows, "during C02 inhalation" findings relate to data collected immediately after removing the hood (+15 minutes).

Healthy Subjects

Carbon dioxide caused dose-related elevations in VAS fear along with VAS indicators in the healthy individuals. Significant placebo-controlled increases in VAS anxiety were seen after inhalation of 5% C02. When taking in 7.5% carbon dioxide, VAS anxiety, nervousness, and terror showed substantial placebo-controlled increases from baseline, but relaxation and mellowness showed a significant decrease.

Patient-Healthy Subject Comparison

The ill group had behavioural effects of 5% CO2 that were larger than those experienced by the healthy patients. Patients experienced VAS anxiety, nervousness, dread, and sadness at much higher levels during 5% C02 inhalation than did healthy participants with placebo-controlled rises from baseline. Concern, anxiety, and apprehension all increased significantly from base after C02 inhalation in patients and dread, indicating that these changes were not exclusively attributable to the patients' bigger declines on the placebo day.

Physiologic Effects

In the healthy participants, carbon dioxide led to measurable dose-related elevations in these parameters. The overall sick group and the healthy patients had the same physiological effects from 5% CO2. Comparisons within the patient population showed that the panicking patients experienced higher increases in pulse rate than the nonpanicking individuals. Systolic blood pressure rose less in the nonpanicking patients compared to the healthy controls. Systolic and diastolic arterial pressures and pulse rates were similar between patients and healthy controls at the outset. There was a significant increase in the patients' first-breath rate.

Biochemical Effects

Plasma MHPG

Healthy subjects showed no change in plasma free MHPG levels in response to either concentration of carbon dioxide. The plasma free MHPG levels of the patients were not significantly affected by the administration of 5% C02. No significant C02 percentage by time interaction was found in the ANOVA comparing the effects of air and C02, whether the exposure was to 5% or 7.5% C02 in the healthy individuals, 5% or 5% in the general patient group, or 5% in either the panicking or nonpanicking patient subgroup. Figure 4 shows that the maximal MHPG increases from baseline were comparable across ill groups and healthy patients on air days, 5% C02 days, and 7.5% C02 days. At first, the levels of free MHPG in the plasma of both patients and healthy controls were similar.

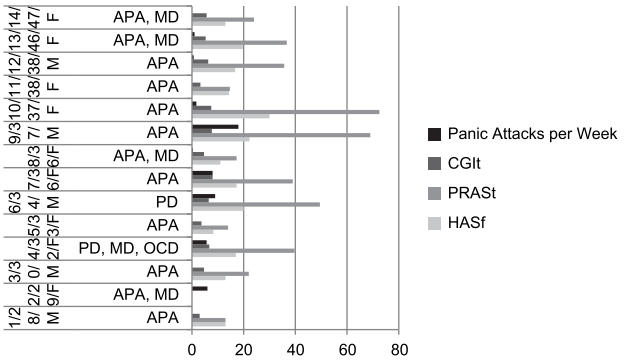

Figure-1. Patient traits and anxiety before an exam

Plasma Cortisol

7.5% of the C02 generated in the healthy participants was 5% C02 did not cause slight increases in plasma cortisol levels. The ANOVA did not find any significant differences between the outcomes of air and 5% C02. significant time-dependent interaction C2%. Significant C02 percent age by duration interactions were found in ANOVAs comparing air and 7.5% C02 and air, 5% C02, and 7.5% C02.

Growth Hormone

Human growth hormone plasma levels were unaffected by inhaling carbon dioxide at either 5% or 7.5% for 15 minutes in either healthy volunteers or patients with panic disorders. Growth hormone changes from baseline after 30 minutes of placebo treatment are seen in Fig. 5. One healthy subject's data and three patients' data were discarded since their development hormonal baseline levels were over 5 ng/mL (5 g/L) on one or more test days.

The values show the placebo-controlled change from the starting point, while the numbers

with bars show the subjects' or patients' statistics. Each group's cortisol and prolactin concentrations peaked at 30 minutes on C02 days. 30-minute measurements of growth hormone are representative of longer-term values. Asterisks denote P.10 for time-point-versus-baseline comparisons.

Rises in plasma prolactin levels caused by carbon dioxide were not dose-related, were not different between patients and healthy people, and did not distinguish between patients who were panicking and those who were not. The patient's prolactin levels weren't accessible. the highest placebo-controlled deviations from the mean (at 30 minutes in all groups). Patients' baseline prolactin values were comparable to those of healthy persons.

