Association between Sexual Abuse Experience and Salivary Stress Biomarkers among Transgender and Gender Non-Conforming Individuals in Chennai – A Cross-Sectional Study

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KEYWORDS
Malondialdehyde (MDA), Interleukin-6, salivary biomarker, gender non-conforming people, Transgender health

ABSTRACT
Introduction: Gender-nonconforming people undergo unimaginable sexual and physical abuse. This leads to stress and other stress-related disorders which can be easily determined by salivary stress biomarkers. Early life stress has an association with the immune system linked with stress. Transgenders and gender nonconforming people are prone to sexual abuse in early life more compared to normal people. This study aims to know about the association between sexual and physical abuse experience and salivary stress biomarkers in Transgender and gender-nonconforming individuals.
Materials and Methods: This study was a cross-sectional epidemiological study. A snowball sampling design was adopted. The study was conducted in various places in Thozhi Shelter for Transgenders, Chetpet, Chennai among Gender non-conforming individuals and Transgenders. This study was conducted in April 2022. MDA and IL-6 levels were determined from their saliva using the passive drool method.
Result: Interleukin L 6 and MDA levels were significantly higher in the GNC Group than in the control group. 63.64% of Transgender and gender non-conforming individuals experienced sexual abuse whereas 73% of them have experienced physical abuse in their lifetime.
Conclusion: Transgender and gender non-conforming individuals who experienced physical/sexual abuse have increased IL 6 levels and decreased MDA levels in saliva when compared to the non-transgender individuals.

1. Introduction

Transgender or gender nonconforming (TGNC) people are those who's sexual orientation or gender expression does not correspond with the sex assigned to them at birth.[1] Gender identity is a deeply felt sense of being male, female, or non-binary, while sex is determined based on biological characteristics like chromosomes, hormones, and reproductive organs. With the phrases male-to-female (MTF) or female-to-male (FTM), this distinction also explains natal sex and gender identity in the medical literature. When referring to transgender and gender non-conforming individuals' person, the terms MTF and FTM are equivalent.[1,2] Despite growing visibility, gay, bisexual, members of the lesbian, transgender, intersex, queer/questioning, and asexual (LGBTQIA) communities still face prejudice and hatred in many civilizations and cultures.[1-3]

The correlations between such physical & sexual assault and documented mental health within communities of trans women are clear. A lifetime incidence of suicide behaviours is significantly correlated with occurrences of self-reported physical and sexual abuse, according to studies released in the last five years.[1-4] Similar findings were found in a recently concluded 3-year prospective study of trans women, showing substantial correlations between physical gender abuse and symptoms of serious depression.[5] According to a second study of 135 trans women, those who had experienced interpersonal trauma and discriminatory life events were 29.9% more likely to engage in self-destructive behaviour and had made 34.8% more suicide attempts.[6] These results show a reliable statistical link between physical and/or sexual abuse suffered by trans women and unfavourable psychological outcomes, even though they do not indicate causative relationships.

Research has also shown that being abused physically or sexually increases the likelihood of getting a serious mental disease, with a focus on symptoms of trauma and/or post-traumatic stress disorder (PTSD). [6,7] Additionally, among sexual minority groups, the experience of childhood maltreatment has been strongly and favourably linked to both mental diagnoses and symptoms, including perceived stress, Generalized Anxiety Disorder, depression, and PTSD. Research from the previous ten years has shown that there is a larger chance that sexual minority groups would be abused as children, which could contribute to a higher prevalence of mental illness in adults.[8] Early research on trans women revealed that, in contrast to sexual minority groups, trans women experience a disproportionate amount of reported physical and sexual violence.

Other possible mental health consequences of prior abuse have mainly been ignored, other than sadness and suicidality. Research on trauma symptoms and their effects on trans women's general functioning is still lacking, in particular.[9] The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) provides a model for trauma. It includes criteria for symptoms such as having experienced an event that was deemed to be a threat to one's life or the lives of others, intrusive symptoms (like past events, disturbing memories/thoughts, episodes of anxiety, and recurrent nightmares), avoidance, negative changes in mood and cognition, and shifts in physiological arousal.[10] In the current studies on the health of trans women, depressive symptoms and suicidal thoughts have largely taken the place of trauma-specific symptoms, especially in relation to experienced abuse.

