Original Research Article

# NON-INVASIVE DIAGNOSTIC MARKERS OF PSYCHOLOGICAL STRESS

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**A B S T R A C T**

**Background:** Psychological stress (PS) leads to different physiological responses including increased heart rate (HR) as well as decreased heart rate variability (HRV) through alterations in the autonomic nervous system (ANS), specifically increased sympathetic nervous system (SNS) activity and decreased parasympathetic (PNS) activity. Emerging evidence indicates that PS advances to certain alteration in the kidneys, which leads to increase in protein excretion and expression of high and low molecular weight proteins in the urine. Currently investigated biochemical markers for confirmation of stress include invasive, cumbersome and costly markers. This issue arises a need to introduce non-invasive and affordable stress markers which can be used globally.

**Materials and Methods:** A total of 120 cases and 106 controls (who were without any obvious stress) belonging to age group of 20 to 60 years were selected. Patients were evaluated for clinical history and assessed by the psychiatric stress scale, perceived stress scale (PSS) and presumptive social life event scale (PSLE). HRV was measured by ECG using lead II configurations using both time domain and frequency domain methods. Urinary protein was analysed by Vitros 5.1 FS from Ortho Clinical diagnostics by Johnson and Johnson USA.

**Results:** PSS was most strongly correlated with Power (ms2) LF/HF followed by Power (nu) HF and Power (nu) LF with a correlation coefficient of 0.805, -0.695 and 0.592 respectively. PSLE had a significant relation with Protein: Creatinine ratio.

**Conclusion:** In short term HRV, frequency domain parameters had significant correlation with perceived stress and proteinuria had significant correlation with PSLE.

**Keywords:** Stress, PSS, PSLE, HRV, Proteinuria.

## INTRODUCTION

Psychological stress is a global problem and every individual experiences it in one stage of their life or another. Being an elemental part of life, stress cannot be isolated from human life. Individuals often do not identify or appreciate the existence of stress with in them and tend to neglect the routine stressors. Negligence can affect the quality of life. It is further documented to serious physical & mental health related problems.[1] It is important to acknowledge the existence of stress. If we can perceive its warning signals promptly, prevention of possible subsequent deleterious effects could be minimised.

Acute stress response, generally do not charge health issues in a young healthy individual except in certain circumstances. However, persistent threat, especially in older or unhealthy individuals, may challenge health due to the cumulative effects of the stress response for longer period.[2] It is well documented that numerous diseases are associated with stress.[3]

Psychological stress (PS) is the equilibrium adjustment induced by psychological factors which may involve several general and emotional stressors.[4] PS is often associated with physiological and biochemical changes.

Methodologies to assess PS have been evolved with the aim of timely diagnosis of stress, to prevent the several adverse outcomes related to it. Stress assessment and its measurement are as important as taking treatment for other disease. The stress can be assessed subjectively by means of classical psychological questionnaires. Different psychiatric tools have been developed for different psychiatric problems. Stress can be measured objectively by biochemical specimens as urine/blood/saliva etc or by other non-invasive physiological markers like heart rate variability (HRV), which makes use of bodily response occurring during stress.[5]

### Stress scales

*Presumptive social life events score (PSLE)-* The PSLE stress scale is a modified form of Holmes and Rahe stress scale which assesses the stress based on different life events every individual confronts in their life.[6] Larger numbers of stressful life events experienced during a specific period of time are associated with a greater susceptibility to illness.[7]

*Perceived stress scale (PSS)-* PSS was developed by Sheldon Cohen.[8] PSS measures the intensity to which situations are perceived as stressful. [9,10] PSS is recommended to make correlation between individual's perceived stress related to contemporary events and is not a diagnostic tool. More advanced PSS score indicates the higher risk factor for few disorders. PSS is not confirmed to particular situation thus, appropriate for current life events.[11]

### Diagnosis of stress

An array of psychiatric problems and other serious illness could arise from stress. Proper diagnosis helps in the prevention and management of these serious outcome. Diagnosis includes proper patient history and subjective questionnaires based psychiatric tools.

