Association of maternal depression and home adversities with infant hypothalamic-pituitary-adrenal (HPA) axis biomarkers in rural Pakistan

Ashley K Hagaman[1,*], **Victoria Baranov**[2], **Esther Chung**[3], **Katherine LeMasters**[3], **Nafeesa Andrabi**[4], **Lisa M Bates**[5], **Atif Rahman**[6,7], **Siham Sikander**[7,8], **Elizabeth Turner**[9,10], **Joanna Maselko**[3]

[1]Department of Social and Behavioral Science, Yale School of Public Health, Yale University, New Haven, Connecticut, USA

[2]Department of Economics, University of Melbourne, Australia

[3]Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

[4]Department of Sociology, University of North Carolina at Chapel Hill, USA

[5]Mailman School of Public Health, Columbia University, USA

[6]Department of Psychological Sciences, University of Liverpool, UK

[7]Human Development Research Foundation, Islamabad, Pakistan;

[8]Health Services Academy, Islamabad, Pakistan

*****Corresponding author**: Ashley.Hagaman@yale.edu, Yale School of Public Health, Department of Social and Behavioral Sciences, Yale University, 60 College St, New Haven, CT 06510, Phone: 248.790.8954.

[9]Department of Biostatistics and Bioinformatics, Duke University, Durham, North Carolina, USA

[10]Duke Global Health Institute, Duke University, Durham, North Carolina, USA

Abstract

Background: Each year, almost 35% of children are exposed to maternal depression and more grow up in persistent poverty, increasing the risk for stress-related disease and other socio-developmental deficits later in life. These impacts are likely related to chronic stress via the hypothalamic-pituitary-adrenal (HPA) axis. However, there is little evidence relating early windows of child HPA axis activity to multiple exposures.

Methods: We investigated chronic measures of hair-derived HPA axis hormones (cortisol and dehydroepiandrosterone (DHEA)) in 104 one-year old infants from rural Pakistan and longitudinal measures of maternal depression, intimate partner violence (IPV), socio-economic status (SES), and the home environment.

Results: Estimates from adjusted linear mixed effects models did not reveal consistent significant associations between infant cortisol and maternal depression or home adversities. By contrast, infants exposed to maternal depression during pregnancy had lower DHEA levels (ß= −0.18 95% confidence interval [CI]: −0.34, −0.02) as did those whose mothers experienced multiple types of IPV (ß=−4.14 95% CI: −7.42, −0.79) within one year postpartum. Higher SES had a significant positive association with infant DHEA levels (ß= 0.77 95% CI: 0.08, 1.47). Depression severity and chronicity at one year postpartum had near significant associations with infant DHEA. Measures of home environment had no observable impacts on infant HPA axis activity.

Limitations: Limitations include the modest sample size and aggregation of hair samples for analysis.

Conclusion: Results point to possible early HPA axis dysregulation driven by changes in DHEA activity, but not cortisol at one year of age. Findings contribute to growing research examining intergenerational transmissions of maternal depression, IPV, and household environment on infant stress-response systems.

Keywords

Introduction:

Early life exposures to chronic stress, including maternal depression and maternal intimate partner violence (IPV), may have deleterious impacts on child health and development that persist into adulthood (Grace et al., 2003; Lupien et al., 2009; Walker et al., 2007). Growing evidence demonstrates that antenatal and postnatal maternal psychopathology has both proximate effects (e.g., birthweight, infectious disease, and preterm delivery) as well as enduring chronic effects (e.g., obesity, mental illness, poor brain development, and chronic disease), suggesting early life biological embedding of adversity (Berens et al., 2017; Staufenbiel et al., 2013; Weinstock, 2005). Specifically, the hypothalamic-pituitary-adrenal (HPA) axis is one pathway through which chronic stressful exposures are hypothesized to

become biologically embedded (Ehlert et al., 2001; Karlén et al., 2013; Laurent, 2017), though other pathways such as oxidative stress are also possible (Hovatta et al., 2010). Prior research has documented that toxic stress, the excessive activation of the stress response system, early in life through factors such as extreme poverty, abuse, or parental mental illness can have damaging impacts on a child's developing brain (National Scientific Council on the Developing Child, 2005). Moreover, experiencing toxic stress early in life can have a cumulative effect on adult physical and mental health (Shonkoff et al., 2012).

