NeuroRegulation

Investigation of Neopterin and Neurophysiological Measurements as Biomarkers of Anxiety and Stress

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Abstract

The aim of this study was to investigate whether the inflammatory marker neopterin and certain neurophysiological measurements could be used as complementary markers for stress and anxiety symptoms as determined by the Depression, Anxiety, and Stress Scale (DASS-21) questionnaire. A cohort of 158 respondents completed the DASS-21 and biographical questionnaire which were used to stratify health sciences university students between Group A (*n* = 20), who had high levels of symptoms, and Group B (*n* = 20) who had normal levels of stress and anxiety. Neurophysiological measurements were taken from these participants, namely heart rate variability (HRV), blood pressure (BP), blood-volume pulse (BVP), electrodermal activity (EDA), and quantitative electroencephalography (qEEG). Each participant also donated a urine sample which was tested for neopterin concentration using an enzyme-linked immunosorbent assay (ELISA). Neopterin positively correlated with the stress and anxiety scores, while HRV and BVP were negatively correlated with these scores. In terms of qEEG, delta and hibeta wave activity increased in the left and frontal brain regions of participants with high mental health scores, whereas alpha wave activity decreased in these regions**.** High DASS scores were associated with elevated neopterin concentration and neurophysiological changes (brain waves, HRV, and BVP).

Keywords: anxiety; biomarkers; brainwaves; heart rate variability; inflammation; neopterin; stress

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Introduction

The number of people who suffer from mental health conditions is increasing. Between 1990 and 2019, cases of mental health disorders increased by 48% (Ferrari et al., 2022). Of these, the cases of anxiety disorders increased by nearly 50% and depressive disorders increased by 64% (Ferrari et al., 2022; Yang et al., 2021). Mental health conditions are among the most costly disorders in terms of projected healthcare expenditures needed to treat them (Tomlinson et al., 2009). Accordingly, the global economic impact of these conditions is estimated to be US$3–7 trillion each year (due to medical costs, disability, and lost productivity; Arias et al., 2022). Despite the pervasive and increasing effects of mental health, the medical resources, interventions, and funding allocated to treating them are not proportional to the actual burden. In many countries, less than 1% of government health expenditure goes towards mental health services, with the average expenditure being only 2.8% (Rajkumar, 2022; Saxena et al., 2003; Whiteford et al., 2013).

Not only is mental health a major concern globally but it is also of particular importance for medical students who have a high incidence of anxiety, depression, burnout, and mental health struggles (Dyrbye et al., 2005; Fares et al., 2016). Studies suggest that medical students often have higher levels of psychological distress than the general population, and their age-matched peers (Dyrbye et al., 2006; Goebert et al., 2009; Maser et al., 2019).

This may have an adverse effect on academic performance, empathy, and the care of their patients, as well as contribute to other negative professional and personal aspects (Dyrbye et al., 2010; Hojat et al., 2004; Thomas et al., 2007).

Stress, Anxiety, and Depression

Stress is commonly defined as the response to a real or perceived threat to homeostasis (Charmandari et al., 2005; Johnson et al., 2019; Smith & Vale, 2006). This response involves physiological, endocrine, and cognitive reactions, which aid in survival (Charmandari et al., 2005; Sapolsky et al., 2000). Although the stress response is a normal and healthy physiological process to help restore homeostasis, prolonged stress can become maladaptive and detrimental. Chronic stress is an important risk factor for the development of many disorders, including anxiety (Pêgo et al., 2010), depression (Kessler, 1997), and cardiovascular disease (CVD; Satyjeet et al., 2020).

Anxiety disorders are a considerable problem worldwide (Buist-Bouwman et al., 2006), and are characterized by excessive worry, fear, and other psychological and physiological alterations (American Psychiatric Association [APA], 2013; 2013; Steimer, 2002; Wilmer et al., 2021). Anxiety can interfere with quality of life, impacting health, emotion regulation, social and occupational function, and the ability to cope successfully with challenges (Steimer, 2002; Wilmer et al., 2021). Aberrations in neurotransmitters, stress hormones, and the autonomic nervous system (ANS) are thought to be involved in its pathophysiology (Bagdy, 1998; Crestani et al., 1999; Gass et al., 2001; Ho et al., 2020; Holwerda et al., 2018; Mitra & Sapolsky, 2008; Nutt & Malizia, 2001; Risbrough & Stein, 2006; Steimer, 2002; Tanaka et al., 2000; Teed et al., 2022; Weinstock, 2001). In addition to stress, genetic, environmental, and experiential factors also contribute to the risk of developing anxiety (Pêgo et al., 2010; Steimer, 2002).

Depression is characterized by feelings of sadness, emptiness, and/or irritability, which are accompanied by somatic and cognitive changes that significantly impact a person's capacity to function (APA, 2013). It should be noted that these symptoms are present every day and are distinguished from normal feelings of sadness or grief, which reduce in intensity over time (APA, 2013). There are many factors that contribute to the risk of developing depression in university students. These include confidence, personality, academic pressure, preexisting conditions, lifestyle choices, social support, and financial struggles (Mohammad, 2021). In addition to these, stress is also a risk factor for depression (Kessler, 1997; Raison & Miller, 2003).

DASS-21 Questionnaire

The Depression, Anxiety, and Stress Scale (DASS) was designed to measure mental health aspects on three scales. The Depression scale reflects self-esteem and motivation, while the Anxiety scale reflects feelings of fear, panic, and arousal. The third scale, Stress, measures tension, irritability, and difficulty relaxing (Lovibond & Lovibond, 1995). Each scale of the DASS assesses unique features of the three conditions, which reduces the overlapping or intercorrelation of the measurements, thus increasing the ability to distinguish between depression, anxiety, and stress. The short-form version of DASS, which consists of 21 questions (DASS-21), was used in this study. The DASS has been found to be a reliable and valid measure in both clinical and nonclinical samples (Akin & Çetin, 2007; Antony et al., 1998; Beaufort et al., 2017; Crawford & Henry, 2003; De Beurs et al., 2001; Dreyer et al., 2019; Henry & Crawford, 2005; Jiang et al., 2020; Tonsing, 2014; Tran et al., 2013).