Patient/ Age, y/Sex	DSM-III Diagnosis	HASf	PRASt	CGIt	Panic Attacks per Week	Seq-uence	Panic With 5% CO2
1/28/M	Anxiety Disorder	13.2	13.1	2.9	0.1	A	Y
2/29/F	Anxiety Disorder, Major Depressive Disorder			6.2	5.3	Y	Y
3/30/M	Anxiety Disorder	13.4	22.1	4.8	0.2	5	Y
4/32/F	Panic Disorder, Major Depressive Disorder, Obsessive-Compulsive Disorder	17.3	39.5	6.6	5.6	5	Y
5/33/F	Anxiety Disorder	8.4	14.1	3.8	0.1	A	Y
6/34/M	Panic Disorder	20.1	49.2	6.4	9.2	5	Y
7/36/F	Anxiety Disorder	17.2	38.5	8.1	8.1	A	Y
8/36/F	Anxiety Disorder, Major Depressive Disorder	11.1	17.2	4.6	0.4	A	Y
9/37/M	Anxiety Disorder	22.5	68.8	7.8	17.9	A	Y
10/37/F	Anxiety Disorder	29.9	72.7	7.6	1.8	5	N
11/38/F	Anxiety Disorder	14.1	14.6	3.2	0.2	A	N
12/38/M	Anxiety Disorder	16.5	35.5	6.2	0.8	5	N
13/46/F	Anxiety Disorder, Major Depressive Disorder	19.5	36.5	5.2	0.9	A	N
14/47/F	Anxiety Disorder, Major Depressive Disorder	12.9	23.9	5.6	0.1	A	N

Table-I. Patient traits and anxiety before an examination

Rating	Healthy Subjects	Patients (5% CO2)
5% CO2	35±18	35±16
7.5% CO2	39±7	61±9
Total	52±8	18±20
Panicking	58±9	49±11
Nonpanicking	16±6	41±8
Anxious	9±5	35±7
Nervous	40±8	52±9
Fearful	4±3	57±10
Calm	22±5	38±6
Mellow	12±7	21±10
Happy	-6±5	14+3
High	4±6	-2±3
Depressed	9±4	17±7
Talkative	-11±5	10±5

Table-II. Relationship between subject health and anxiety during assessments

Subject/Age	y/Sex	Sequence	Stress Out At 7.5% CO2
1/19/F	A	5, 7	YES
2/20/M	5	A, 7	NO
3/20/F	A	5, 7	YES
4/22/F	5	A	
6/33/M	A	7	
7/34/F	5	A	
8/40/F	5	A	NO
9/43/F	A	5, 7	YES
10/46/F	7	5, A	NO
11/49/M	5	7, A	NO

Table-III. Analyzing the impact of carbon dioxide on behavioral in healthy individuals and panic disorder patients

DISCUSSION

The following is what we found to be true: For three reasons: (1) the physiological effects of 5% carbon dioxide are the same in patients with

panic disorder as in healthy subjects; (2) a subset of patients experience carbon dioxide-induced panic attacks, and this portion is to blame for the greater behavioral response in the entire patient group; and (3) the behavioral effects of 5% carbon dioxide in individuals with anxiety disorder are identical to the behavioral consequences of 7.5% carbon dioxide in healthy subjects. (5) Serum cortisol levels are somewhat higher during C02-induced panic episodes compared to those measured on placebo days as well as higher pulse rate rises than those seen in nonpanicking patients.[14]

The design flaw of this research is that steady-state end-inspiratory percentages were matched between patients and controls, but end-tidal (end-expiratory) percentages were not. In most cases, brain tissue CO2 concentrations and endtidal CO2 percentages are same[15] In an experiment like this one, the end-tidal CO2 percentage is mostly governed by the inspired CO2 %. However, it is also influenced by other factors, There may be differences between patients and controls in a number of aspects, including initial end-tidal CO2 pressure, the rate at which the body generates CO2[16] and the ventilatory response to CO2. It was decided against measuring the end-tidal CO2 % since doing so would have required the participants to wear a nose clip and a mouthpiece though under the hood, which might have contributed to increased anxiousness on placebo day.[17]

Finding that healthy participants exposed to 5% and 7.5% C02 breath for 15 minutes suffer dose-related rises in anxiety lends credence to the idea that this concentration and duration render C02 anxiogenic.[12] In the literature, there has been considerable debate over whether CO2 increases110 or decreases 51”6 anxiety. According to statistics on panic attacks, VAS, and somatic symptoms, Those who suffer from panic attacks are especially vulnerable to C02’s anxiety-inducing properties.

Individuals experiencing anxiety-related conditions may be classified into two groups, one of which is more anxiogenic to carbon dioxide than the other. Disparities between patients and healthy subjects in C02’s effects on anxiety and somatic symptoms were driven by those patients whose symptoms met the diagnostic criteria for a panic attack[18] In terms of anxiety brought on by C02 and somatic symptoms, the no panicking patients matched the healthy participants. It’s probable that the individuals who have panic attacks and those who don’t have diseases with various underlying pathologic causes[19,20] Another explanation that fits the results is that they share a problem that, at the time of testing, was more severe in the panicked group.

CONCLUSION

The panic disorders are more likely to experience anxiety in response to exposure to CO2 than people without panic disorders. The inhaling CO2 may trigger panic episodes and cause more intense behavioral and physiological reactions in those with panic disorder. No significant correlation was seen between CO2 inhalation and shifts in plasma levels of MHPG, cortisol, growth hormone, or prolactin, however. The limitation of study was to further understand the exact effects of carbon dioxide on neurotransmitter systems in panic disorder, more study is required.