The HPA axis is the central stress response system apparatus that regulates the response of, and adaptation to, perceived stressors and threats. The HPA axis responds to stress by stimulating the adrenal release of hormones, of which, cortisol and dehydroepiandrosterone (DHEA) are two end products (Crowley and Girdler, 2014; Staufenbiel et al., 2013). Recent less invasive techniques have validated hair-derived HPA axis hormones as a reliable index of long-term HPA axis function (Kirschbaum et al., 2009). Several studies find both positive and negative correlations between stressors and cortisol, and negative correlations between DHEA and stress (Goodyer et al., 1996; Qiao et al., 2017; Staufenbiel et al., 2013). Research suggests that DHEA is an antagonist to cortisol, reducing its physiological impacts (Buoso et al., 2011; Pinto et al., 2015). In efforts to examine multiple HPA axis indicators and their relative concentrations, researchers recommend the cortisol/DHEA ratio as a useful marker, where lower levels are thought to indicate HPA axis dysfunction (Goodyer et al., 2003; Qiao et al., 2017). Such relationships, however, have yet to be elucidated in infants and young children using hair derived biomarkers, and the effects of perinatal psychopathology and stress, alongside the infant's home environment, on longer-term infant HPA axis activity is largely unknown.

Previous literature points to the early impacts of adverse social environments on infant cortisol levels including low socioeconomic status (Ursache et al., 2017; Vliegenthart et al., 2016), household income (Flom et al., 2017; Ursache et al., 2017), maternal psychosocial distress (Karlén et al., 2013; Mughal et al., 2018; Palmer et al., 2013), maternal IPV (Boeckel et al., 2017), and parental education (Bates et al., 2017; Rippe et al., 2016). However, few studies have examined chronic stress in infants using chronic measures of cortisol, and fewer examine the interrelationships of glucocorticoids (e.g., DHEA and cortisol) that constitute the HPA axis environmental system. Most of this literature focuses on maternal past trauma, uses crude measures of maternal exposure, and is often limited to children older than 2 years (Simmons et al., 2016; Slopen et al., 2018). Moreover, there is a need for understanding the role of DHEA in early development, especially in the stress response pathway and the risk for psychopathology (Kamin and Kertes, 2017). There is growing evidence for the importance of DHEA in neural development of infants (Quinn et al., 2018). Shifts in infant DHEA levels following the intergenerational transmission of maternal trauma may increase the risk of these children for suboptimal development and have enduring effects. Additionally, recent evidence suggests significant impacts of maternal psychopathology and stress on offspring cortisone dysregulation (Molenaar et al., 2019), but this has not been replicated in infants living in low-income environments. Therefore, much is left to be learned about a child's current environment (maternal exposures, home environment, and parenting) and its impact on child HPA-axis activity.

Determining exposure windows in early life that may have effects on HPA-axis function is an important step towards early identification and intervention of health and developmental risk in young children. The plasticity of the brain during infancy and early childhood makes it vulnerable to chemical influences, especially due to chronic stress (Shonkoff et al., 2012). Early life stressors such as exposure to abuse and neglect, have been associated with observable and permanent changes to the developing brain's architecture (McEwen, 2006; McEwen and Gianaros, 2011). Additionally, there is a paucity of research in low and middle income countries (LMIC), environments where families have increased vulnerabilities and are more likely to be exposed to persistent poverty (Gonzalez et al., 2018; Liu et al., 2017b). Due to time, travel, cultural considerations, and cost, measuring physiological stress in infants and young children can be methodologically challenging in LMIC. Thus, there is limited research among young children and in particular among infants in such settings (Braig et al., 2016; Braig et al., 2017; Gray et al., 2018; Ursache et al., 2017). Steroid biomarkers measured in hair is a novel and new measurement approach and hair cortisol is a promising marker of chronic stress (Liu et al., 2017b; Schury et al., 2017; Vliegenthart et al., 2016) that may be feasible to conduct in such settings. Given that methods extracting hair-derived markers of chronic HPA-function are relatively novel (Kirschbaum et al., 2009; Slopen et al., 2018), HPA-axis associations with varying types of stressful exposures (e.g., extreme poverty, exposure to maternal IPV, exposure to environments with low household stimulation, etc.) have yet to be elucidated (Gonzalez et al., 2018; Ursache et al., 2017; Zhu et al., 2019). Children who grow up in extreme poverty are often exposed to multiple stressors such as overcrowding, substandard housing, and possibly violence and often have elevated stress levels (National Scientific Council on the Developing Child, 2005). The impacts on early stress response symptoms are often worsened when mothers experience depressive symptoms (Essex et al., 2002; Lupien et al., 2009). The quality of relationships between children and caregivers early in life plays a crucial role in the development of stress hormone regulation (Blair et al., 2011).

This analysis seeks to fill these gaps by examining associations between infants' exposures to adverse maternal, social, economic, and home environments and markers of chronic HPA activity at 12 months of age. In order to gain a more comprehensive understanding of HPA-axis activity and infant environment, we will examine multiple HPA-axis outputs including cortisol, cortisone, DHEA and their interrelationships. We explore environment at two levels, maternal and household. First, we examine whether maternal depression and IPV exposure are associated with biological indicators of her infant's chronic stress markers, measured through hair cortisol, DHEA, cortisone, and cortisol/DHEA ratio. Second, we focus on several household and family level adversities and their association with the same biological indicators of persistent stress.