The DASS questionnaire is a dimensional rather than a categorical measure. As such, it should not be used to diagnose participants into discrete categories proposed in classification systems such as the *Diagnostics and Statistical Manual of Mental Disorders*, but rather should be used as a screening tool to assess symptom severity.

Although mental health aspects can be determined through the administration of self-assessment questionnaires, suitable physiological measurements may be necessary to complement and substantiate the questionnaire facets surveyed. Adjunct physiological biomarkers may contribute to the scientific understanding of mental well-being and may be advantageous in improving the management thereof. For example, some but not all depressed patients present with elevated inflammation (Osimo et al., 2020), as such an inflammatory biomarker could be useful in identifying this subset of patients and thus prescribing an appropriate course of treatment.

The body of research investigating the links between mental health and inflammation is growing. Studies involving the effects of proinflammatory cytokines on the brain suggest that inflammation may have a pivotal role in the pathophysiology and symptom severity of stress, anxiety, and depression (Bankier et al., 2008; Bauer & Teixeira, 2019; Dowlati et al.,

2010; Hoge et al., 2009; Osimo et al., 2020; Pace & Miller, 2009; Pace et al., 2007; Valkanova et al., 2013; Von Känel et al., 2007).

Neopterin: A Biomarker of Inflammation

Neopterin is a molecule that forms part of the pteridine family. It is also known as 2-amino-4-hydroxy-(erythro-1,2,3-trihydroxypropyl)-pteridine (Hamerlinck, 1999). Neopterin is synthesized from guanosine-5′-triphosphate (GTP) and forms part of the tetrahydrobiopterin (BH4) synthetic pathway (Ghisoni et al., 2015).

The main source of neopterin in humans is monocytes and macrophages, which produce neopterin when stimulated by interferon-γ (IFNγ); a cytokine produced by immune cells (Huber et al., 1984). Activated T-lymphocytes, particularly Th1 cells, produce IFNγ which stimulates macrophages, resulting in the production of neopterin (Huber et al., 1984; Maggi et al., 1992). Therefore, neopterin is reflective of immune activation and considered to be a nonspecific biomarker of cell-mediated immunity because it reflects the production and effects of IFNγ, in addition to Th1 cell and macrophage activity (Berdowska & Zwirska-Korczala, 2001; Dunbar et al., 1992). As such, neopterin has been used as a marker of immune activation during inflammation in a broad range of conditions, including cancer (Berdowska & Zwirska-Korczala, 2001), CVD (Fuchs et al., 2009; Pacileo et al., 2007), and infectious diseases (Eisenhut, 2013).

Neopterin may provide a link between mental health and inflammation, as it could reflect one mechanism by which immune system activation can affect neurotransmitters (Klaus et al., 2021). In addition, neopterin levels have been found to change significantly during periods of psychological stress, suggesting a correlation between mental state and alterations in cell-mediated immunity (Dunbar et al., 1993). Furthermore, inflammation-induced stimulation of indoleamine 2,3-dioxygenase (IDO) and the kynurenine pathway can contribute to tryptophan depletion and decreased serotonin, which has been associated with depression (Albert et al., 2012; Maes et al., 1994; Müller & Schwarz, 2007; Myint et al., 2013; Myint & Kim, 2003; O'Connor et al., 2009) and anxiety (Bagdy, 1998; Blokland et al., 2002). This relates to neopterin as tryptophan depletion and increased kynurenine have been found to be correlated with neopterin (Brown et al., 1989; Maes et al., 1994). Despite the potential mental health effects, tryptophan depletion by the immune system is purposeful, as it can reduce microbial proliferation (Gao et al., 2020). Considering these findings, neopterin has potential to be an immunological marker for mental health conditions.

In terms of assessing immune activity, cytokines, such as IFNγ, can be measured. However, monitoring neopterin instead may be superior as it is biochemically inert and has a longer half-life. These properties allow neopterin to reach and stay in circulation, unlike other cytokines, which have a short half-life and may not reach circulation (Fuchs et al., 2009). Once in circulation, neopterin levels can be measured with ease due to its unchanged excretion by the kidneys (Berdowska & Zwirska-Korczala, 2001), allowing it to be quantified in the urine using validated assays such as an enzyme-linked immunosorbent assay (ELISA; Fuchs et al., 2009; Gieseg et al., 2018).

Measures of Autonomic Activity

Fluctuations between the dominance of the sympathetic and parasympathetic nervous system is part of normal and healthy responses to maintain homeostasis; however, when stress becomes chronic, an imbalance between these two systems can result in negative health outcomes. Namely, chronic stress and the imbalance of the ANS are implicated in the pathogenesis of anxiety and depression (Godoy et al., 2018). As such, measuring aspects of ANS activity and finding suitable biomarkers thereof may contribute to the management of mental health symptoms.

Heart rate variability (HRV) is a measure of ANS activity; it measures fluctuations in heartbeat intervals (Hourani et al., 2020). As stated by Shaffer and Ginsberg, “a healthy heart is not a metronome; the oscillations of a healthy heart are complex and constantly changing, which allow the cardiovascular system to rapidly adjust to sudden physical and psychological challenges to homeostasis” (Shaffer & Ginsberg, 2017). Therefore, HRV is indicative of ANS flexibility in response to stressors and can be used to assess the links between the stress response and neuropsychological parameters (Hourani et al., 2020). Abnormalities in HRV may serve as a biomarker for various mental health disorders and stress-related variables (Hourani et al., 2020; Shinba, 2017). For example, there is an association between reduced HRV and mental health conditions, such as anxiety and depression (Hourani et al., 2020; Schiweck et al., 2019). This is not entirely surprising given the high comorbidity observed between CVD and depression (Cohen et al., 2015). Conversely, higher HRV is associated with increased resilience, greater recovery from

acute stressors, changes in cognitive performance and emotional regulation, and less vulnerability to depressive-like states (Hourani et al., 2020). Due to this, HRV could serve as a measure of neuropsychological parameters and ANS activity. However, HRV should not be used as a single indicator or as a diagnosis.