Psychological stress, neuronal sensitivity to oxidative stress and neurotoxic peptides, are potential contributors to higher interleukin 6 levels in depression. Gained proinflammatory IL-6 activity is also linked to the hypothesis of glucocorticoid resistance-mediated depression and hyperfunction of the hypothalamic-pituitary-adrenal axis.[11] Cells in the central nervous system both produce IL-6 and exhibit its receptors. Serotonin transporter levels are regulated by interleukin 6, which also affects synaptic processes like synaptic plasticity and transmission.[12] It has been demonstrated that central infusion of IL-6 causes depressive-like behaviour and that long-term low-grade inflammation causes depression through altering brain plasticity. Anti-inflammatory medications have also been found to lessen the symptoms of depression. All of the aforementioned points to a variety of potential causes for interleukin concentration variations.[13]

A naturally occurring consequence of lipid oxidation in living organisms is malondialdehyde (MDA). Lipid oxidation happens in plant or animal cells under oxidative stress [14]. Certain fatty acids undergo oxidation and break down into a convoluted array of substances, one of which being MDA. MDA is also produced by some other biochemical reactions within the organism, such as thromboxane synthase, which can also be catalyzed, but changes in the level of lipid oxidation can be observed as long as the appropriate control is set at the time of measurement. With the help of these salivary stress biomarkers, many underlying stress disorders, diseases can be detected and it can also be associated with early life traumas due to sexual, physical and mental abuses faced by an individual, especially gender non-conforming people. This study aims to know about the association between sexual and physical abuse experience and salivary stress biomarkers in transgender and gender non-conforming individuals.

2. Materials & Methods

This study was a cross-sectional epidemiological study. A snowball sampling design was adopted. The study was conducted in various places in Thozhi Shelter for Transgenders, Chetpet, Chennai among Gender non-conforming individuals and Transgenders. This study was conducted in April 2022. All the people belonging to the LGBTQIA community who are willing to participate were included in the study and also Self-Identified Transgender/Gender non-conforming who were aged 18-65 years were included. Individuals undergoing hormonal therapy and individuals with Systemic Illness were excluded from the study. For the control group, healthy non-transgender individuals who were aged 18-65 years and willing to participate were included after administering the SPAQ, they were included if there was no sexual/physical abuse experience in their lifetime. Individuals who have a history of psychological/psychiatric problems, those who were not willing to participate, and individuals with severe illness are excluded.

Ethical Approval

The study was authorised by the institution's institutional ethical board (**SRB/SDC/PHD-2101/22/046)**. After explaining the goal of the study, we asked each participant to sign a written consent form. Consent forms were read aloud to those who had trouble reading them. The Declaration of Helsinki was followed when conducting the study.

Sample size calculation

Using G power software 3.1.9.7, the sample size was determined based on a prior study conducted by Arvind et al., [15] The final sample size was 44, 22 transgender and gender non-conforming individuals and 22 non-transgender individuals.

Sampling and data collection

Transgender and gender nonconforming residents at Thozhi shelter in Chennai were selected through convenience sampling utilising inclusion and exclusion criteria until the necessary sample size was reached, according to the researcher's convenience and the logistics that were accessible.

Questionnaire

Prior to collecting salivary samples, the lead investigator collected data using a questionnaire to gather socio-demographic information, Participants' sexual and physical abuse experience was recorded using The Sexual and Physical Abuse Questionnaire (SPAQ) which is a pre-validated questionnaire.[16]

Saliva collection and analysis

All appointments were made in the afternoon to reduce diurnal variance and promote a more consistent evaluation of biomarkers. Participants were asked to refrain from smoking, or consuming dairy, caffeine or alcohol and cleaning their teeth at least for 30 minutes before sample collection. In order to account for potential temporary increases in stress while travelling to the appointment, stress biomarkers were assessed before the survey and after listening to a 10-minute relaxation tape. To minimise potential bias, researchers in the laboratory were made blind to the source of the sample. Using the passive-drool method, unstimulated saliva was collected into a polypropylene vial with a straw. Within an hour of collection, all samples were labelled with participant identity numbers and kept at 70 °C until the batch test of the analyte panels. The clear supernatant from the centrifugation of the samples at 3000 rpm for 15 min was used for analysis.

