Association of childhood adversity with hair cortisol level in a sample of Egyptian primary school children

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Abstract

Background: Childhood adversities have detrimental effects on children's development with psycho- bio- social consequences throughout life. Children who are abused, bullied, mistreated or witnessed any form of violence, will react to all these experiences by developing emotional, learning, or behavioral disorders or even deleterious physical health outcome. How these early life experiences affect physical and mental health, is a point of concern and endless research till now. The most widely discussed biological basis for these effects is the glucocorticoid cascade hypothesis, where chronic stress evokes persistent hyperactivity of the hypothalamic- pituitary- adrenal (HPA) axis leading to hypercortisolemia. Identification of cortisol level in hair is a promising, non- invasive technique for assessment of role of stress and, to fill the methodological void of long- term cortisol assessment.

Aim: To evaluate the association of exposure to early adverse childhood experiences with hair cortisol level in a sample of Egyptian primary school children.

Subjects and Methods: This study was carried out on 114 students aged between 8- 10 years, divided into: Group A: (55 students) who exposed to one or two adverse experiences, Group B: (56 students) with multiple exposure to more than three adverse experiences, the rest of the students were not exposed (3 students). The children of the two groups were matched as regards their age, sex, and socioeconomic status. Assessment of chronic alteration of hypothalamic- pituitary- adrenal axis was done through measurement of hair cortisol level with modified an immunoassay originally developed for measuring cortisol in saliva.

Results: The median hair cortisol level was 33.7 pg. /mg of hair, IQR (20- 37.5) which is considered high level when compared to normal reference validation ranges from 6.8 to 8.5 pg/mg. Median hair cortisol level showed non- significant statistical difference between the two studied groups.

Conclusions: Early exposure to adverse life experiences is associated with high hair cortisol level rather than the multiplicity of exposure.

Key Words: Childhood Adversity- hypothalamic pituitary adrenal axis- Hair Cortisol.

دراسة مدى ارتباط اساءة معاملة الأطفال بمستوى الكورتيزول فى الشعرفى عينة من أطفال المدارس الابتدائية المصرية

مقدمة: إن مرور الطفل بخبرات مؤلمة فى سنواته الأولى له تأثير سلبى على صحته البدنية والنفسية ويظهر هذا التأثير على المدى القريب أو البعيد. لوحظ أن المحور الأساسى لهذا التأثير هو الخلل فى الترابط بين غدة تحت المهاد والغدة النخامية والكظرية والذى قد يؤدى إلى زيادة إفراز هرمون الكورتيزول وما يترتب عليه من آثار ضارة على صحة الطفل النفسية والجسدية.

الهدف: تحديد العلاقة بين التعرض لخبرات سيئة ومستوى الكورتيزول فى الشعر.

طرق وعينةالدراسة: تمت الدراسة على ١١٤ طفلا من تلاميذ المدارس الابتدائية تتراوح أعمارهم من ٨ إلى ١٠سنوات وتم اختيارهم بطريقة عشوائية من المدارس الحكومية تم تقسيم الاطفال المدرجين بالدراسة الى مجموعتين تبعا لأنواع الخبرات المؤلمة التى يتعرض لها الطفل (أصدرته منظمة الصحة العالمية فى ٢٠١١) المجموعة الأولى: مجموعة الأطفال الذين تعرضوا لنوع واحد أو نوعين على الأكثر (٥٥ طفلا)، بينما شملت المجموعة الأخرى: الأطفال الذين تعرضوا لثلاثة أنواع أو أكثر من الاساءات أو الخبرات المؤلمة (٥٦ طفلا) وقد تم قياس نسبة هرمون الكورتيزول فى الشعرعن طريق طحنه ووضعه مع الميثانول لمدة ١٦ ساعة لاستخلاص الكورتيزول ثم تبخير الميثانول وقياس الكورتيزول فى الراسب.

النتائج: كان متوسط عمرالأطفال بالسنين (٩,٩٣ ±٠,٩٣) وكانت نسبة الذكور ٤٦,٥% والإناث ٥٣,٥% وأظهرت الدراسة أن تعرض الأطفال للإساءة أو للخبرات المؤلمة فى الصغر قد أدى الى خلل فى الية تنظيم هرمونات التوتر بالجسم والذى تم الاستدلال عليه بارتفاع نسبة الكورتيزول بالشعر عن المعدل الطبيعى فى مثل هذا السن. واتضح أن هذا الارتفاع لا يتأثر بتعدد أنواع الاساءات حيث لم تثبت فروق ذات دلالة احصائية واضحة بين مستوى الكورتيزول فى المجموعتين.