1. Methods:

1.1 Sample:

Data were collected from a randomly selected sub-sample of mother-infant dyads (n=104) participating in the Bachpan Cohort, a population representative birth cohort with a nested perinatal depression cluster randomized controlled trial (cRCT) in rural Pakistan that has

followed mothers and their child (n=1,154) starting in their last trimester of pregnancy. The study's detailed procedures are described elsewhere (Turner et al., 2016). In brief, all married pregnant women residing in the study area's 40 village clusters were screened for depression. Depressed women were invited to participate and for every woman recruited, one non-depressed woman from the same village was also included. Data were elicited beginning in the last trimester of pregnancy and again at 3, 6, 12, 24 and 36 months. The study is ongoing and thus, for this analysis, we use data from baseline through the child's first year. In 2016, at the one-year data collection wave, a randomly selected subsample of 177 mothers and their infants were approached for HPA-axis hormone biomarker collection through hair. Of the 177 dyads approached, 158 consented, and 104 met inclusion criteria (at least 1 centimeter of infant hair was available). Women who consented were not statistically different in baseline characteristics to those who refused or did not have enough hair. Additionally, there is no evidence that the resulting sample of infants with hair biomarkers is not representative of the entire study sample of 1154 women.

1.2 Maternal mental health:

All measures underwent extensive and rigorous validation and piloting prior to administration. Maternal depression was assessed in two ways. First, a clinical diagnosis of Major Depressive Episode (MDE) was assessed using the Structured Clinical Interview (SCID) for DSM-IV diagnosis. The SCID generates a yes/no diagnosis for Major Depressive Episode (MDE) and has been used previously in Pakistan (Rahman et al., 2003). The SCID was administered at baseline, 3, 6, and 12 months postpartum. Second, the Urdu validated Patient Health Questionnaire (PHQ-9) was used as an indicator of depression symptom severity and was included at baseline, 3, and 6 months postpartum (Gallis et al., 2018). A cumulative measure of depression chronicity was created by generating an index across the three evenly spaced waves (last trimester and six and 12 months postpartum), with a range of 0 to 3. Five individuals were not present at the sixth month wave and their SCID was imputed from their third month wave. Three were not present at either the 3 or 6-month wave and, therefore, these cases were dropped from relevant analyses (see supplementary tables for sensitivity analyses comparing these three women to the biomarker and larger cohort samples). History of intimate partner violence (IPV) in the past year was assessed with the World Health Organization's Violence Against Women Instrument at baseline and 12 months (Ellsberg et al., 2008). This strategy assesses for three types of IPV: psychological, physical, and sexual within the past 12 months. A three-level IPV indicator variable was constructed to indicate if a woman experienced any of the three types within the past 12 months (any, none, or did not respond). We report the non-response associations in supplementary tables. A count variable was created totaling the number of types a woman endorsed, with a range between zero to three.

1.3 Sociodemographic and household measures:

A composite measure of household assets was used to assess SES. The measure was derived using a polychoric correlation principal component analysis of baseline asset data where only assets with sufficient variability were considered, a common and robust strategy employed in LMIC and resource-poor settings (Maselko et al., 2018; Rutstein, 2008). Home environment was evaluated using the Home Observation for Measurement of the

Environment (HOME), a 45-item inventory that assesses the quality of the child's household environment, including available stimulation, emotional support, and adverse exposures (e.g., physical punishment, shouting, etc) and collected at 12 months (Bradley and Caldwell, 1977). Higher scores indicate more favorable and stimulating environments for positive child development. The HOME has been used extensively in Pakistan and other LMIC environments (Jeong et al., 2016; Linver et al., 2004). The HOME scores were categorized into tertiles.

1.4 HPA-axis markers:

Our outcomes of interest were infant HPA-axis biomarkers at 12 months of age. A standard protocol for hair sample collection was employed so that approximately 200 hair strands were clipped from the infant's posterior vertex (Kirschbaum et al., 2009). Evidence suggests that a 1cm segment of human hair reflects the HPA-axis hormone output for the past month. The segment closest to the scalp is reflective of the most recent month. This required infants to have at least 1cm of hair to be included in the study, and as much as 3 cm. For hair hormone extraction (including cortisol, DHEA, and cortisone), the entire sample of infant hair was analyzed as one segment. Therefore, this dataset allows for the examination of between 1–3 months of HPA-axis hormone production in infants. Mothers reported any applications of hair products, hair treatment, and oral/topical steroids for their infant, but no women reported such use. Hormones were extracted and measured by Dresden LabService using standard liquid chromatography mass spectrometry (LC-MS) procedures (Gao et al., 2013). We considered potential confounders of child hair derived glucocorticoids including child age (days) at the time of hair collection and child sex (Gray et al., 2018).

