ORIGINAL ARTICLE

STRESS HORMONE LEVELS AMONG CHILDREN BEREAVED BY TERROR ATTACK

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Background: Terrorism is characterized by the use of violence against civilians, with the expressed desire of causing terror or panic in the population. Since the Soviet invasion of Afghanistan in 1979, the Khyber Pakhtunkhwa (formerly NWFP) province of Pakistan has suffered the most from unrest and terrorist activities. Terror related bereavement is more stressful and difficult to recover compared from that of bereavement from natural death. **Methods:** This retrospective study was carried out in the Department of Physiology, IBMS, Khyber Medical University, Peshawar, Pakistan. Salivary Cortisol Levels were estimated in Non-Bereaved and Bereaved Groups of children. **Results:** Children in the Bereaved Group showed a statistically significant (p=0.005) higher levels of salivary cortisol in the morning sample (Log Cortisol M=14.43) as compared with the children in the Non-Bereaved Group (Log Cortisol M=6.44). Children in the Bereaved Group showed a statistically non-significant (p=0.164) decrease in the levels of salivary cortisol in the post-dexamethasone sample (Log Cortisol M=8.85) as compared with the children in the Non-Bereaved Group (Log Cortisol M=4.25). **Conclusion:** Terror bereavement causes long-term activation of the Hypothalmo-Pituitary-Adrenal (HPA) axis, elevating the cortisol levels even 3–4 years after the traumatic bereavement. Chronic elevation of cortisol results in the manifestations of depression related signs and symptoms in bereaved children.

Keywords: Terrorism, Bereavement, Salivary Cortisol levels, Depression

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INTRODUCTION

Terrorism is characterized by the use of violence against civilians, with the expressed desire of causing terror or panic in the population.[1] The Global Terrorism Index (GTI) defines terrorism as 'the threatened or actual use of illegal force and violence by a non-state actor to attain a political, economic, religious, or social goal through fear, coercion, or intimidation.' This definition recognizes that terrorism is not only the physical act of an attack but also the psychological impact it has on a society for many years after. The intensity of global terrorism has decreased since the peak of terror activity in 2014. Pakistan was among five countries which accounted for three quarters of all deaths from terrorism in 2016. As regards the deaths related to terrorism, 2016 was the third deadliest year since 2000.[2]

Since the Soviet invasion of Afghanistan in 1979, the Khyber Pakhtunkhwa (formerly NWFP) province of Pakistan has suffered the most from unrest and terrorist activities.[3] Bereavement is a state of sadness or mourning after the death of some loved one, close relative, sibling or even a friend.[4] Terror related bereavement is more stressful and difficult to recover compared from that of bereavement from natural death.[5] Childhood bereavement due to terrorism is associated with increased emotional and behavioural problems, depression, suicide, post-traumatic stress disorder (PTSD), alcohol or substance use in adulthood.[6]

Trauma and psychological stressors are some of the most potent stimuli of the endocrine stress response, i.e., non-specific activation of the hypothalamic-pituitary-adrenal (HPA) and sympatho-adreno-medullary (SAM) axes occur.[7] In humans, both high and low levels of HPA activity had been related to stress sensitive mental disorders, which responded well while treating some patients by exogenously giving glucocorticoid.[8]

Dexamethasone, a corticosteroid, shows similar effects to those of cortisol and provides an estimate of corticosteroid feedback sensitivity. In normal individuals, the morning salivary cortisol levels were diminished due to negative feedback exerted on HPA axis by giving exogenous dexamethasone. However, this level will remain higher in chronically depressed persons due to dysfunctional HPA axis negative feedback mechanism.[9] It was hypothesized that bereaved children due to terror related deaths would have greater HPA axis activation as compared with the non-bereaved children. That hyper-activation in bereaved children would be evidenced by higher basal salivary cortisol levels. It was also hypothesized that there would be less dexamethasone suppression of basal salivary cortisol in bereaved children.

PARTICIPANTS AND METHODS

This study was conducted in the Institute of Basic Medical Sciences (IBMS), Khyber Medical University

(KMU), Peshawar, Pakistan during 2015–18. Approval was taken from the Institutional Ethical Review Committee of the Khyber Medical University. Written informed consent was obtained from all participants. A planned questionnaire was used for interview which was done face to face to obtain the correct psychological and behavioural information. A General Physical Examination was also carried out to obtain the anthropometric data.