Human serum, plasma, urine, or cell culture medium can all contain human interleukin-6 (Hu IL-6), which can be measured using an ELISA. Both native and recombinant Hu IL-6 will be specifically recognised by the assay. The goal of the enzyme-linked immunosorbent test (ELISA) for Human IL-6 solid-phase sandwich is to quantify the amount of target bound between two complementary antibodies. The microplate's wells have been pre-coated with an antibody specific to the target. These wells are subsequently filled with samples, standards, or controls that bind to the immobilised (capture) antibody. A substrate solution is added once the sandwich has been put together using the second antibody, which is the detector. The combination of the enzyme, antibody, and target reacts with this solution to create a detectable signal. The quantity of target in the original specimen directly correlates with the signal's strength.

A two-site sandwich ELISA is used in the Human Malondialdehyde (MDA) ELISA Kit to quantify MDA in samples. A microplate has been pre-coated with an antibody that is particular to MDA. Any MDA that is present is bound by the immobilised antibody as standards and samples are pipetted into the wells. HRP-Conjugated Human MDA Detection Antibody is added to the wells after eliminating any unattached compounds. Chromogen solution is added to the wells after a wash to remove any unattached HRP reagent, and colour develops proportionally to the amount of MDA bound in the first phase. The growth of the colour is halted, and the colour's intensity is gauged.

For healthy, age-matched non-transgender controls those who haven’t undergone any physical and sexual abuse (History of sexual and physical abuse was assessed by administering the SPA Questionnaire), who reported to Saveetha Dental College's outpatient department, the same saliva collection and analytical techniques were used.

Statistical Analysis

Data management and analyses were conducted using SPSS 26. 0 software (SPSS, Inc, Chicago, Ill., USA). Mean and standard deviation was used to express the values of IL6 and MDA levels in both the groups. To compare the differences in the mean values of IL6 and MDA levels between the groups, an independent t-test was used. Also to establish the relationship between the abuse scale and IL6 and MDA levels, the Kendall tau_b correlation test was used with significant levels (p-value) at <0.05.

3. Results

The mean IL6 value in the control group is 109.1708 ± 86.343 but in the GNC group it is eightfold higher than 799.4351±97.322 and the difference is also statistically significant (p=0.001). Meanwhile, MDA mean levels in the Control and GNC groups don't exhibit major variation and the values are represented respectively, 0.8±0.49 and 2.12±0.12 and were also statistically significant (p=0.000). All the study participants are between 25 to 50 years of age group (Figure *1*). The responses to the Sexual and Physical abuse questionnaire were represented in the graph (Figure *2*). All the Transgender and Gender non-conforming individuals in the study had sexual abuse experience at least once in their lifetime. 82% of Transgender and Gender non-conforming individuals in our study, had been forced to touch the abuser's private parts sexually against their will. Almost 86% of transgender and Gender non-conforming individuals in our study had been forced by abusers to have sexual intercourse against their will. All the Transgender and Gender non-conforming individuals in our study have experienced physical abuse in their lives.

There is a significant strong positive correlation between Sexual/Physical abuse and IL6 levels in the control group whereas there was a weak positive correlation between Sexual/Physical abuse and MDA but it was not significant (Table 1). The Kendall's tau correlation in the Gender non-conforming group has significant strong positive correlation between Sexual/Physical abuse and IL6 levels whereas there was a weak negative correlation between Sexual/Physical abuse and MDA but it was not significant (Table 2).

Table 1: Correlation between SPAQ and salivary stress biomarker levels in the control group using Kendall's tau b test.

Correlations			CONTROL IL6	CONTROL MDA
Kendall's tau_b	Abuse scale	Correlation Coefficient	0.639	0.248
		p value	0.001	0.183

Table 2: Correlation between SPAQ and salivary stress biomarker levels in GNC group using Kendall's tau b test.

Correlations			GNC IL6	GNC MDA
Kendall's tau_b	Abuse scale	Correlation Coefficient	0.527	-0.263
		p value	0.005	0.157

Table 3: Independent Sample t test for IL-6:

	Groups	N	Mean	Std. Deviation	t	Sig. (2-tailed)
IL 6	Control group	22	109.1708	86.34327	-3.404	0.001
	GNC group	22	799.4351	97.32202		

Table 4: Independent Sample t test for MDA:

	Groups	N	Mean	Std. Deviation	t	Sig. (2-tailed)
MDA	Control group	22	.8097	0.49165	-5.701	0.000
	GNC group	22	2.1289	0.12753		

The results of independent t test show the difference in the interleukin and MDA levels among control and GNC group respectively, In both IL 6 and MDA the levels were significantly higher in GNC Group than that of the control group (Table *3* & 4). In the lifetime of transgenders, maximum number of people at the age of 16 years and above have experienced sexual abuse (63.64%). Most of the Transgenders experienced physical abuse between 12-16 years, nearly 73% had experienced physical abuse.