Other measures of ANS activity include blood volume pulse (BVP) and electrodermal activity (EDA), which have been used as biomarkers of psychological arousal (Kushki et al., 2011) and emotional states such as depression (Sarchiapone et al., 2018). BVP is a measure of the volume of blood in the arteries, which is related to the constriction and dilation of the vessels (Sarchiapone et al., 2018). Greater vasoconstriction leads to lower volume of blood in the vessels, so BVP decreases. Greater vasodilation leads to a greater blood volume, so BVP increases. Therefore, BVP is reflective of ANS activity, as autonomic activation of adrenergic receptors on blood vessels can cause vasoconstriction (Gordan et al., 2015; Peper et al., 2007; Sarchiapone et al., 2018). Of relevance, ANS activity changes with emotions, thus an emotion like fear can lead to vasoconstriction (Kreibig, 2010).

Previous research using BVP as a biomarker found it to be useful in measuring anxiety levels, although it was more accurate when combined in a model with other physiological measures (Šalkevicius et al., 2019). Another study used BVP to create a model for short-term anxiety recognition (Handouzi et al., 2014). Little research has been done on the use of BVP as a biomarker, particularly in the area of mental health, thus warranting further investigation.

EDA (also known as skin conductance) depends on the electrical conductivity of the skin, which is altered by sweat levels. ANS activity affects the amount of sweat on the skin due to eccrine sweat glands having sympathetic innervation. Thus, EDA can be used to measure sympathetic activity of the ANS (Kushki et al., 2011; Sarchiapone et al., 2018). EDA has potential as a biomarker for mental health as studies have found electrodermal hypoactivity in depression. Thus, EDA can be useful in distinguishing depressive patients from healthy patients (Sarchiapone et al., 2018).

Blood pressure (BP) can also be used to assess ANS activity. For example, hypertension may be indicative of ANS abnormalities and imbalance (Edwards et al., 2011). In addition, chronic stress has been shown to increase heart rate and BP (Torpy et al., 2007). Given that many physiological systems influence BP, it clearly cannot be used in isolation as a single biomarker for mental health conditions. This warrants further studies for its link and usefulness when combined with other measures.

Quantitative Electroencephalography (qEEG)

Electroencephalography (EEG) is a medical imaging technique that measures the electrical activity of the brain (Rojas et al., 2018; Teplan, 2002).

The electrical currents detected by an EEG are referred to as brain waves which are measured from the peak of one wave to the peak of another (Teplan, 2002). There are four main categories of brain waves: delta (1–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), and beta (12–38 Hz). Different brain regions do not simultaneously produce the same frequency of brain waves, they produce varying amounts of each frequency. Therefore, signals between EEG electrodes consist of many waves that have differing characteristics (Teplan, 2002).

Different brainwaves are associated with different states. For example, when an individual's eyes are open, beta waves are usually dominant. When an individual is relaxed or drowsy, alpha activity rises. As an individual moves into a sleep state, lower frequency waves such as theta and delta increase (Teplan, 2002).

Quantitative EEG (qEEG) involves the digitalization of raw EEG measurements. Complex algorithms then allow for the creation of brain maps using EEG readings. These maps can be used to examine the power, amplitude, coherence, and lag phase of different brain waves. There are two types of power measured by qEEG: absolute power (the electrical power at each site of measurement) and relative power (the distribution of power at one site compared to other sites; Neurofeedback Alliance, 2021).

Quantification of EEG recordings may give further insight into mental health and potential markers. For example, qEEG allows the identification of abnormalities, such as frontal alpha asymmetries that are often observed in depressed patients (Kanda et al., 2009). A preliminary study also found that participants with higher activity in the right anterior of the brain reported a greater tendency to feel anxious a year later. Thus, right frontal EEG activity may act as a vulnerability marker and predict the future onset of anxiety symptoms (Blackhart et al., 2006). Other studies have also found greater relative right frontal EEG activity in those with

anxious or depressive symptoms (Blackhart et al., 2006).

Materials and Method

Study Design and Participants

This study was noninterventional, observational, and cross-sectional. The participants were students from the Faculty of Health Sciences at the University of Pretoria, South Africa, who were recruited online via a biographical questionnaire and the DASS-21.

A total of 158 respondents completed the online questionnaires. A cohort of 78 respondents met the inclusion requirements for physiological measurements. A total of 40 participants were recruited for the measurements and were divided into Group A (n = 20) and Group B (n = 20) based on their DASS questionnaire scores. Participants qualified for Group A if they scored Moderate, Severe, or Extremely Severe in the Anxiety and/or Stress categories of the DASS questionnaire. Participants qualified for Group B if they scored Normal or Mild on all three DASS categories.

Participants were not included if they did not sign informed consent, complete the questionnaires, and/or withdrew from the study at any time, thus not completing the measurements (qEEG, HRV, BP, BVP, EDA, and donate a urine sample), or did not have qualifying DASS scores. Other exclusion criteria included having epilepsy, use of recreational drugs, use of medication that may alter EEG readings (e.g., barbiturates, antidepressants, antipsychotics, antihypertensives), use of anti-inflammatory drugs, a chronic or recent infection, or an inflammatory disorder.

Institutional Review Board Statement

This study was conducted in accordance with the recommendations of the Research Ethics Committee at the University of Pretoria (210/2022) and with approval of the Dean of the Health Sciences Faculty. Furthermore, this study complied with the Declaration of Helsinki, and with South African privacy law. Participation was voluntary, and participants could withdraw at any time without any negative consequences. All data were stored only by using an anonymous ID for each participant and the data obtained were used solely for scientific purposes.

Measures

The electrodes for the qEEG were placed according to the 10–20 electrode placement protocol. The measurements from the 19 active electrodes were transferred to the qEEG Pro program (BrainMaster Technologies Inc., Bedford, OH). This program analyzed and compared the recordings to the qEEG Pro normative database.