الخلاصة: إن مجرد التعرض لإحدى الخبرات المؤلمة فى الطفولة المبكرة يؤدى الى خلل فى محور الترابط بين غدة تحت المهاد والغدة النخامية والكظرية والذى يؤدى بدوره إلى زيادة إفراز هرمون الكورتيزول بغض النظر عن عدد هذه الخبرات.

الكلمات الافتتاحية: إساءة معاملة الأطفال- الغدة النخامية- مستوى الكورتيزول فى الشعر.

Introduction:

Positive and negative Childhood experiences have a tremendous impact on future physical and mental health, hence, early experiences are an important public health issue. (CDC, 2016)

The experience of trauma, loss and bereavement during childhood have both immediate and long- term consequences for health and general wellbeing (Cerel et al., 2006).

Children who have experienced the death of a parent or witnessed violent and/or traumatic events or exposed to physical and sexual abuse and maltreatment during childhood have consistently been linked to an increased likelihood of depression (Chapman et al., 2004), low self-esteem, alcohol and drug abuse during adolescence and adulthood (Diaz et al., 2002).

Moreover, cumulative and/ or concurrent exposure to a number of these adversities (i.e. multiple adversities) has been linked to exponentially poorer outcomes for children compared to their exposure to single adversity (Zubrick et al., 2005; Jaffee et al., 2007).

The most widely discussed biological basis for these effects is the glucocorticoid cascade hypothesis (Ladd et al., 1996)

The normal physiologic stress response occurs through hypothalamic pituitary adrenal (HPA) axis and sympathetic adreno- medullary system, resulting in increased level of corticotrophin releasing hormone (CRH), epinephrine, norepinephrine, and cortisol. (Shonkoff& Garner, 2012).

Although the normal stress response includes increased level of stress hormones which are vital for life and have protective functions, excessive and prolonged high level of these hormones is harmful or may be fatal and any alterations or defect in this physiologic network can lead to chronic wear and tear effect on different body systems and organs including the brain (McEwen, 2005).

Elevated glucocorticoids impair neuronal growth and survival (Duman, 2009), diminish neutrophils and modify immune functions (Epel, 2009), and accelerate cellular aging (Ceccatelli et al., 2007; Epel, 2009).

Present biological cortisol markers like saliva, urine and blood suffer from the drawback of only covering spot time periods of up to 24 hours. Therefore, there is a need in stress research for biological markers reflecting long term and extended exposure to stress. Cortisol in hair has the potential of becoming a new indicator for stress exposure of major life stressors over periods of months meaning that in a retrospective fashion. (Karlen et al., 2011); as hair grows approximately 1 centimeter per month (Loussouarn et al., 2005)

An additional advantages of analyzing cortisol in human hair is the ease of sample collection and transportation as well as being non- invasive technique (Karlen et al., 2011).

Aim Of The Study:

This study was conducted to evaluate the association of exposure to early adverse childhood experiences with hair cortisol level in a sample of Egyptian primary school children.

Subjects And Methods

The current study is a cross sectional study, was conducted along the period from 2013 to 2015.

Subjects of the study were recruited from public primary schools in El-Dokki district, Giza Governorate. Students of Public primary schools in Egypt have limited variation in socioeconomic status. The age of the students under the study ranged from 8- 10 years old, to be aware to answer the interviewer's questionnaire and to avoid the possible psychological effects of sexual maturation. So, the sample population included the 3rd, 4th, and 5th grade students in the primary schools.

Apparently healthy prepubescent students were recruited in the study.

Orphans, intellectually deficit children, children who had history of chronic or endocrinal disease or who were receiving corticosteroid medication, were excluded from the study.

Methods:

All subjects were subjected to the following:

1. A well- organized questionnaire for gathering relevant personal data: This questionnaire was filled out by the caregiver, Data included: name, age, sex, residence, maternal and paternal education and occupation, monthly income, and number of family members 'for assessment of socioeconomic standard'. In addition, detailed medical and developmental history were explored including perinatal history and progress of developmental milestones.

2. Adverse Childhood Experiences International Questionnaire (ACE-IQ): It is a screening questionnaire designed by WHO for assessment of different types of adverse childhood experiences (ACEs) as abuse with its different types (sexual, emotional, and physical), neglect (physical and emotional), and household, peer, and community violence.

In the current study, the questionnaire was translated into Arabic language using the back translation method (Brislin, 1980; Harkness, 2003). some modifications were done on the questionnaire to be compatible with age group of our selected sample and to be easily applicable. Furthermore, some questions were omitted to exclude uncontrollable conditions like wars and death of one or both parents.