1.5 Statistical Analysis:

Outliers and distributions were assessed for each of the outcome biomarker variables. To correct for skewness, cortisol, cortisone and the cortisol/DHEA ratio were log transformed whereas DHEA was not transformed as it was symmetric. A single DHEA value of 43.74 pg/mg exceeded four standard deviations from the mean and two standard deviations from the next highest observation and was therefore identified as an outlier. As a consequence, it was dropped from DHEA related analyses. As per recommended protocol (Kirschbaum et al., 2009), the five infants whose DHEA was below the lower limit of detection of 0.24 pg/mg were recoded as 0.24. Analysis of the ratio of cortisol to DHEA was performed on the log-ratio scale (Saczawa et al., 2013). This is consistent with previous literature (Gray et al., 2018). For exploratory analyses of mother-level and household-level stressful exposure impacts on infant chronic HPA-axis hormone levels, we estimated separate linear mixed-effects models (to account for the clustered study design for each outcome (i.e., log-cortisol, DHEA, log-cortisone, and the log-cortisol/DHEA ratio) accounting for our survey design and clustering at the village-level. As described in the sample description above, an embedded cluster randomized control trial of a low-level peer-delivered maternal depression intervention was embedded within the Bachpan cohort study design. As a consequence, all models included an indicator for treatment arm. We also adjusted all models for child age (in days) and child sex, because previous evidence has demonstrated associations of these variables with HPA-axis hormone levels (Gray et al., 2018). Additional analyses were conducted to explore differences between the biomarker subsample (n=104) and the full

sample (n=1,154) as well as individuals missing clinical depression and/or IPV data at one or more timepoints compared to those with complete data (see Supplemental Appendix). STATA version 15 was used for data analysis.

1.6   Ethical considerations:

all study procedures were approved by ethics committees in the US (Duke University, UNC) and Pakistan (Human Development Research Foundation's Ethics Committee).

2.   Results

2.1   Descriptive statistics

Data on cortisol, cortisone, DHEA, and cortisol/DHEA ratio concentrations in infant hair at 12 months as well as maternal and household characteristics at baseline are reported in Table 1. About 54% of our infant sample was female and had a mean age of 372 days (SD=16). The mean age of mothers was 26.8 years, with 7.0 years of education (SD = 4.0). Twenty-six percent had a positive SCID diagnosis of MDE at 12 months postpartum. The majority of families owned their house (88.46%) with, on average 1.9 (1.6) living children at baseline. The average child hair HPA axis biomarkers were as follows: cortisol 28.16 pg/mg (SD = 34.95), DHEA 11.33 pg/mg (SD = 5.82), cortisone 51.03 pg/mg (SD = 34.16), and cortisol/DHEA ratio 7.74 (SD = 32.03). We present analyses in supplementary tables that document the differences between our biomarker sub-sample and the rest of the cohort and those missing data for exposures including SCID and IPV. Women in the full sample had significantly lower parity and, therefore, this was adjusted for in all subsequent regression analyses.

2.2   Maternal mental health and infant HPA axis activity

Table 2 illustrates the results of the adjusted linear mixed-effects regression models of maternal-specific exposures, controlling for SES, maternal parity, child age, child sex, and treatment arm. The maternal exposures of interest included clinical depression in pregnancy and 12 months postpartum, clinical depression chronicity over the perinatal period, depression symptom severity in pregnancy and post-partum, and IPV at baseline and within the child's first year of life (elicited at 12 months postpartum). Increased exposure to maternal depression did not significantly or consistently correlate with infant cortisol, as all 95% CI crossed the null. More consistent patterns were detected with DHEA. Increased depressive symptoms at baseline (e.g., fetal exposure), where a one unit increase on the PHQ-9 was associated with lower DHEA (adjusted B= −0.175 95% CI −0.33, −0.02) and slightly higher infant cortisol/DHEA ratio (adjusted B= 0.04 95% CI 0.003, 0.08). While a clinical diagnosis of MDE at any single timepoint was not associated with any child HPA biomarkers, there was evidence of a dose-response association among children with mothers with a MDE diagnosis and DHEA levels. Each additional instance of maternal MDE is associated with lower child DHEA levels by 1.22 units (95% CI −2.30, −0.14).

We similarly find no detectible or consistent associations between maternal IPV exposure at any timepoint and infant hair cortisol. While there were no associations with maternal any IPV exposure and IPV count measured at baseline or any IPV exposure within one-year

postpartum, mothers who reported experiencing the most types of IPV within the first postpartum year (i.e. all three types) had children with significantly lower infant DHEA (adjusted B=−4.140 CI: −7.42, −0.79) compared to those with no IPV exposure. As we found with increased counts of MDE diagnoses, a similar dose-response relationship is observed for IPV counts, where infants of mothers with more types of IPV exposure have lower DHEA levels (Table 2). While we fitted a series of models with cortisone as an outcome, because the results did not indicate statistically significant associations with the predictors of interest, we present the findings in a supplementary appendix.