4. Discussion

In this study, The Sexual and Physical Abuse Questionnaire (SPAQ) was used to record the sexual and physical abuse experience, the first time it had happened to the individual and with/by whom. Totally the questionnaire comprises of 9 questions, the first 5 questions were regarding sexual abuse and the next 4 questions were regarding physical abuse. Nearly 63.4% GNC had experienced sexual abuse at 16 years of age or older, 36.6% had experienced between 12 to 16 years. Physical abuse has happened between 12 to 16 years to 73% of GNC, 27% older than 16 years. Almost none of them has shared about physical and sexual abuse to others.

In both control and GNC group, Kendall tau correlation tests of MDA levels with sexual/physical abuse exhibits weak positive correlation (Correlation Coefficient = 0.248) and strong negative correlation (Correlation Coefficient = -0.263) respectively. This proves that lesser the MDA levels the stress due to physical/ sexual abuse might be more, but it is not statistically significant (p = 0.183 in control and p=0.157 in GNC group).

Interleukin 6 levels were found to have strong positive correlation in GNC group (Correlation Coefficient = 0.527) and in control group (Correlation Coefficient = 0.639) and is also statistically significant p = 0.005 for GNC Group and p = 0.001 for control group. With increase in stress due to physical /sexual abuse, Interleukin 6 level also increases in saliva. These results are contradictory to results from other studies which showed no correlation between stress biomarkers (Interleukin 6) and chronic psychological stress. Interleukin 6 has correlation towards Body mass Index and age but not with the stress the individual underwent [13]. Interleukin 6 levels were increased in people who had experienced physical and sexual abuse in their life. [17] IL 6 levels might be a bridge between childhood abuse and development of depression and its related disorders in future. Early detection of IL6 might lead to detection and prevention of depressive disorders. [18] Study by Islam MR et al revealed positive association between MDA and stress & stress related disorders, but the similarity between serum and salivary stress biomarkers should be evaluated. [19-20]

All the participants in the GNC group had experienced sexual abuse once in their lifetime. Study done at Chelsea and Westminster Hospital NHS Foundation Trust, London, UK has also revealed that the LGBTQ+ people are at high risk for domestic abuse [21]. Extra care should be taken during regular checkups to identify the victims and also to eliminate stress caused by Sexual / physical abuse which will prevent the individual from developing stress related disorders and suicidal thoughts/attempts. [22]

Violence to these minority people can be by their intimate partners, friends, relatives and even family members. Policies can be developed to support the gender non-conforming community as they are the major victims. [23-27] The main limitation of this study is the smaller sample size, further studies to be done with larger sample size to get accurate pictures about association between salivary stress biomarkers and sexual/physical experience.

5. Conclusions

It is evident that transgender individuals face heightened stress levels, primarily stemming from sexual and physical abuse experiences. This vulnerable population encounters unique challenges that exacerbate their stress levels, leading to adverse effects on their mental and physical well-being. The use of interleukin-6 (IL-6) as a salivary biomarker to detect stress shows promise as a potential tool for assessing stress in transgender individuals.

However, the limitations of using malondialdehyde (MDA) levels as a stress indicator suggest that further research is required to fully understand the complexities of stress in this context. To obtain more accurate and conclusive results, future studies must be conducted with larger sample sizes, providing a comprehensive understanding of the factors contributing to stress in transgender individuals and informing the development of effective interventions and support mechanisms.