So items included in our study were 6 main items:

a. Protection (3 questions).

b. Neglect (3 questions).

c. Household dysfunction (7 questions).

d. Abuse (8 questions).

e. Peer violence (1 question).

f. Witnessing community violence (3 questions).

Each student was face to face interviewed to answer the questionnaire. Duration of interview ranged from 20 to 30 minutes.

The questionnaire comprised 25 questions with 4 responses for each question indicating the degree of exposure during the last year. The questions' responses include: never, once, a few times, and many times. The student is considered exposed to a certain adversity when

the response to the question concerned with this adversity is many times except for sexual abuse.

Accordingly, subjects were classified into two groups:

¤ Group A: (55 students) who exposed to one or two adverse experiences,

¤ Group B: (56 students) with multiple exposure to more than three adverse experiences.

The rest of the students were not exposed to any adversity (3 students).

3. Clinical examination and anthropometric measurement:

    a. Thorough physical examination was performed including assessment of vital signs, general examination and specific examination of all body systems.

    b. Anthropometric measures: weight in kg and height in cm were measured and compared with standard charts and the approximate age- percentiles according to the standards of (WHO, 2009).

    c. Assessment of puberty using Tanner staging (Tanner, 1998).

4. Assessment of chronic alteration of hypothalamic- pituitary- adrenal (HPA) axis Through measurements of hair cortisol level: Steps of hair cortisol level analysis:

    a. A hair sample of approximately one centimeter was collected from the vertex posteriorly using scissors as close to the scalp as possible just above the nape, the scalp end of the hair sample is carefully indicated.

    b. Each hair sample was minced finely with scissors 'to increase the exposed surface to methanol' each weighing 10 mg.

    c. Each sample incubated overnight (about 16 hours) in 1 ml methanol in 50 ° C.

    d. The methanol was removed from the hair and evaporated by nitrogen stream.

    e. The residue was reconstituted in 250 µl phosphate buffer saline (PBS) pH 8.0.

    f. The cortisol concentration in the resulting buffer solution was determined using a commercial Cortisol Saliva Luminescence Immunoassay according to the manufacturer instructions (RE52611 I B L international G M B H Flughafenstrasse 52a D-22335 Hamburg- Germany), performed at National Research Centre. Thomson et al. (2010)

Because the kits used in this method was originally designed to measure cortisol level in liquid samples such as plasma or saliva, so the result of our assessment should be converted to amount of cortisol per unit of powdered hair.

The following formula was used for this conversion from µg/dl to pg. cortisol per mg hair:

$$(A/B) * (C/D) * E * 10.000 = F$$

Where:

A= µg/dl from assay output; B= weight (in mg) of hair subjected to extraction; C= vol. (in ml) of methanol added to the powdered hair; D= vol. (in ml) of methanol recovered from the extract and subsequently dried down; E= vol. (in ml) of assay buffer used to reconstitute the dried extract; and F= final value of hair CORT concentration in pg./mg. Meyer et al. (2014)

Ethical Aspects:

Approval by the Ethics Committees of the National Research Centre, the Institute of Postgraduate Childhood Studies and the Egyptian Ministry of Education, in addition to parental informed consent were obtained.

Statistical Analysis:

The data was collected& analyzed on personal computer using the Statistical Package for the Social Science (SPSS) version number 18. Description of quantitative (numerical) variables were in the form of mean± standard deviation and range. Qualitative (nominal) variables were in the form of number and percentage. Student's t- test of 2 independent samples were used to compare 2 quantitative variables. Pearson correlation coefficient test (r- test) was used to rank different variables against each other either directly or indirectly. A p- value of <0.05 was considered significant.

Results:

One hundred fourteen students were enrolled in this study, the studied sample composed of 53 males (46.5%) and 61 females (53.5%) Figure (1). The age of the students ranged from 7.5 to 11 years with a mean 9.93+ 0.93 years table (1).

In the current study: The mean weight for age Z- score (WAZ) was 0.2+ 1.13, the students were distributed according to WHO reference range into: (93%) had normal weight for age, (1.7%) were underweight and (5.3%) were overweight. The mean height for age Z- score (HAZ) was- 0.16+ 0.93. The majority of students (94.7%) had normal height for age while (5.3%) were stunted. The mean body mass index for age Z score (BAZ) was 0.35+ 1.42. Most of the students (85.1%) had normal BMI for age while (4.4%) were wasted and (10.5%) were obese students. (Table 2)

As regard the exposure to different adverse childhood experiences, it was observed that the majority of the studied sample (90.4%) exposed to witnessing household member treated violently while emotional neglect is coming after by percentage of (88.6%). Sexual abuse came as the least adverse experience with percentage of (3.5%). Table (3), Figure (2)

As Regard Hair Cortisol Level, There Was Statistically Non-Significant Difference Between The Medians Of Hair Cortisol Levels In The Exposed And Highly Exposed Groups As P >0.05 Table (4).