Eighty-eight children were randomly recruited from the local population. They were divided into two groups, i.e., Bereaved Group and Non-bereaved Group. Bereaved Group had 49 children from 35 families and Non-bereaved Group had 39 children from 33 families. The bereaved participants were inducted through local '*Jirga*' (assembly of some elders) of the North-West part of Khyber Pakhtunkhwa province which was badly affected area of terrorist activities. Non-bereaved children were selected from the same socio-demographic background from settled areas of Peshawar. Ages of the participants ranged between 8 and 18 years. The bereaved children included 46 (93.9%) boys and 3 (6.1%) girls. Time was calculated as time between the terror event (incidence) and time of filling questionnaire and taking blood. In the case of controls (non-bereaved) time was calculated as time after natural death of parent or any other loved one.

Bereaved children were included if they suffered from bereavement due to terror attack associated loss of parents or close loved ones or if they were themselves injured in the attack. Time since bereavement was about 3–5 years in the Bereaved Group. In case of loss (death) of multiple loved ones, nearest one was reported in data as the cause, e.g., if one parent and uncle were lost, only parent was reported as cause of bereavement.

All the non-bereaved children were apparently healthy, normal subjects and did not experience terror attack related sudden loss of their parents or loved one. Bereaved children having psychopathology before the terror attacks were excluded from the study; children showing symptoms of any disease on physical examination were also excluded from the study. Children using any drug which alters hormonal levels such as antidepressants, antipsychotic and anti-anxiety drugs were also excluded from this study.

For salivary cortisol collection microfuge tubes and a real cotton balls were used. At the time of sample collection children were instructed to pop the cotton ball into their mouths and roll it with the help of their tongue and gently chew to saturate the cotton ball completely with the saliva for one minute. After complete saturation, the saliva from the cotton ball was squeezed in to the microfuge tube. The saliva thus collected was kept in ice box and rapidly transferred and stored at -80 °C till analysis. All the participants were restricted to exercise or eat for at least 30 minutes prior to sample collection. Three salivary samples were collected at different time intervals, i.e., morning (M, 8.00 AM), evening (E, 6.00 PM) and post-dexamethasone sample (PD at 7.00 PM). A dose of 1 mg dexamethasone tablet was given orally to each child in the presence of trained medical person soon after collecting the evening samples at 6.00 PM and post-dexamethasone samples were collected after one hour of dexamethasone treatment, i.e., at 7 PM. The analysis of sample was done within one month of collection.

Salivary cortisol concentration was measured by Enzyme Linked Immunosorbent Assay (ELISA) method using human cortisol saliva kit (Calbiotech Inc., 10461 Austin Dr, Spring Valley, CA) according to manufacturer's instructions. Duplicate samples were run for analysis and mean values for each sample were calculated. Cortisol values were log-transformed to correct for deviations from normality. Assay detection limit for cortisol was 0.01 ng/ml. Statistical analyses were carried out by using SPSS-19. The normality of the data was tested using Kolmogorov-Smirinov and Shapiro-Wilk tests and histograms. Summary statistics were calculated and data were expressed as Mean±SD. Comparison of categorical data between bereaved and non-bereaved was analysed using Independent Sample *t*-test, and $p \leq 0.05$ was accepted as significant.

RESULTS

Table-1 shows the anthropometric and behavioural parameters in children of the Non-Bereaved and the Bereaved groups. Time since bereavement was about 3–5 years in the Bereaved Group. Time since bereavement or death of any relative in Non-Bereaved was significantly different in bereaved (4.49±1.71 years) as compared to Non-Bereaved (0.28±1.23) ($p<0.001$). Ages of the participants in the two groups showed non-significant ($p=0.97$) differences. Body Mass Index (BMI) showed a statistically significant ($p=0.001$) lower values among children of Bereaved Group as compared with the Non-Bereaved group. All behavioural parameters showing signs of depression (Isolation, Suicidal Thought, Crying and Sighing, Difficulty in Sleeping, Fatigue, Headache, Appetite Loss) were present in higher number of children among the Bereaved Group as compared with the Non-Bereaved Group and the difference was statistically significant ($p=0.001$).

Children in the Bereaved Group showed a statistically significant ($p=0.005$) higher levels of Salivary Cortisol in the morning sample (Log Cortisol M=14.43) as compared with the children in the Non-Bereaved Group (Log Cortisol M=6.44). Children in the Bereaved Group showed a statistically non-significant ($p=0.512$) higher levels of Salivary Cortisol in the evening sample (Log Cortisol M=11.46) as compared

with the children in the Non-Bereaved Group (Log Cortisol M=8.96). Children in the Bereaved Group showed a statistically non-significant (p=0.164) decrease in the levels of Salivary Cortisol in the Post-Dexamethasone sample (Log Cortisol M=8.85) as compared with the children in the Non-Bereaved Group (Log Cortisol M=4.25). Although the dexamethasone suppressed the cortisol level (Log Cortisol PD=8.85) in the Bereaved Group as compared with their Morning levels (Log Cortisol M=14.43), their cortisol levels were higher than the morning levels in the Non-Bereaved Group (Log Cortisol M=6.44). (Table-2).