2.3 Household adversity and infant HPA axis activity

Table 3 focuses on household and family level correlates of child chronic HPA-axis hormone levels, controlling for maternal parity, child age, child sex, and treatment arm. We find no associations between either HOME total score or any of the HOME subscales and child cortisol, DHEA, and cortisol/DHEA ratio. However, children in the highest HOME tertile (indicating those with the most stimulating and positive household environments) had significantly lower hair log-cortisol (adjusted B= −0.66 95% CI −1.27, −0.04). We found a significant positive association between higher SES and DHEA (adjusted B=0.77 95% CI 0.08, 1.47). No significant associations were found between cortisol or cortisol/DHEA ratio and measures of household adversity. We share findings for statistically insignificant associations of adversity exposures and cortisone, in supplementary appendix. All sensitivity analyses can be found in the supplementary appendix.

3. Discussion

3.1 Key findings

In our sample of one-year old infants from rural Pakistan, we found no evidence of associations between infant hair cortisol levels and exposure to any of the maternal or household adversities. This is in line with previous findings that suggest hair-derived cortisol may not be a sensitive marker of stress exposure at one year and that infant HPA axis systems are robust to adverse exposures at this young age (Slopen et al., 2018). For example, Slopen et al (2018) found that children of mothers with the greatest lifetime trauma exposure before their birth prior had elevated hair cortisol levels at ages three and four, but not one and two, compared to mothers with the lowest trauma exposure (Slopen et al., 2018). Similar results have been found in other child studies with small samples (Karlén et al., 2013; Rippe et al., 2016; Simmons et al., 2016).

We found that specific maternal and household characteristics (i.e. increased depressive symptoms in pregnancy, IPV within the first year postpartum, and the child's household socioeconomic assets) were associated with altered HPA-axis hormone levels, particularly DHEA levels. Our findings contribute to the growing literature of infant HPA dysregulation and possible intergenerational impacts of maternal depression, poverty, and violence. Importantly, vulnerable populations exposed to persistent poverty are under-represented in the psychoneuroendocrinology literature. Thus, our findings point to possible early HPA axis dysregulation driven by changes in DHEA activity, but not cortisol at 12 months of age.

3.2 Depression

We find no associations with depression and cortisol or cortisone, which adds to the variable findings in the literature. For example, Palmer et al (2013) found higher maternal postpartum depression symptoms to be associated with lower cortisol levels in 12 month olds while higher pregnancy psychological distress was associated with higher cortisol (Palmer et al., 2013). Yet, Slopen et al found that depression was not associated with cortisol (Slopen et al., 2018). To date, very few intergenerational transmission studies explicitly investigate clinical levels of depression and instead rely on broader batteries of psychological distress. Moreover, previous studies have not examined the effects of sustained depression from pregnancy through the first year of life. Our findings, then, suggest that more chronic depression may have significantly different effects on child HPA-axis regulation than episodic, or isolated instances. In Pakistan, it may be that the child is buffered from episodic events due to high rates of living in multigenerational families. But, perhaps this buffering may be insufficient to protect against persistent maternal depression.

We do see significant associations of infant DHEA with greater maternal depressive symptoms during pregnancy, and with chronic maternal depression (diagnosed with clinically relevant depression at two or more time points). More longitudinal research is needed to further untangle the effects of chronic maternal depression on infant HPA regulation.

3.3 IPV

Neither cortisol nor cortisone were found to be associated with any measures of IPV. For DHEA, we find that children whose mothers experienced all three types of IPV (psychological, physical, and sexual) in the child's first year of life had lower DHEA. This finding is in line with growing evidence of the intergenerational transmission of trauma between mothers and their offspring (Condon et al., 2018; Simmons et al., 2016; Slopen et al., 2018). While the directionality of DHEA levels trend in the expected direction (e.g., lower levels with more increased maternal IPV exposure), findings should be interpreted with caution as these associations may indicate varying HPA axis response and regulation capability at varying child ages (Laurent et al., 2013; Wetherall et al., 2018). Importantly, any dysregulation, regardless of direction, may be a more salient marker of stress than hormone quantity alone, particularly within early stages of development (Laurent, 2017). However, more work is needed to explore patterns with markers beyond cortisol. The timing of trauma may also be important. For example, we do not see similar patterns in children with mothers that experienced IPV in the year before childbirth, but we do in the year postpartum. Yet, Schury et al found that mothers with histories of maltreatment had infants with higher DHEA, but this effect was observed in newborns within days of birth (Schury et al., 2017). Additionally, other research has found significant effects of inutero exposure to maternal IPV, indicating that more evidence is needed to further untangle these associations and exposure windows of importance (Davis et al., 2018; Schury et al., 2017). More research on specific infant and child age windows is needed to grow an improved understanding of maternal traumatic exposures and their associations with fluctuations of HPA-axis hormones in the first years of life.