Abbreviations

TGNC - Transgender or gender nonconforming

MTF - Male-to-Female

FTM - Female-to-Male

LGBTQIA - Lesbian, Gay, Bisexual, Transgender, Queer/Questioning, Intersex, and Asexual

PTSD - Post-Traumatic Stress Disorder

IL 6 - Interleukin 6

MDA - Malondialdehyde

SPAQ - The Sexual and Physical Abuse Questionnaire

TGNC - Gender nonconforming

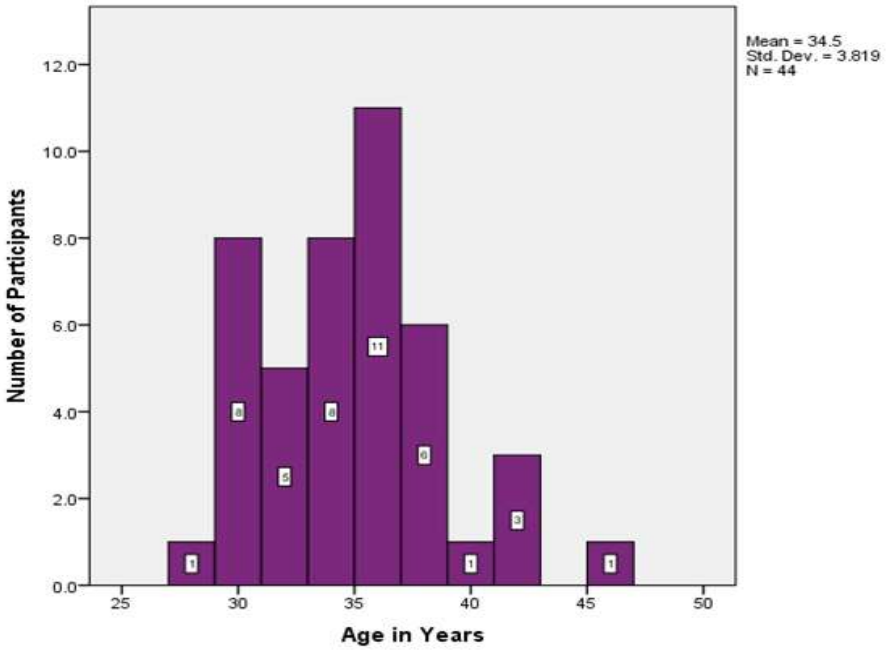

Figure 1: Demographic distribution (Age) of control and experimental group

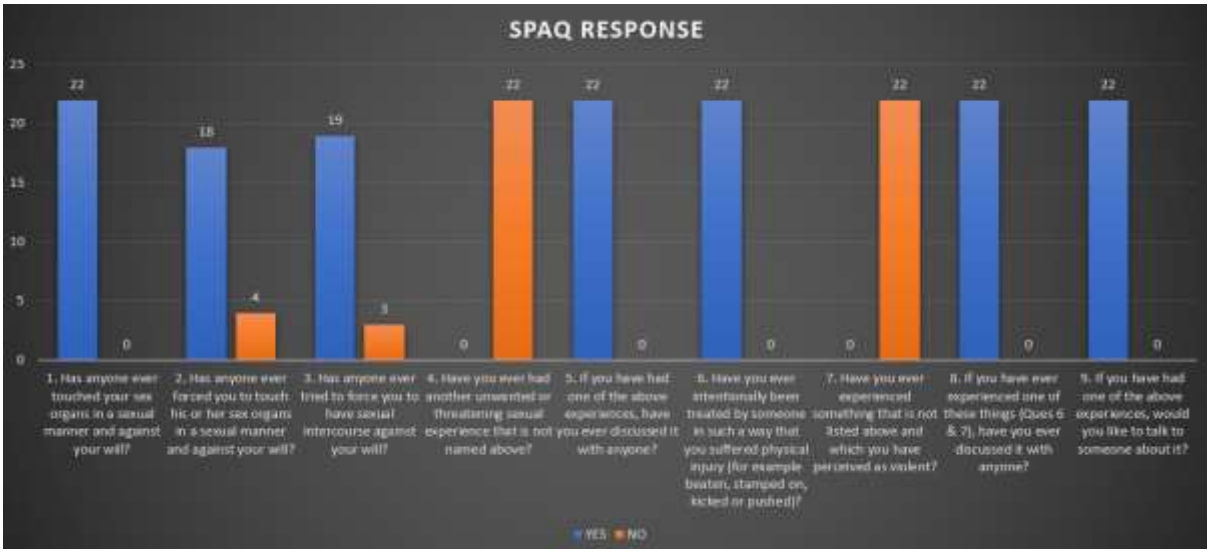

Figure 2: The Sexual and Physical Abuse Questionnaire Response

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