Although, for comprehensive evaluation of PS, physiological and biochemical parameters appear to be more reliable and objective.

Currently investigated biochemical stress markers include Cortisol, Prolactin, T3, T4, TSH, etc. [3,12] Measurement of physiological parameter- Heart Rate Variability (HRV) also seems to be a promising approach.[13]

### Proteinuria and stress

Proteinuria is the appearance of proteins > 150 mg/dL in urine. kidney's glomerular membrane act as an ultra-filter for plasma proteins. Large protein molecules are restricted by the charge/size barrier. Relatively small protein molecules are absolutely reabsorbed by renal tubules. The limited amount of protein present in urine predominantly is albumin. Tubular proteinuria is identified by the presence of low molecular weight protein in the urine. It may either appear alone or related with glomerular proteinuria generally. Specific tests are required to detect tubular proteinuria.

Emerging evidence indicates that PS develop certain alteration in the kidneys, which leads to the expression of high and low molecular weight proteins in the urine. [8,14,15]

### Heart rate variability (HRV)

Psychological stress alarms body by generating stress responses for instance, increased heart rate (HR) and decreased heart rate variability (HRV) via influencing autonomic nervous system (ANS). These changes are outcome of over sympathetic nervous system activity and decreased parasympathetic nervous system (PNS) activity. If continued, such alterations can lead to different cardiovascular diseases. [8,14,15,16]

HRV is the beat-to-beat differences in either heart rate or in the R-R interval duration - the heart period. HRV is generally measured by electrocardiogram (ECG). HRV marks psychological well-being, cardiovascular health and can also predicts mortality. [17,18,19]

There are two primary methods for the analysis of HRV, Time domain and frequency domain. In short term HRV, frequency domain method is preferred.[20] HRV appears to be an optimistic proposition for assessment of stress (acute and chronic).[21]

The search for stable stress biomarkers remains a challenging task for researchers and clinicians due to several restrictions. Each stress marker has its own pros and cons. Most of them are invasive for e.g. serum cortisol, DHEA, etc.

Non-invasive markers are expensive and require tedious pre-processing like as in catecholamines, which is also challenging. At present, there is no universally recognized standard markers for stress evaluation. Such issues arise need of non-invasive and affordable markers for global use.

The purpose of the present study was 1) To examine the relation among perceived stress, life event stressors, proteinuria and HRV in individuals suffering from psychological stress.

2) To introduce urinary Protein & HRV as cheap and non -invasive stress diagnostic markers.

## MATERIALS AND METHODS

The present study was hospital-based case control study conducted in the Biochemistry department in collaboration with research lab of Physiology department and Psychiatry department of the Subharti Medical college.

A total of 120 cases of stress and 106 age and sex matched controls (without any obvious stress) belonging to age group of 20 to 50 years were selected. Ethical clearance was taken from the institutional ethical committee and research council. After obtaining the informed consent patients were evaluated for detailed past history, clinical history,

family history and assessed by the psychiatric stress scales, PSLE and PSS.

**Experimental protocol** – After psychiatric assessment by perceived stress scale, patients with significant stress scores were included and asked to attend the research lab in department of physiology for ECG. Also, their urine sample were collected, centrifuged and stored at -20°C and -80°C in aliquots for presence of biochemical markers i.e. Microalbumin/protein/creatinine which were later estimated on Vitros 5.1 FS chemistry analyzer from Ortho Clinical diagnostics by Jhonson and Jhonson USA.

For analysis of HRV, ECG was recorded in supine position for 7-10 minutes using a standard limb lead II electrode configuration. Button electrodes were placed at right arm, left arm and left leg to obtain recording of heart beat interval.

The analysis of HRV was done by frequency domain (HF, LF and VLF) method using RMS Polyrite D version 2.4.