3.4 Household Environment

We found that higher SES infants had significantly higher DHEA levels. While there was no association between any HPA-axis hormones and the continuous HOME score, we did find that infants in higher HOME tertiles had significantly lower cortisol levels. Prior research has documented the importance of quality early care and education environments on child cortisol levels (Gunnar et al., 2010; Nachmias et al., 1996). To date, to our knowledge, there are no studies exploring hair derived infant DHEA concentrations and associations with SES and the quality of their household stimulation environment. Zhu et al found that low-SES children are more vulnerable to anxiety and have altered cortisol awakening response (measured from saliva), most likely mediated through increased parental anxiety (Zhu et al., 2019). However, saliva is a different, more acute, indicator of HPA-axis function from the chronic stress captured by hair cortisol and its associations with maternal and household adversity are different than hair derived measures (Malanchini et al., 2019).

Our findings preliminarily suggest that disparities may exist between infants in environments of very low household SES compared to their counterparts in relatively higher SES households. Importantly, our SES measure is asset based and is a robust, appropriate, and multifaceted indicator of SES in our LMIC setting, particularly where elicitations of income are unreliable and/or inappropriate due to informal work (Rutstein, 2008). It likely captures different components of social stratification than measures of income or education, which is typically used in studies in higher income countries (Bates et al., 2017; Flom et al., 2017; Ursache et al., 2017).

Taken together, we see some modest evidence of disruptions in HPA activity in infants exposed to chronic maternal and household-level stressors, including SES, in a low-resource environment. Such changes early in life can lead to impairments in the ability to regulate stress hormones and affect learning and memory development (National Scientific Council on the Developing Child, 2005). Alongside literature indicating that young infants have the ability to process positive and negative facial emotions and other environmental stimuli (Johnson et al., 2003; Martin et al., 1999), further exploration of infant development and cortisol as a complicated signal of arousal, stress, and emotion dysregulation is warranted. More research is also needed to further untangle relationships between various types of stressful exposures and biomarkers of the developing HPA-axis that extend beyond only examining a single marker.

3.5 Strengths, limitations, and future directions

We used data from a population-representative sample of households, rich with standardized measures of adverse exposures at timepoints including both fetal exposure and infant exposure at 12 months. Additionally, we explored multiple chronic measures of HPA axis glucocorticoids. Additionally, our sample is situated within a low-income environment, extending the infant HPA axis literature to understudied, chronically disadvantaged, populations. Our analytic design is also a strength in that previous literature (Bates et al., 2017) has largely identified between-group differences; however our study employed linear mixed effects regression models to control for possible confounders. There are, however, several limitations to discuss. First, as this is an exploratory descriptive study with a modest

sample size, we are unable to comment on causality and have limited generalizability. However, we drew a random sample from the larger Bachpan cohort, which is a representative sample of the population of interest. Second, given our small sample size, we have limited capability of detecting meaningful differences. Similarly, there is likely variability in the experiences of individuals within our sample that we are unable to assess (e.g., sickness episodes). Third, infant hair samples varied in length and were analyzed as one combined segment, so samples could be capturing slightly different time periods. Fourth, we are unable to adjust for unmeasured confounds—such as chemical and microbiological factors—related to both exposure and HPA hormones in infant hair. Fifth, as hair cortisol is a novel biomarker of stress within young child research, it requires additional validation to confirm that it measures cumulative cortisol secretion robust to unwanted influences in children (Slopen et al., 2018). Lastly, there is the possibility for recall bias with mothers' reporting on IPV and the standard limitations that exist with any self-reported data. Despite these limitations, our study contributes to the literature by focusing on one-year old children in rural Pakistan, an important, yet understudied population.

In sum, we find that infant DHEA is associated with 1) maternal depressive symptoms prenatally and with chronic depression postpartum, 2) maternal IPV postpartum, and 3) household SES, indicating that infants' maternal and home environments early in life are likely related to chronic stress and that DHEA may be the most sensitive of the HPA-axis hormones at 12 months of age. Our study, combined with the current literature, indicates several avenues for further research. For example, infants may need a longer temporal exposure to chronic stress for us to see relationships between such stress and infant biomarkers. Additionally, existing relationships may become stronger or may change over time (Karlén et al., 2013; Slopen et al., 2018). Specifically, the stress response system may mature over time, so infants' biomarker data may become less variable in subsequent years (Karlén et al., 2013). Related, potential moderation from environmental or genetic factors should be explored as well as individual and dyadic level variables such as maternal sensitivity, social supports, and child temperament, which may be related to child cortisol level should be assessed in future research (Liu et al., 2017a).