HRV was measured using the Zephyr BioHarness and Ominisense software (Medtronic PLC, Midrand, South Africa). Further analysis was conducted using Kubios software (Kubious Oy, Kuopio, Finland). Eleven HRV parameters were investigated: mean HRV, mean RR, standard deviation of normal-to-normal (SDNN), root mean square of successive differences between normal heartbeats (RMSSD), low-frequency (LF) peak, high-frequency (HF) peak, LF power, HF power, LF/HF ratio, sympathetic nervous system (SNS) index, and parasympathetic nervous system (PNS) index (SD1 and SD2).

BVP and EDA were measured using the Infiniti Pro biofeedback apparatus (Thought Technology Ltd., Montreal, Canada), whereby sensors were placed on the fingers. BP was measured using an automated BP monitor (Clicks Retailers [Pty] Ltd., Woodstock, Cape Town).

The Demeditec ELISA kit (Demeditec Diagnostics GmbH, Kiel, Germany) was used for the determination of urine neopterin concentrations according to the manufacturer's protocol.

Statistical Analysis

Data was analyzed using IBM SPSS Statistics version 28.0.1.0 software. Spearman's Rank Correlation was used as the measure of association between two variables. The independent samples t-test was used to determine if there was a significant difference in means between Group A and B. For variables that did not have a normal distribution, the Mann-Whitney U test was also performed. A p-value of less than .05 was considered significant.

For variables that were not normally distributed, the median, interquartile range (IQR), and p-value for the Mann-Whitney U test were also reported. Normality was determined through having a Shapiro-Wilk p-value of less than .05. For variables where no median, IQR, or Mann-Whitney p-value is reported, normality was assumed.

Table 1

Comparison of Means Between Group A and B

	Group A	Group B		Group A	Group B	
	Mean ± SD		*t*-test *p*-value	Median (IQR)		M-W *p*-value
Demographics						
Year of Study	3.45 ± 1.90	4.00 ± 3.60		-	-	-
Age	22.35 ± 3.27	23.85 ± 3.60		-	-	-
DASS Scores						
Stress Score	24.10 ± 6.79	9.20 ± 5.09	<.001**	-	-	-
Anxiety Score	20.60 ± 8.03	4.65 ± 3.28	<.001**	20.00 (11.00)	4.00 (7.00)	<.001**
Depression Score	13.22 ± 7.87	5.20 ± 3.07	<.001**	12.00 (13.00)	6.00 (6.00)	<.001**
Neopterin						
Neopterin (µmol neopterin/ µmol creatinine)	33.81 ± 22.80	13.22 ± 10.52	<.001	31.40 (38.39)	10.47 (15.47)	.002**
Heart Rate Variability						
PNS Index	2.64 ± 1.09	2.12 ± 1.24	.171	-	-	-
SNS Index	−1.80 ± 0.63	−1.49 ± 0.84	.204	−1.83 (0.80)	−1.64 (1.38)	.242
Mean HRV	57.07 ± 15.36	74.21 ± 15.61	.002**	59.74 (27.14)	80.68 (33.54)	.001**
Mean RR (ms)	1381.33 ± 196.74	1259.49 ± 235.71	.084	-	-	-
SDNN (ms)	54.48 ± 12.42	63.91 ± 14.27	.032*	-	-	-
RMSDD (ms)	60.92 ± 15.51	67.15 ± 14.87	.202	-	-	-
Peak LF (Hz)	0.074 ± 0.038	0.066 ± 0.027	.430	0.05 (0.07)	0.06 (0.03)	.845
Peak HF (Hz)	0.186 ± 0.034	0.186 ± 0.022	.993	0.17 (0.04)	0.18 (0.04)	.614
LF Power (ms^2)	1601.71 ± 993.97	2031.41 ± 1306.90	.254	1013.15 (1895.61)	2142.80 (1876.49)	.351
HF Power (ms^2)	956.67 ± 644.01	1254.97 ± 643.07	.151	778.82 (983.69)	1036.40 (1045.70)	.149
LF/HF	1.79 ± 0.78	1.78 ± 1.18	.991	1.54 (1.15)	1.68 (2.03)	.940
SD1 (ms)	43.26 ± 11.02	47.69 ± 10.55	.202	-	-	-
SD2 (ms)	62.53 ± 14.66	75.97 ± 19.40	.018*	-	-	-
Blood Pressure						
Systolic BP (mmHg)	116.08 ± 8.45	121.43 ± 13.88	.149	-	-	-
Diastolic BP(mmHg)	81.30 ± 7.19	81.10 ± 7.98	.934	-	-	-
Blood-Volume Pulse						
Mean BVP Amplitude (%)	8.81 ± 4.18	11.84 ± 6.60	.091	-	-	-
Min BVP Amplitude (%)	3.57 ± 2.27	4.3 ± 2.28	.278	3.22 (3.64)	3.88 (3.69)	.267
Max BVP Amplitude (%)	19.90 ± 10.22	18.54 ± 10.02	.672	-	-	-
Mean BVP FFT Peak Frequency (Hz)	0.16 ± 0.07	0.13 ± 0.07	.240	-	-	-
Min BVP FFT Peak Frequency (Hz)	0.04 ± 0.06	0.04 ± 0.05	.775	0.02 (0.00)	0.02 (0.00)	.820
Max BVP FFT Peak Frequency (Hz)	0.28 ± 0.08	0.25 ± 0.08	.106	-	-	-
Electrodermal Activity						
Mean EDA (µSiemens)	1.15 ± 0.86	1.31 ± 1.18	.647	0.99 (1.64)	1.15 (1.18)	.988

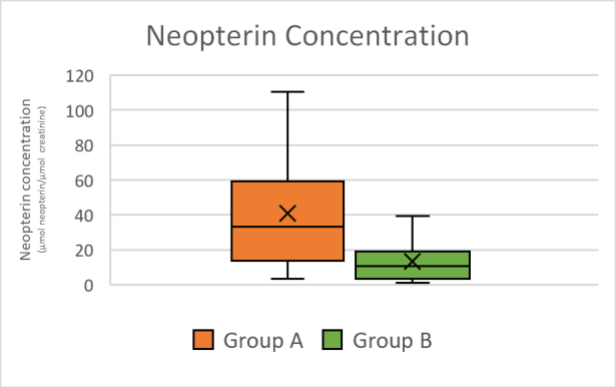
* = Difference in means is significant at the .05 level; ** = Difference in means is significant at the .01 level. The two-sided independent samples *t*-test was performed. *Df* = 38; *n* = 40; The nonparametric Mann-Whitney U test was also performed for variables that were not considered to be normally distributed (determined using the Shapiro-Wilk test).