**Statistical Analysis**

Spearman rank correlation coefficient was used to assess the association of various parameters with each other. Quantitative variables were compared using independent T test/Mann-Whitney test (when the data sets were not normally distributed) between the two groups. Qualitative variables were correlated using Chi-Square test. A p value of $< 0.05$ was considered statistically significant. The data was entered in MS Excel spread sheet and analysed by statistical package for social sciences (SPSS version 21.0).

# RESULTS

In our study the mean age of the cases was $32.89\pm8.6$ years and the mean age of the controls was $31.91\pm 8.7$ years. Age of the cases and controls were comparable ($p>0.05$).

On analysing the correlation of ACR with the other parameters among the cases in our study, it was found that there existed a significant correlation of ACR with PSLE, PSS, RMSSD and SDNN ($p<0.05$). ACR was most strongly correlated with PSLE (correlation coefficient r = 0.548). On analysing the correlation of PCR with other parameters among the cases in our study, it was found that there existed a significant correlation of PCR with PSLE ($p<0.05$). PCR was strongly correlated with PSLE with a correlation coefficient of 0.768 and was negatively correlated to LF. On analysing the correlation of PSS with other parameters among the cases in our study, it was found that there existed a significant correlation of PSS with ACR (mALB) HF, LF & LF/HF ($p<0.05$). PSS was strongly correlated with LF/HF power (ms2) followed by HF power (nu) and LF power (nu) with a r value of 0.85, -0.695 and 0.592 respectively. The correlation of PCR with all the

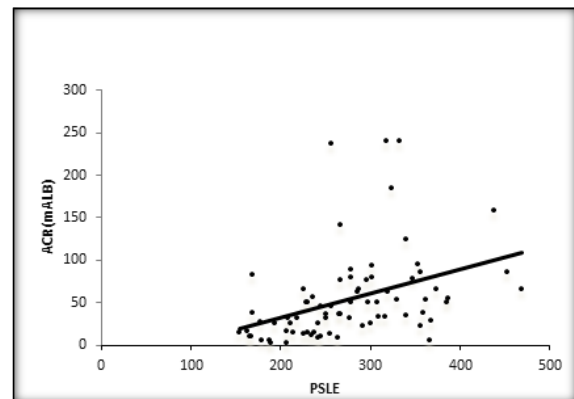other parameters has been depicted in the scatter plots shown in figures.

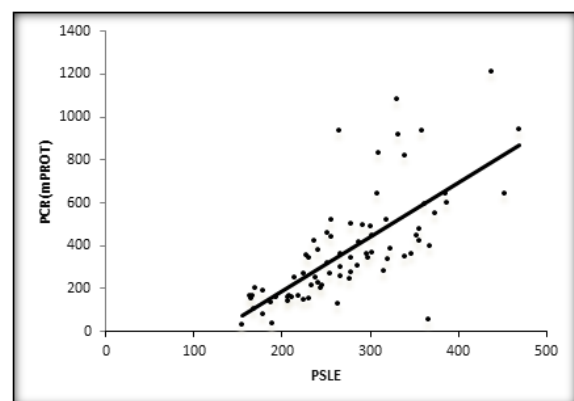

**Figure 1: Correlation between PSLE and ACR (mALB)**



**Figure 2: Correlation between PSLE and PCR (mPROT)**
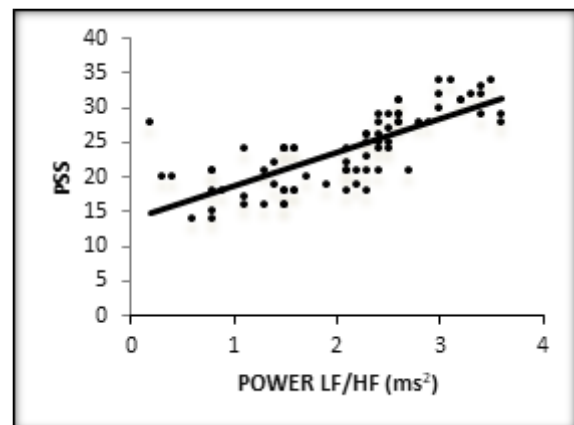


**Figure 3: Correlation between POWER (ms2) (LF/HF) and PSS**

# DISCUSSION

The present case control study was aimed primarily to explore the resting HRV, microalbuminuria and proteinuria as non-invasive psychological stress marker. The stated aim was fulfilled by examining the correlation between the perceived stress, life events, urinary proteins & resting HRV. The study demonstrated a significant relationship between