It Was Found Also That Hair Cortisol Level Didn't Correlate With Multiple Exposure To Adverse Experiences Table (5).

Table (1) Age And Sex Of The Studied Sample (N= 114 Students):

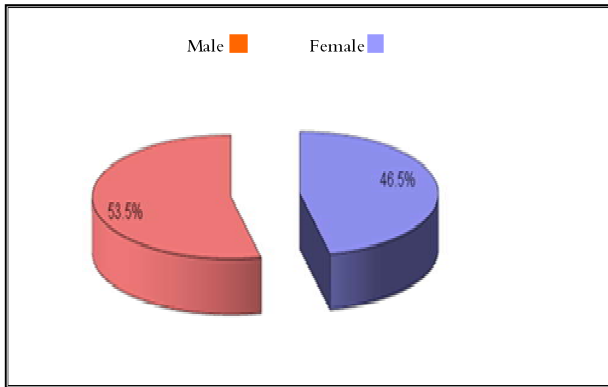|		
Age In (Years)	Mean ± SD	9.39 ±0.93
	Range	7.58- 11.0
Sex	Males	53 (46.5%)
	Females	61 (53.5%)

Figure (1) Shows sex distribution of the studied sample.

Table (2) Anthropometric data and nutritional status of the studied sample according to WHO reference range (N= 114 student):

Variable	Mean± SD	Nutritional Status	No.	%
Weight for age Z score (WAZ)	0.2+ 1.13	Normal Weight Students	106	93%
		Underweight Students	2	1.7%
		Overweight Students	6	5.3%
Height for age Z score (HAZ)	-0.16+0.93	Normal Height Students	108	94.7%
		Stunted Students	6	5.3%
Body mass index for age Z score (BAZ)	0.35+ 1.42	Normal Baz Students	97	85.1%
		Wasted Students	5	4.4%
		Obese Students	12	10.5%

Table (3) Distribution of students by type of adverse childhood experience with sex:

| Type Of Adversity	Total Sample N (%)	Male Students N (%)	Female Students N (%)
Emotional Abuse	15 (13.2%)	7 (13.2%)	8 (13.1%)
Emotional Neglect	101 (88.6%)	48 (90.6%)	53 (86.9%)
Physical Abuse	25 (21.9%)	12 (22.6%)	13 (21.3%)
Physical Neglect	13 (11.4%)	8 (15.1%)	5 (8.2%)
Sexual Abuse	4 (3.5%)	2 (3.8%)	2 (3.3%)
Family Separation	39 (34.2%)	18 (34%)	21 (34.4%)
Witnessing Household Member Treated Violently	103 (90.4%)	48 (90.6%)	55 (90.2%)
Bullying	58 (50.9%)	37 (69.8%)	21 (34.4%)
Community Violence	77 (67.5%)	39 (73.6%)	38 (62.3%)
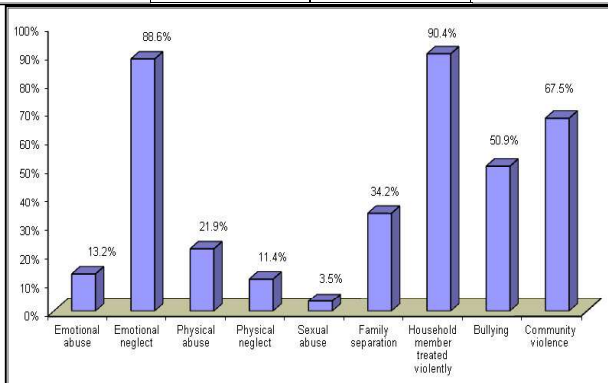
Figure (2) Distribution of studied sample by type of ACE

Table (4) Comparison Between Exposed And Highly Exposed Groups As Regard Hair Cortisol Level

Hair Cortisol	Exposed	Highly Exposed	Mann- Whitney Test	
	N= 40	N= 33	Z	P- Value
Median (IQR)	33.75 (20- 36.25)	30 (12.5- 35)	0.507	0.612
Range	2.5- 150	2.5- 150		

Table (5) Relation between hair cortisol level and number of ACEs

Number Of Adversities	Hair Cortisol		Kruskall- Wallis Test	
	Median (IQR)	Range	K	P- Value
One	35 (22.5- 57.5)	7.5- 150	1.188	0.552
Two	28.75 (20- 35)	2.5- 50		
Three Or More	30 (12.5- 35)	2.5- 150		

Discussion:

The hypothesis that early adversity may influence the HPA axis which in turn leading to high cortisol level in children was supported by the current study, as it

Assess the exposure to many types of adverse childhood experiences such as neglect (physical and emotional), abuse (physical, emotional, and sexual), paternal separation, witnessing household member violence or community violence, and bullying. It was observed that there is association between exposure to any of these adverse experiences and high hair cortisol level.