Results

Group A consisted of 18 females and 2 males. Group B consisted of 12 females and 8 males. There was a significant difference between the DASS scores of the Group A and B (p < .001 for each category). For Group A, the mean scores were moderate for stress (24.10 ± 6.79), extremely severe for anxiety (20.60 ± 8.03), and mild/moderate for depression (13.22 ± 7.87). The mean scores for participants in Group B were Normal for the categories of stress (9.20 ± 5.09), anxiety (4.65 ± 3.28) and depression (5.20 ± 3.07). Group B had less variation in scores than Group A, as can be seen in Table 1.

There was a significant difference in the concentration of neopterin between Group A and B. Group A had a higher concentration of neopterin than Group B (33.81 ± 22.80, 95% CI [22.47, 45.15] vs. 13.22 ± 10.5, 95% CI [8.29, 18.14]; p < .001). The distributions can be seen in the neopterin boxplot (Figure 1).

Figure 1. *Boxplot of Neopterin Concentration*.



Note. The boxplot shows the concentrations of neopterin between the Group A (n = 20) and Group B (n = 20).

The mean values for the power of hibeta, beta, and theta at each electrode were not significantly different between the groups. Only frequencies and electrodes that showed a significant difference are shown in Table 2. None of the electrode positions in the theta, beta, or hibeta band showed a significant difference in mean.

Table 2

Comparison of Mean Power at Various Electrodes Between Group A and B

	Group A	Group B		Group A	Group B	
	Mean ± SD		*t*-test *p*-value	Median (IQR)		M-W *p*-value
Absolute Power						
Delta FP1	0.38 ± 0.48	−0.34 ± 0.72	.002**	-	-	-
Delta FP2	0.63 ± 0.68	−0.26 ± 0.73	<.001**	-	-	-
Relative Power						
Delta FP2	−1.39 ± 1.08	−2.73 ± 1.71	.005**	-	-	-
Delta Fz	−1.74 ± 2.11	−3.98 ± 3.75	.025*	−1.65 (2.80)	−4.15 (4.38)	.038*
Alpha FP1	−1.40 ± 1.37	−0.48 ± 1.53	.051	-	-	-
Alpha T3	−2.03 ± 1.09	−1.20 ± 1.29	.033*	-	-	-

* = Difference in means is significant at the .05 level; ** = Difference in means is significant at the .01 level. The two-sided independent samples *t*-test was performed. *Df* = 38; n = 40; The nonparametric Mann-Whitney U test was also performed for variables which were not considered to be normally distributed (determined using the Shapiro-Wilk test).

In terms of a difference in mean or correlation with DASS scores, the only notable electrodes were FP1, FP2, F7, F3, Fz, and T3, and the only notable frequencies were delta, alpha, and hibeta. No significant correlation or difference in mean was found at any of the other electrodes or frequencies (theta and beta). All notable correlations are presented in Table 3.

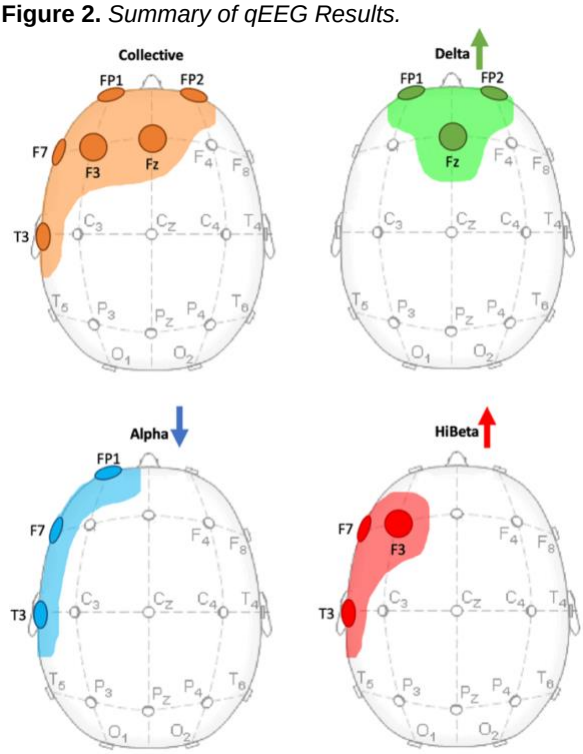
Table 3
Correlations Between Parameters.

	Spearman's Correlation Coefficient	*p*-value
Stress Score and:		
Anxiety Score	0.829	<.001**
Depression Score	0.732	<.001**
Neopterin	0.588	<.001**
Mean HRV	−0.433	.005**
FP1 Z-Delta Absolute Power	0.373	.027*
FP2 Z-Delta Absolute Power	0.344	.030*
T3 Z-Alpha Relative Power	−0.379	.016*
F3 Z-HiBeta Relative Power	0.323	.042*
T3 Z-HiBeta Relative Power	0.333	.036*
Anxiety Score and:		
Stress Score	0.823	<.001**
Depression Score	0.642	<.001**
Neopterin	0.426	.006**
Mean HRV	−0.365	.021*
Min BVP Amplitude	−0.366	.020*
Mean BVP Amplitude	−0.344	.030*
FP2 Z-Delta Absolute Power	0.374	.018*
F7 Z-Alpha Relative Power	−0.324	.042*
T3 Z-Alpha Relative Power	−0.399	.011*
F7 Z-HiBeta Relative Power	0.325	.041*
F3 Z-HiBeta Relative Power	0.359	.023*
Depression Score and:		
Stress Score	0.732	<.001**
Anxiety Score	0.642	<.001**
Neopterin	0.451	.003**
Age	−0.315	.048*
Mean HRV	−0.383	.015*
BVP Min Amplitude	−0.400	.011*
BVP Mean Amplitude	−0.368	.019*
BVP Max FFT Peak Frequency	0.387	.014*

Note. Only correlations that had $p < .05$ are presented.
* = Correlation is significant at the 0.05 level (2-tailed);
** = Correlation is significant at the 0.01 level (2-tailed).
BVP – blood-volume pulse; FFT – fast Fourier transform; HRV – heart rate variability; $n = 40$.