4. Conclusion:

The results from this study advance the literature of maternal and environmental effects on offspring biology in several ways. Hair derived HPA-axis hormones are relatively novel within child research, particularly those beyond cortisol (e.g., DHEA, cortisone, and the cortisol/DHEA ratio). We contribute important evidence of variabilities in markers beyond cortisol and cortisone in relation to adversity, highlighting that DHEA appears to be more sensitive than cortisol at 12 months of age. As many studies have large age ranges, our precise sample is an important contribution to build evidence around specific windows within child development. Further studies are needed to elucidate vulnerable windows and specify important risky exposures in order to build effective interventions for children to meet their full potential.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

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Highlights:

- Intergenerational impacts on DHEA levels were detected within infants exposed to maternal depression during pregnancy

- Infants whose mothers experienced multiple types of IPV in the first year postpartum

- No intergenerational impacts of maternal exposures to IPV or depression were found on infant hair cortisol

- SES is associated with early HPA axis dysregulation in infants, driven by changes in DHEA activity

- Measures of the household environment had limited impact on infant hair derived HPA axis markers

Table 1.

Descriptive characteristics for children and their mothers (*N*=104)

Characteristics	n OR mean (range)	Percent or SD
Maternal and Household Data		
Age	26.83 (18– 40)	4.95
Living children (baseline)	1.9 (02013–6)	1.6
Education (Mom)	6.99 (0–16)	4.02
None	15	14.42
Primary	24	23.08
Middle	28	26.92
Secondary	24	23.08
More than secondary	13	12.5
SES Tertile		
lowest	29	27. 88
middle	30	28.85
highest	45	43.27
HOME (Total Score)	30.81 (10–42)	5.52
HOME (Subscale Scores)		
Responsivity	9.64 (4–11)	1.31
Acceptance	5.89 (1–8)	1.18
Involvement	4.27 (0–6)	1.32
HOME Tertiles		
lowest	23	22. 12
middle	62	59.62
highest	19	18.27
Maternal Psychosocial Data		
Baseline depression severity (PHQ9)[1]	11.22 (0–23)	6.8
Current MDE[1] (12 months)	27	25.96
MDE Diagnosis count[2]		
0	36	35. 64
1	36	35.64
2	18	17.82
3	11	10.89
Baseline IPV[3]		
Any	50	51. 02
Total Count (Sexual, Physical, Psychological)[3]		
0	54	52. 88
1	17	16.35
2	16	15.38
3	17	16.35
12 months post-partum IPV		

Characteristics	n OR mean (range)	Percent or SD
Any	49	51. 46
Total Count (Sexual, Physical, Psychological) [†]		
0	55	51. 92
1	18	17.31
2	17	16.35
3	14	13.46
Child Data		
Female	56	53.9
Age (days)	372 (351–466)	16.73
Hair steroids [4]		
Cortisol	28.15 (1.24–196.2)	34. 95
DHEA	11.32 (0.24–29.52)	5.83
Cortisol/DHEA ratio	7.74 (0.063–303)	32.02
Cortisone	51.03 (5.54–160.74)	34.16

[*] outlier of 43.74 dropped from DHEA related analyses, minimum value set to 0.24

[†] missing in any category treated as 0

[1] Major Depressive Episode (MDE) determined by the Structured Clinical Interview for DSM-IV and depression severity determined by the PHQ-9

[2] Three children were dropped due to missingness. Differences were explored between these children and the biomarker sample in supplementary tables.

[3] IPV variables contain a dummy category for non-response, these estimates are reported in sensitivity analyses.

[4] All biomarkers were measured in pg/mg and cortisol, cortisol/DHEA ratio, and cortisone were log-transformed. One outlier was identified for DHEA and dropped.

Table 2.

Linear mixed effects models examining associations between mother characteristics on child 12-month HPA axis biomarkers.