Cortisol level measurement in blood or urine was used for many years, and in recent years it can be measured in saliva and feces also as non-invasive techniques to assess hypothalamic pituitary adrenal axis function. (Meyer& Novak, 2012)

The current method of measuring cortisol in saliva has an extra-advantages rather than non- invasiveness, as it is cost effective, less stressful, can be collected by non- medical persons, and finally it can be done in different environments. (Karlen et al., 2011)

Although these all methods have many advantages, but unfortunately there are some limitations which are: They give temporary idea about cortisol level, meaning that they give feedback about level of cortisol at the time of sampling only not longitudinally or retrospectively. (Karlen et al., 2011), they are affected by any environmental disturbances (Meyer& Novak, 2012), so any physical or physiological stress will affect accuracy of cortisol level. Hence, affect accurate judgment (Thomson et al., 2010; Manenschijn et al., 2011), the circadian rhythm pattern and pulsatile nature of cortisol release is considered an obstacle in the interpretation of results, and finally, the method of assessment of cortisol level in the blood is considering only with the free part of the hormone although it represents only 10% of circulating cortisol, and ignoring the binding part whether to globulin (about 75%) or to albumin (about 10%). (Carroll et al., 2011).

Due to all these limitations and disadvantages, so there is urgent need for a method which give broad and extended idea about chronic exposure to stress.

Identification of cortisol level in hair is a promising, valuable, non-invasive technique in the research field for assessment of role of stress in life and, to fill the methodological void of long- term cortisol assessment (Lambert et al., 2013).

As the rate of hair growth is about 0.35 mm/day which equivalent to 1 cm/ month in average, so the length of the hair can be considered reliable index of stress exposure over time. Thus, the distance between the scalp and the examined segment of hair gives an idea about time of cortisol incorporation. Meaning that the proximal 1 cm to the scalp contain

cortisol incorporated during last month and so on, every 1 cm distal indicating longer duration (Lambert et al., 2013; Russell et al., 2012).

In the current study, the median hair cortisol level was 33.75 pg. /mg, IQR (20- 36.25) in the exposed group, while the median level was 30 pg. /mg, IQR (12.5- 35) in the highly exposed group which is considered high level when compared to normal reference validation ranges at this age group which ranges from 6.8 to 8.5 pg. /mg (Noppe et al., 2014).

High cortisol level has many hazards in children such as Suppressed immunity, Hypertension, High blood sugar (hyperglycemia), Insulin resistance, Carbohydrate cravings, Metabolic syndrome and type 2 diabetes, and Fat deposits on the face, neck, and belly. (Whiticomb, 2011)

Median hair cortisol level showed non- significant statistical difference between the two studied groups. This could be explained by that the early exposure to adverse life experiences is associated with high hair cortisol level rather than the multiplicity of exposure.

Concomitant with these findings, study of (Rietschel et al., 2016) which found positive association between hair cortisol concentration (HCC) and perceived stress which was measured with the Perceived Stress Scale and/or the Daily Life and Stressors Scale. Added that, this association became stronger whenever cortisol level increase, the study explained that by the genetic effects which influence both the HCC and the perceived stress.

In the same context, study of (Groeneveld et al., 2013) who assessed (HCC) in children before and after elementary school entry. Hair analysis reflected that HCC were higher after school entry than before, especially for fearful children. Alterations in HCC were not documented in daycare centers before school entry. Thus, HCC suggests that starting elementary school is accompanied by increased stress hormone levels in young (in particular fearful) children.

Study of (Simmons et al., 2016) also came with results in the same context, as it revealed that exposure to early life trauma was associated with elevated HCC in 70 children (with mean age= 9.5). The study suggested that hair cortisol may represent a biomarker of exposure to trauma in this age group.

In conclusion, Early exposure to adverse life experiences is associated with high hair cortisol level "with its negative impacts on physical and mental health" rather than the multiplicity of this exposure.

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