The significant results regarding the qEEG measures are summarized in Figure 2. The results suggest that delta power increased, alpha power decreased, and hibeta increased with stress and anxiety symptoms.

Figure 2. *Summary of qEEG Results.*



Note. Electrodes at which there was a significant correlation with DASS score or difference in mean were included. The first image (Collective, in orange) shows all the electrodes with a significant association. The inclusion of FP1 in the alpha frequency should be treated with caution given that the *p*-value for the difference in means was .051 and not $p < .05$.

Discussion

Subjectivity, misdiagnosis, and social stigma can interfere with the detection, prevention, and treatment of mental health problems (Forgione, 2018; Rössler, 2016; Wakefield, 2010). Therefore, more objective measures such as biomarkers are necessitated to aid in the identification and treatment of mental health disorders (García-Gutiérrez et al., 2020; Guest, 2017; Macaluso & Preskorn, 2012; Roffman, 2011). As the ANS is implicated in stress and anxiety (Chu et al., 2022; Godoy et al., 2018; Ho et al., 2020; Holwerda et al., 2018; Teed et al., 2022), biomarkers measuring ANS activity were investigated in this study, namely HRV, BP, BVP, EDA, and qEEG. Additionally, inflammation appears

to have a bidirectional relationship with stress and anxiety (Bauer & Teixeira, 2019; Hodes et al., 2015; Maes et al., 1998; Maydych, 2019; Silverman et al., 2005; Von Känel et al., 2007). Therefore, the inflammatory marker neopterin was also investigated as a possible biomarker of stress and anxiety scores. The study aimed to determine whether the aforementioned biomarkers could be used as complementary markers for stress and anxiety scores as determined by the DASS-21 questionnaire.

Neopterin

Neopterin concentration was found to be significantly higher in Group A, compared to Group B, and was positively correlated with scores for stress, anxiety, and depression. This is in line with other studies that found increased neopterin in depression (Dunbar et al., 1992; Klaus et al., 2021; Maes et al., 1994; Widner et al., 2002), PTSD (Atmaca et al., 2002), and psychological stress (Dunbar et al., 1993). Increased inflammatory markers have also been found in panic disorder (Hoge et al., 2009) and generalized anxiety disorder (Bankier et al., 2008). It should be noted that the neopterin concentrations found in the present study correlated with symptom severity and not diagnosed disorders.

Our results also support other studies that found inflammation to be connected to stress (Maydych, 2019; Raison et al., 2006), anxiety (Bankier et al., 2008; Hoge et al., 2009; Maydych, 2019; Vogelzangs et al., 2013), and depression (Dowlati et al., 2010; Inserra et al., 2019; Raison et al., 2006; Valkanova et al., 2013).

The exact nature of the association between neopterin and mental health symptom severity requires further research. However, macrophage activation, IFNγ, and oxidative stress may provide potential mechanisms, as neopterin is associated with the levels of these (Fuchs et al., 2009; Gieseg et al., 2018; Huber et al., 1984; Maes et al., 1998; Monteiro et al., 2016; Nathan, 1986). In addition, the aforementioned all contribute to or have been associated with mental health symptoms (Bouayed et al., 2009; Inserra et al., 2019; Monteiro et al., 2016; Salim, 2014). For example, macrophage infiltration into the brain can increase neuroinflammation, which contributes to anxiety and depression (Dunn, 2006; Haroon et al., 2012; Maes et al., 1992; Quagliato & Nardi, 2018; Raison et al., 2006; Reader et al., 2015; Torres-Platas et al., 2014; Wohleb et al., 2013). Neurotransmitter abnormalities due to IFNγ may also be involved, as this cytokine can impact levels of serotonin, dopamine, and glutamate by stimulating the activity of the enzymes IDO and GTP-cyclohydrolase I (Capuron & Castanon, 2016; Dantzer et al., 2008; Ghisoni et al., 2015; Lanser et al., 2020; Miller et al., 2009; Müller & Schwarz, 2007; Weiss et al., 1999), which can contribute to mental health disorders (Myint et al., 2013). Additionally, the absence of IFNγ in the hippocampus, a region involved in memory and learning, has positive neuronal effects. These neuroplastic changes have been associated with improved performance in learning and memory tasks (Monteiro et al., 2016), which is of particular importance in the context of university students. Therefore, IFNγ could be a therapeutic target for treating or preventing cognitive dysfunction associated with inflammation (Monteiro et al., 2016). Considering these factors, neopterin may provide a connection between inflammation and mental health by linking immune system activation and neurotransmitter abnormalities (Dunbar et al., 1992; Klaus et al., 2021). However, since neopterin can be influenced by other factors, it should not be used solely as a marker or to discriminate severity but should be combined with other mental health measures.

ANS Measures

The HRV results of certain indices were significantly lower in Group A. This is suggestive of having reduced ANS flexibility and resilience to stress (An et al., 2020) and that HRV is decreased among those with high mental health scores. Higher variability in heart rate has been associated with better health, self-regulation, adaptability, and resilience. Although the “normal” range for an individual is based on age and sex, it should be noted that females tend to have a higher mean heart rate, which means smaller NN intervals, and lower SDNN when compared to males. In addition, HRV time-domain measurements decrease with age (Shaffer & Ginsberg, 2017).