perceived stress, major life events with urine albumin-creatinine ratio (UCAR) and urinary protein: creatinine ratio (UPCR). A significant relationship was found between the stress scores with a few HRV parameters too. The major findings of the present study were the strong and significant association between proteinuria with PSLE scores and HRV with PSS scores. A moderate association was found between UACR and PSLE score. Previous supportive studies have reported increased proteinuria and albuminuria (Ratnakar & Dalui et al), [15,22] and decreased HRV (Chandola et al, Michelis et al), [23,24] in stressed individual.

The present study demonstrated that high scores of life event stressor was associated with raised urinary proteins. It also demonstrated correlation between certain HRV parameters and perceived stress in cases, as compared to controls. Ratnakar et al,[15] demonstrated depression, anxiety and proteinuria amongst primary caregivers of patients with cancer. Similarly, Dalui et al,[22] demonstrated depression, anxiety and albuminuria amongst primary caregivers of patients with mental illness. PS thus can be associated with caregiving to mentally ill and cancer patients and can be one of the determinants of protein excretion rate in otherwise healthy subjects. The UPCR was significantly higher among the cases than controls as shown in earlier studies. [15,22]

Ratnakar et al,[15] in their study indicated that urinary protein excretion might be employed as potential marker for stress in subjects who did not suffer from any obvious kidney disease. They found that HADS (measure of stress) and UPCR were significantly high in the caregivers as compare to controls. They further concluded that psychological stress leads to increased oxidative stress and glomerular permeability to proteins. A few other studies supported this observation. [14,25] Spearman rank correlation coefficient in cases demonstrate a correlation between UPCR, UCAR ratio and PSLE scores. PSLE was more strongly correlated with UPCR as compared to UACR. Hence it is evident from the results that an increase in stress factors (assessed by PSLE) is associated with an increase in UPCR. This finding can be explained by the fact that major life events act as stressors, leading to significant PS that effects the renal system. PS generates transient alterations in the kidney function probably by affecting glomerular/tubular function, resulting in excretion of proteins in general, without significantly affecting albumin excretion in particular. Cases as well as controls were extensively screened for other physiological as well pathological causes of proteinuria. Hence, this marked difference between the two could be due to stress induced by major life events acting as stressors.

In our study none of the controls had microalbuminuria or significant proteinuria. Most of the cases had microalbuminuria values at a lower side. Few cases did not show microalbuminuria at all but showed significant proteinuria. The cases were apparently normal and did not report any obvious illness. With the above findings and facts, we propose a hypothesis that stress can lead to the efflux of proteins by alteration in charge distribution across the membrane Na-K-ATPase pump. Urinary microalbuminuria estimation is cost intensive for routine screening purposes. Urinary microprotein estimation is cost effective and easy to perform even in a small laboratory set up. Also, it has a potential to be used as a routine diagnostic aid for assessment of PS. It was evident from the results that increased major life events and its perception may lead to excretion of urinary proteins that are not present in urine of healthy subjects. Further our study involved the correlation between the life event stress score, perceived stress and resting HRV. Major findings of this aspect of the study were a significant reduction in the HF, increases in the LF component and increased LF/HF ratio in stressed individuals. Difference in HF, LF component and LF/HF ratio was statistically more in cases as compared to other study parameters. Our findings were consistent with previous studies done by Chandola et al, Michelis et al, Punita et al, [23,24,26] which also reported similar findings. As expected, difference in HRV parameters between the cases and controls were seen. Frequency domain parameters showed strong and significant correlation with PSS. PSLE appears to be weakly associated with HRV parameters. It is obvious from our study that despite the significant major life event stressors, it is our perception for the stress, which probably leads to imbalance in the sympatho-vagal activity and reduction in HRV. LF/HF ratios increased significantly in stressed subjects. This tendency towards an increase could be explained by sympathetic over activity under stressful conditions, of which diminished HRV could be one such marker. HRV had less significant correlation with proteinuria. Life events stressors and overall perception to stress in cases might increase LF component of HRV. As increased LF shows sympatho-vagal predominance and reduced vagal tone it suggests over activity of sympathetic nervous system. Blood supply of kidney is subject to central nervous system control. Under psychological strain vasoconstriction in the renal vascular bed occurs in normotensive subjects.[25] Sympathetic activation leads to vasoconstriction and other related biochemical changes in kidneys which probably leads to proteinuria.