Mother's Characteristics	Cortisol[1] (n=104)	DHEA[2] (n=103)	Cortisol/DHEA[1] (n=103)
	Beta. Coefficient (CI beneath in parentheses)		
MDE[3] Diagnosis (Baseline)	0.19	−1.63	0.52
	(−0.23 – 0.61)	(−3.816 – 0.56)	(−0.012 – 1.05)
Depression Severity (Baseline)[3]	0.02	−0.18*	0.04*
	(−0.01 – 0.05)	(−0.34 – −0.02)	(0.01 – 0.08)
MDE Diagnosis (12 months)	−0.31	−2.10	0.05
	(−0.75 – 0.14)	(−4.42 – 0.23)	(−0.53 – 0.63)
MDE Diagnosis count (never is referent)			
Once	0.20	0.19	0.26
	(−0.31 – 0.71)	(−2.43 – 2.80)	(−0.39 – 0.91)
Two	0.03	−2.38	0.66
	(−0.55 – 0.62)	(−5.46 – 0.70)	(−0.11 – 1.42)
Three	−0.11	−3.50	0.29
	(−0.81 – 0.60)	(−7.11 – 0.11)	(−0.599 – 1.18)
MDE Diagnosis count (continuous)			
	−0.03	−1.22*	0.17
	(−0.23 – 0.18)	(−2.30 – −0.14)	(−0.09 – 0.44)
Sample Size for MDE count[4]	Cortisol (n=101)	DHEA (n=101)	Cortisol/DHEA (n=101)
Any IPV[5] Baseline	0.10	−0.88	0.13
	(−0.32 – 0.52)	(−3.16 – 1.39)	(−0.41 – 0.67)
IPV Count One Year Baseline			
One Type	0.16	−1.33	0.23
	(−0.41 – 0.74)	(−4.30 – 1.64)	(−0.51 – 0.96)
Two Types	0.23	0.31	0.09
	(−0.37 – 0.82)	(−2.75 – 3.37)	(−0.66 – 0.85)
Three Types	0.06	−2.05	0.27
	(−0.51 – 0.63)	(−5.06 – 0.96)	(−0.48 – 1.02)
IPV Count One Year Baseline (continuous)			
	0.04	−0.48	0.08
	(−0.14 – 0.22)	(−1.42 – 0.46)	(−0.15 – 0.31)
Any IPV One Year	−0.27	−1.81	−0.18
	(−0.67 – 0.13)	(−3.90 – 0.28)	(−0.70 – 0.34)
IPV Count One Year PP			
One Type	−0.06	−0.61	−0.21
	(−0.61 – 0.49)	(−3.43 – 2.22)	(−0.92 – 0.50)
Two Types	−0.39	−1.504	−0.19

	(−0.95 – 0.17)	(−4.38 – 1.37)	(−0.91 – 0.54)
Three Types	−0.47	−4.10*	−0.19
	(−1.10 – 0.16)	(−7.42 – −0.79)	(−1.02 – 0.64)
IPV Count One Year PP (continuous)			
	−0.17	−1.16*	−0.08
	(−0.35 – 0.01)	(−2.10 – −0.22)	(−0.31 – 0.16)

***p<0.001,

**p<0.01,

*p<0.05

[1] All biomarkers were measured in pg/mg and cortisol and cortisol/DHEA ratio were log-transformed

[2] One outlier was identified for DHEA level and dropped

[3] Major Depressive Episode (MDE) determined by the Structured Clinical Interview for DSM-IV and depression severity determined by the PHQ-9

[4] Three children were dropped due to missingness. Differences were explored between these children and the biomarker sample in supplementary tables.

[5] IPV variables contain a dummy category for non-response, these estimates are reported in sensitivity analyses.

[6] All models controlled for SES, Child Age in Days, child sex, study arm, and maternal parity

Table 3.

OLS Regression Modeling of Household Characteristics on child 12-month HPA-axis functioning[2]

Household Characteristics		Cortisol (n=104)[1]	DHEA (n=103)	Cortisol/DHEA (n=103)
		Beta. Coefficient (CI beneath in parentheses)		
HOME (Total Score)		−0.03	−0.02	−0.03
		(−0.06 – 0.01)	(−0.22 – 0.18)	(−0.08 – 0.019)
	Responsivity	0.018	−0.18	0.05
		(−0.14 – 0.18)	(−1.00 – 0.65)	(−0.15 – 0.25)
	Acceptance	−0.06	0.14	−0.13
		(−0.24 – 0.11)	(−0.76 – 1.04)	(−0.35 – 0.09)
	Involvement	0.02	−0.37	0.0
		(−0.14 – 0.17)	(−1.18 – 0.44)	(−0.15 – 0.25)
HOME Tertiles (lowest is referent)				
	Middle	−0.50	1.84	−0.61
		(−1.06 – 0.05)	(−1.07 – 4.76)	(−1.34 – 0.11)
	Highest	−0.66*	−0.82	−0.60
		(−1.28 – −0.04)	(−4.09 – 2.44)	(−1.41 – 0.22)
SES Total Asset Score		0.05	0.77*	−0.06
		(−0.08 – 0.18)	(0.08 – 1.47)	(−0.24 – 0.11)
SES Tertiles (lowest is referent)				
	Middle	0.06	3.55**	−0.50
		(−0.42 – 0.55)	(1.01 – 6.10)	(−1.13 – 0.14)
	Highest	0.39	3.37*	−0.06
		(−0.13 – 0.9)	(0.68 – 6.06)	(−0.74 – 0.61)

*** p<0.001,

** p<0.01,

* p<0.05

[1] All biomarkers were measured in pg/mg and cortisol and cortisol/DHEA ratio were log-transformed. One outlier was identified for DHEA level and dropped

[2] All models controlled for SES, Child Age in Days, child sex, study arm, and maternal parity