Lower SDNN and SD2 in Group A suggest increased SNS activity (or reduced PNS activity) in individuals with mental health struggles. This is supported by other studies that found increased sympathetic activity in depression (Singla et al., 2020) and anxiety (Holwerda et al., 2018), and reduced autonomic flexibility in patients with anxiety disorders (Hoehn-Saric & McLeod, 1988). Furthermore, it has been suggested that ANS imbalance is involved in the pathogenesis of anxiety and depression (Godoy et al., 2018).

Mean HRV correlated with stress, anxiety, and depression scores. This is supported by meta-analyses which also found that both anxiety disorders (Chalmers et al., 2014) and depression (Kemp et al., 2010) are associated with decreased HRV. HRV has been found to be even more reduced in patients with comorbid anxiety and depression (Kemp et al., 2012). Additionally, measures like RMSSD and HF reflect parasympathetic activity, and the present study only found differences in parameters that reflect sympathetic activity. Yet, neither the SNS Index nor LF/HF ratio was significantly higher in Group A, which would indicate sympathetic dominance (Shaffer & Ginsberg, 2017). Thus, the involvement of sympathetic and parasympathetic activity in HRV parameters and mental health needs to be further investigated.

In addition to being a potential marker for mental health, reduced HRV has also been associated with other negative health outcomes, such as diabetes and obesity (Karason et al., 1999; Kudat et al., 2006). More importantly, HRV has been associated with CVD and risk thereof, and as such HRV could be a predictor of CVD (Hillebrand et al., 2013; Kubota et al., 2017). This provides a link between mental disorders and the high rate of comorbid CVD observed, in that both anxiety and depression increase the risk of CVD. In fact, anxiety can be considered a predictor or early marker of CVD risk (Chalmers et al., 2014). Furthermore, comorbid anxiety and depression increase the risk of mortality and CVD by two to threefold (Vogelzangs et al., 2010). Given that CVD is the leading global cause of death (Roth et al., 2018), this relationship is important to note. Addressing mental health problems, particularly in student populations, not only impacts the present but may also help prevent future health problems.

The present study only found significant associations between mean HRV, SDNN, SD2, and mental health, which are inconsistent with previous findings. This highlights that there is some kind of relationship between HRV and mental health, but that further research is required. In addition, the relationship between sympathetic and parasympathetic balance in HRV parameters needs to be further investigated. Despite the discrepancies in the results between the present study and other studies, HRV should not be discounted as a viable biomarker as there is a large body of evidence to suggest otherwise.

In terms of BVP, minimum and mean BVP amplitude negatively correlated with the anxiety and depression scores, but not with the stress score. BVP decreases with increasing sympathetic activity, and increases with decreasing parasympathetic activity (Gordan et al., 2015; Peper et al., 2007; Sarchiapone et al., 2018). Therefore, the results of the present study suggest that BVP may decrease (and sympathetic activity may increase) as symptoms of anxiety and depression increase. This is supported by the correlations found with minimum BVP amplitude, which suggests that the lower the minimum value recorded, the greater the anxiety and depression scores. This is of interest considering that stress is usually more acute and is regularly associated with activation of the sympathetic activity, yet BVP only correlated with anxiety and depression scores and not stress.

The results from the present study suggest that BVP could be a potential biomarker for anxiety and depression scores. However, not much research has been done concerning BVP and mental health, thus further investigation is required.

qEEG Power

Of the 19 electrodes used in the EEG, six electrodes showed a significant association with the DASS scores. These electrodes resided in the frontal and left side of the brain, five of which were on the frontal lobe and the other one was located at the left midtemporal lobe. These findings suggest altered function in the prefrontal cortex, which is involved in emotion, cognitive function, and motivation (Perlstein et al., 2002).

The power of delta frequencies showed the greatest associations. The power of delta at the FP1, FP2, and Fz electrodes was significantly higher in Group A than in Group B. These electrodes measure activity from the left and right Brodmann area 10 and the left Brodmann area 8, respectively. The location of FP2 appears to be largely involved and could be a predictor of the severity of the symptoms, given that the absolute power of delta at FP2 had a significant positive correlation with both the stress score and the anxiety score. This is suggestive of delta power in the prefrontal cortex increasing as stress or anxiety symptoms increase, which might make focusing and performing tasks difficult as the FP1, FP2, and Fz electrodes are associated with executive function (e.g., planning, decision-making, working memory), self-regulation, regulation of emotions, and social behavior (Warner, 2013). Additionally, Brodmann area 8 includes the frontal eye field, which is involved in visual attention and control of eye movements; therefore, visual disturbances may also be present, exacerbating

feelings of detachment from the surroundings (Watanabe, 2017), due to higher delta activity (Sroykham & Wongsawat, 2019; Warner, 2013).

Significant associations were also found in the alpha frequency band. The relative power of alpha was significantly lower at the T3 electrode in Group A, and negatively correlated with stress and anxiety scores. As alpha waves are associated with alertness and relaxation, low alpha can be indicative of anxiety (Warner, 2013). The T3 electrode records activity from the left temporal lobe, which is usually the dominant side in most people. It is involved in memory, learning, perception, hearing, speech, and understanding language (Guy-Evans, 2021). Damage to this area can result in impaired memory, executive function, learning, speech and understanding thereof. Other effects include apathy, memory loss, and poor impulse control (Guy-Evans, 2021). Specifically, the T3 electrode records activity from Brodmann areas 41 and 42 which form part of the primary auditory cortex. This area is involved in speech perception, sound intensity, pitch, auditory working memory, and the processing of auditory information. There is sparse information on the effect of alpha oscillations in the auditory cortex; however, some research suggests that alpha waves are involved in selective auditory attention, speech processing, and tinnitus (Malekshahi et al., 2020; Schlee et al., 2014; Strauß et al., 2014). In relation to this, almost half of tinnitus patients also have a mental disorder, mostly anxiety and depression, which correlates with the severity of tinnitus symptoms (Pinto et al., 2014; Zöger et al., 2006).