**Limitations:** the major limitations of this study were the different education background in the subjects of this study. Extracting accurate information and history from less literate patients were fairly challenging. Besides, it was also not possible to collect urine for the three times on different days due to feasibility issues. We tried to compensate for this diurnal variation by estimating both albumin: creatinine ratio, as well as the protein: creatinine ratio. Another lacuna of the present study includes the absolute lack of data generated pertaining to the urinary protein profile. This makes

it impossible for us to throw a molecular size/chart insight into the possible mechanism involved in the entire process. Without performing electrophoresis, immunological tests and protein sequencing etc, one cannot identify the type of proteins involved. Again, in case of HRV, we faced the anxiety in a few subjects on putting electrodes during HRV assessment, which could influence HRV readings. Although in such cases recordings in the initial few minutes were edited to remove ectopic beats or artefact, generated due to anxiety. One of the considerable strengths of this study was the use of disposable button electrodes, which were more accurate than the three pin wires-based electrodes and another strength of this study was significantly longer durations (7-10 min) of measurement as compared to studies done in past. Other strengths of the study included the estimation of urinary microalbuminuria and microprotein, using a fully automated analyser. This decreases the possibility of analytical errors and variations during standardization. Collaborated use of psychological, biochemical and physiological measures to assess the psychological stress further broadened our view of understanding stress, thus making the study more impactful.

## CONCLUSION

Psychological stress is subject of immense interest in modern times as more and more clinical conditions get extrapolated to it. It is well documented that chronic stress, if left undiagnosed, can lead to serious outcomes in form of physical or psychological disorders. Hence early diagnosis and management of stress is crucial to prevent such deleterious outcomes.

Stress assessment per se is important for assessment of its own magnitudes, as well as for planning an overall therapeutic approach to several other conditions. Stress can be assessed subjectively by use of psychological questionaries or objectively by using biochemical markers present in blood/urine or saliva. Currently employed markers are either invasive and costly or difficult to standardize.

The present study was primarily intended to:
- Introduce cheap and non-invasive diagnostic markers of psychological stress

**The major findings of the study were**
- Significant correlation between PSLE and Urinary protein and microalbumin.
- Significant correlation between PSS and frequency domain parameters (LF, HF and LF/HF ratio)

Urinary Microprotein measurement may provide superior diagnostic information, in the context of stress analysis. It also has the potential of being used as a marker for stress in population studies.

However, in order to fine tune our understanding of proteinuria in these cases, including the likely mechanism involved, we propose the identification of the proteins excreted.

In short term HRV, frequency domain parameters had significant correlation with perceived stress. Diminished HF, increased LF and LF/HF ratio can be used as diagnostic marker for stress in otherwise healthy individuals.

We all are exposed to stressors but the way we perceive these stressors ultimately defines our stress levels. Routine check-up using the two markers (UPCR & HRV) could help individuals to identify the early stress signals and thus help to make appropriate pre-emptive modifications in their lifestyle.

In our study it was practically not feasible to repeat HRV and urine testing. Hence, further extensive studies need to be carried out with literate and aware subjects, with repeated HRV (or with long term HRV) and Urinary Protein, Albumin and Creatinine estimations, to get more conclusive and accurate results.

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