Table-1: Anthropometric and behavioural parameters in Non-bereaved and Bereaved Groups

Parameter	Non-Bereaved Control (n=39)	Bereaved Cases (n=49)	*p*
Time since Bereavement (Yrs)	0.28±1.23	4.49±1.71	<0.001
Age SQRT*	14.76 (15.60–13.83)	14.78 (15.58–13.90)	0.97
BMI (Kg/m^2)	20.26±3.40	17.72±3.28	0.001
Isolation	3 (7.7%)	41 (83.7%)	<0.001
Suicidal thought	0 (.0%)	23 (46.9%)	<0.001
Crying & sighing	0 (.0%)	47 (95.9%)	<0.001
Difficulty in sleeping	2 (5.1%)	45 (91.8%)	<0.001
Fatigue	3 (7.7%)	41 (83.7%)	<0.001
Headache	2 (5.1%)	40 (83.3%)	<0.001
Appetite loss	1 (2.6%)	44 (89.8%)	<0.001

SQRT=*Squared for analysis, values are geometric means

Table-2: Salivary Cortisol Levels in Non-Bereaved and Bereaved Groups [Mean (Range)]

Parameter	Non-Bereaved Control (n=39)	Bereaved Cases (n=49)	*p*
Log cortisol M*	6.44 (4.60–8.99)	14.43 (9.71–21.44)	0.005
Log cortisol E*	8.96 (6.38–12.56)	11.46 (5.80–22.63)	0.512
Log cortisol PD*	4.25 (1.84–9.80)	8.85 (4.58–17.11)	0.164

*Log transformed for analysis, values are geometric mean.

DISCUSSION

Over the past more than a decade, terrorists killed an average of 26,000 people worldwide each year. The global death toll from terrorism over the past decade ranged from 8,200 in 2011 to a high of 44,600 in 2014.[10] In Pakistan, terrorism is increasing during the last three years.[11] Pakistan has already endured over US $ 126 billion economic losses and 83,000 people, including armed forces personnel, policemen, and other martyred.[12]

Although bereavement is always difficult, most children and young people learn to adjust and live with their loss over time. However, some are unable to process their grief if the traumatic way they perceive the death leaves them feeling profoundly unsafe. As a result, they are more likely to develop mental health problems and to have difficulties in areas such as relationships and school attainment. These children need significant support.[13]

This study reports the neuroendocrine alteration in parentally bereaved children who have participated in this retrospective study of the impact of sudden unexpected parental or close relative death during terrorist attacks during the global war against terrorism in the North-West part of Pakistan.

Bereaved children had significantly higher rates of psychiatric problems compared to non-bereaved children. Our results are in line with previous longitudinal studies on the levels of cortisol response and stress induced depression in bereaved children.[14,15] BMI of Bereaved Group children showed a lower value as compared with the Non-Bereaved Group. This may be because bereaved children had decreased appetite.

Salivary cortisol levels remained high in Bereaved as compared with the Non-Bereaved children. The level of morning salivary cortisol in bereaved children was significantly higher compared to non-bereaved children (p=0.005). The levels of evening and post dexamethasone salivary cortisol response were also high in bereaved children to non-bereaved control. The salivary cortisol levels remained high despite dexamethasone suppression suggesting an impaired central feedback. Our results are in line with previous longitudinal studies on the levels of cortisol response and stress induced depression in bereaved children.[14,15] The non-significant change in salivary cortisol response in the evening and post-dexamethasone group might be due to the small number of subject included in our study. However, discrepancies still exist in different studies and showed inconsistent salivary cortisol levels measured at different time intervals. Another study observed a significant increase in the evening salivary cortisol level rather than morning cortisol among children exposed to stressful events compared to control children not exposed to such events.[16] Similar variations in salivary cortisol measurement were also reported to be dependent upon gender, age and time of sampling.[17]

CONCLUSION

Terror bereavement causes long-term activation of the HPA axis, elevating the cortisol levels even 3–4 years after the traumatic bereavement. Chronic elevation of cortisol results in the manifestation of depression related signs and symptoms.

RECOMMENDATION

Further studies are warranted to examine changes in the HPA axis after exposure to chronic stress to understand how the evolving change in stress response and neuroendocrine alteration correlate to mental and physical health of bereaved children.

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