In terms of both delta and alpha waves, a study found increased delta power and decreased alpha power in elderly patients with mild cognitive impairment (Sroykham & Wongsawat, 2019). Therefore, it could be interpreted that participants in the present study with high DASS scores, which were associated with increased delta and decreased alpha, might be suffering from cognitive impairment, a symptom of mental health struggles (Trivedi, 2006). An alternative interpretation is that changes in brain waves could help explain the cognitive impairment observed in patients with mental health problems. If cognitive impairment is related to the increased delta and decreased alpha, then this might negatively impact the academic performance of those with mental health symptoms; mental health struggles impact academic achievement (Awadalla et al., 2020; Eisenberg et al., 2009; Jamil et al., 2022; Vitasari et al., 2010; Wagner et al., 2022). However, caution should be exercised with these interpretations given the age difference between the present study and the study with elderly patients.

Hibeta was found to increase in the positions of F3 and T3 as the stress score increased, and hibeta increased in the positions of F3 and F7 as the anxiety score increased. Excess hibeta has been associated with being tense and anxious, and it can be indicative of inefficient frontal alpha activity in areas associated with emotional control (Warner, 2013). Considering that decreased alpha and increased hibeta were found at both the T3 and F7 positions in Group A, difficulties with emotional control could be associated with their mental health scores. Furthering this, decreased frontal alpha and increased hibeta is thought to be indicative of agitation, anxiety, feeling overwhelmed, and impulsivity (Warner, 2013). As there may be an inverse relation between alpha and hibeta, increased hibeta and concomitantly decreased alpha at T3 might produce alterations in auditory processing.

The F7 electrode measures activity from Brodmann area 47, which is in the orbitofrontal cortex. The function of this area involves motivation, social behavior, and emotional reactions. Interestingly, the orbitofrontal cortex has been implicated in disorders involving thinking, feeling, or fear, with altered activity during sadness, depression, and distress (Mayberg, 1997). In conjunction with F3, activity at F7 is thought to regulate engagement, mood, processing of positive emotions, and conscious awareness. Alterations in alpha and hibeta activity in these areas may be linked to aberrations in these processes in mental health conditions. Specifically, increased hibeta at the F3 electrode is thought to indicate that a patient is hiding emotions and feelings, although this effect occurs with a simultaneous increase at FP2 (Warner, 2013)

The activity at three electrodes (FP2, F3, and T3) correlated with both stress and anxiety scores, which suggests that these regions are involved in both stress and anxiety symptoms. This is supported by previous research which found that brain regions involved in anxiety, such as the prefrontal cortex, are also implicated in the stress response (Shin & Liberzon, 2010).

In summary, people with mental health struggles, particularly those with high stress and anxiety scores, might present with increased delta and hibeta, and decreased alpha activity, in the frontal and left side of the brain. Differences at the T3 and F7 locations occurred in more than one frequency,

which might be related to difficulties with attention, focus, cognition, emotional regulation, and visual and auditory processing. Therefore, delta, alpha, and hibeta frequencies could potentially be used as biomarkers for stress and anxiety scores.

Potential Biomarkers

The most promising biomarker in this study was neopterin concentration, as it showed a significant difference between Group A and B, and crucially neopterin showed a strong positive correlation with all three of the DASS score categories. Therefore, neopterin could be of use in aiding the measurement of stress, anxiety, and depression scores. An advantage of using neopterin as a biomarker is that it can be obtained simply and noninvasively via a urine sample.

There appears to be a negative relationship between mental health and HRV (mean HRV, SDNN, and SD2). Although the present study did not find the same associations in HRV parameters as other studies, HRV should not be discounted as a biomarker. More research is required to define the best parameters and ranges thereof that can be considered normal or at-risk. These definitions will need to produce consistent, reliable, and reproducible results. HRV may be a particularly important marker in students as it has been related to attention, emotional processing, and executive function (McCraty & Shaffer, 2015), which are important for university success.

Due to the number of frequencies and electrodes that showed a difference with DASS scores, qEEG could also be a viable biomarker. However, there are some factors to consider such as recordings can vary depending on the state of the individual and EEG can be quite sensitive. Further research is required to validate the findings of the present study and to verify changes in brain activity in relation to mental health scores and increase the validity of measuring such differences.

Limitations

The measurements in the present study were taken from one moment in time and may not reflect the average lives of the participants. The recording time was relatively short, and no follow-up measurements were taken at a later stage.

Other lifestyle factors were also not recorded, such as exercise, supplements, sleep, diet, and alcohol consumption. Participants were also not asked if they had participated in intense exercise on the day or if they had an upcoming academic assessment, which could have affected perceived anxiety.

All the biomarkers used in this study can be affected by biological factors other than mental health. Therefore, studies using such biomarkers should consider their results to be interpreted with caution and with professional psychological assistance.

Future Directions

Further investigation into the differences in neopterin, HRV, BVP, and qEEG parameters in comparison to mental health scores in different groups of students, different ages, and the differences between sex is required. Furthermore, studies that consider comorbidity and lifestyle should also be conducted to consolidate associations. Finally, studies that investigate the exact mechanisms that are involved are necessary to establish causation and not just correlation. With regard to neopterin, determining whether oxidative stress, IFNγ, macrophages, the neopterin molecule itself, or inflammation in general are the cause(s) of correlation with mental symptom severity, may aid in developing therapeutic targets.

Conclusion

Social stigma, together with misdiagnosis and subjectivity can interfere with the detection, prevention, and treatment of mental health issues. This study found that neopterin and certain neurophysiological measures could be used as complementary markers for stress and anxiety symptom scores as determined by the DASS-21 questionnaire.

Measurements such as HRV, BVP, qEEG, and neopterin may have potential to be used as biomarkers in conjunction with existing measures such as questionnaires. The inflammatory and noteworthy neurophysiological changes associated with increased stress and anxiety contribute to our understanding of mental health. Identifying physical changes associated with mental health conditions could be useful in the prevention, identification, and treatment of these struggles. This is prudent considering that anxiety is the leading mental health disorder, and that stress and anxiety are associated with inflammation, another major contributor to disease.

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Author Declaration

The authors declare no conflict of interest.

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