REFERENCES

1. Savulich G, Hezemans FH, van Ghesel Grothe S, Dafflon J, Schulten N, Brühl AB, et al. **Acute anxiety and autonomic arousal induced by CO2 inhalation impairs prefrontal executive functions in healthy humans.** Translational Psychiatry. 2019; 9(1):296.

2. Attwood AS, Ludwig CJ, Penton-Voak IS, Poh J, Kwong AS, Munafò MR. **Effects of state anxiety on gait: a 7.5% carbon dioxide challenge study.** Psychological Research. 2021; 85:2444-52.

3. Kyriakoulis P, Kyrios M, Nardi AE, Freire RC, Schier M. **The implications of the diving response in reducing panic symptoms.** Frontiers in Psychiatry. 2021; 12:2157.

4. Klevebrant L, Frick A. **Effects of caffeine on anxiety and panic attacks in patients with panic disorder: A systematic review and meta-analysis.** General Hospital Psychiatry. 2022; 74:22-31.

5. Khan H, Kalia S, Itrat A, Khan A, Kamal M, Khan MA, et al. **Prevalence and demographics of anxiety disorders: A snapshot from a community health centre in Pakistan.** Ann Gen Psychiatry. 2007; 6:30.

6. Kinkead R, Ambrozio-Marques D, Fournier S, Gagnon M, Guay LM. **Estrogens, age, and, neonatal stress: panic disorders and novel views on the contribution of non-medullary structures to respiratory control and CO2 responses.** Frontiers in Physiology. 2023; 14:1183933.

7. Rabipour S. **Air chemical particles and pollution quality association with mental health: A case for depression and anxiety among Iranian university students in Iranian cities with lower air quality.** Chem Rev Lett 6 (2023) 66-78.

8. Havel L, Mehta P, Gautam A, Danielyan E, Saxena K. **Neurochemistry of Childhood Anxiety Disorders.** Handbook of Child and Adolescent Anxiety Disorders: Springer; 2022. p. 55-72.

9. Province VM. **Ventilatory dynamics and exertional dyspnea during respiratory challenges in young adults with anxiety:** Appalachian State University; 2021.

10. Banushi B, Brendle M, Ragnhildstveit A, Murphy T, Moore C, Egberts J, et al. **Breathwork interventions for adults with clinically diagnosed anxiety disorders: A scoping review.** Brain Sciences. 2023; 13(2):256.

11. Tural U, Iosifescu DV. **A systematic review and network meta-analysis of carbon dioxide provocation in psychiatric disorders.** Journal of Psychiatric Research. 2021; 143:508-15.

12. Yandım MB. **Bilişsel Davranışçı Terapi ve Yeni Kuşak Bilişsel Davranışçı Terapilerde Yaygın Kaygı Bozukluğunun Tedavisi:** Fatih Sultan Mehmet Vakıf Üniversitesi, Lisansüstü Eğitim Enstitüsü; 2022.

13. Aessopos A, Karabatsos F, Farmakis D, Katsantoni A, Hatziliami A, Youssef J, et al. **Pregnancy in patients with well-treated β-thalassemia: Outcome for mothers and newborn infants.** American journal of obstetrics and gynecology. 1999; 180(2):360-5.

14. Barlow DH. **The development of an anxiety research clinic.** The Neurotic Paradox, Volume 1: Routledge; 2020. p. 122-4.

15. Klosko JS, Barlow DH, Tassinari R, Cerny JA. **A comparison of alprazolam and behavior therapy in treatment of panic disorder.** The Neurotic Paradox, Volume 1: Routledge; 2020. p. 105-21.

16. Liu K, Droncheff B, Warren SL. **Predictive utility of symptom measures in classifying anxiety and depression: A machine-learning approach.** Psychiatry research. 2022; 312:114534.

17. Ng YY. **Treatment of Generalized Anxiety Disorder (GAD): A randomized controlled trial of an internet self-help program with minimal non-therapist support:** The Chinese University of Hong Kong (Hong Kong); 2021.

18. Perwez S, Jamal R. **Comparison of anxiety and depression among epileptics and non-epileptics in Peshawar.** Clinical and Counselling Psychology Review. 2021;3(2).

19. Khan FU, Rahman Z, Rahman F, Ahmad W, Muhammad W, Nazir N, et al. **Mental health problems & its causes: A case study of University Students in Pakistan.** resmilitaris. 2023; 13(2):2852-61.

20. Rowther AA. **Power, gender, and trust in experiences of pediatric emergency physician teleconsultation and maternal antenatal anxiety in Pakistan:** Johns Hopkins University; 2022.

AUTHORSHIP AND CONTRIBUTION DECLARATION

No.	Author(s) Full Name	Contribution to the paper	Author(s) Signature
1	Fazeelat Hajra Karim	Study design, Data collection.	
2	Muhammad Razaq	Study design, Supervision, Draft preparation.	
3	Fouzia Qadir	Data collection and data analysis.	
4	Ali Ahsan Mufti	Practical work, Data collection and Writing.	
5	Sikandar Ali Khan	Practical work, Data analysis and writing.	