

Inflammatory parameters in sexually abused children

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ABSTRACT

الأهداف: لمعرفة ما إذا كانت مستويات المصل من الكورتيزول، انترلوكين 6 و انترلوكين 10 تختلف بين الأطفال الذين يعانون من أو بدون صدمة جنسية.

الطريقة: وقد أجريت هذه الدراسة الوصفية في قسم خدمة حماية الطفل، مستشفى بورسا يوكسك إهتيساس للتدريب والبحوث، بورصة، تركيا، خلال الفترة من مايو 2016م إلى يوليو 2016م. واستخدمت 3 علامات مختلفة (الكورتيزول، انترلوكين 6 و انترلوكين 10) لتنفيذ هذه التجربة. وأدرجنا 17 طفلاً تقل أعمارهم عن 18 عاماً واعتدي عليهم جنسياً، و 10 أطفال لم يتعرضوا لسوء المعاملة كمجموعة ضابطة. تم قياس مستويات الكورتيزول، انترلوكين 6، والانترلوكين 10- في عينات الدم باستخدام طريقة الفحص المناعي المرتبط بالإنزيم. تم تحليل نتائج التجربة إحصائياً.

النتائج: وتشير النتائج إلى أن مستويات مصل الدم من الإنترلوكين 6 كانت أعلى لدى الأطفال الذين تعرضوا للإيذاء الجنسي مقارنة مع المجموعة الضابطة ($p=0.002$). لم تكن هناك فروق ذات دلالة إحصائية في مستويات مصل الكورتيزول ($p=0.604$) والانترلوكين 10 ($p=0.835$) بين الأطفال المعتدى عليهم جنسياً والمجموعة الضابطة.

الخاتمة: لم تظهر اختلافات كبيرة بين المرضى الذين تعرضوا أم لم يتعرضوا للاعتداء الجنسي من حيث مستويات الكورتيزول والانترلوكين 10. وجد أنه بعد ضبط العمر مع المجموعة الضابطة، كانت مستويات الإنترلوكين 6 أعلى لدى الأطفال الذين تعرضوا للإيذاء الجنسي.

Objectives: To investigate whether serum levels of cortisol, interleukin-6 and interleukin-10 differed between children with or without a sexual trauma.

Methods: This descriptive study was conducted in the Department of Child Protective Service (CPS), Bursa Yuksek Ihtisas Training and Research Hospital, Bursa, Turkey, between May 2016 and July 2016. Three different markers (cortisol, interleukin-6 and interleukin-10) were used to perform this experiment. We included 17 children age less than 18 years old and sexually abused, and 10 children who were not

abused as a control group. The levels of cortisol, interleukin-6, and interleukin-10 were measured in blood samples using enzyme-linked immunosorbent assay method. The results of the experiment were statistically analyzed.

Results: The findings indicate that serum levels of interleukin-6 were higher in the sexually abused children compared with the control group ($p=0.002$). There were no significant differences in the serum levels of cortisol ($p=0.604$) and interleukin-10 ($p=0.835$) between the sexually abused children and the control group.

Conclusion: No significant differences were seen between the patients with or without sexual assault in terms of cortisol and IL-10 levels. It is found that in age-matched controls, SAC had higher IL-6 levels.

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The term sexual intercourse with the minor refers to 'any person who enters, without any force, threat or deceit, into sexual intercourse with a minor who has completed 15 years of age shall be sentenced to a penalty of imprisonment for a term of 6 months to 2 years, upon complaint according to the Turkish Law.¹ A minor means a boy or girl who is under the age of 18 years, in general. Sexual abuse can be performed through oral or genital contact, attempted or made intercourse, fondling of genitals or through clothing, exhibitionism

or having sustain to sexual behavior of an adult or pornography.² Sexual abuse of children is not seen rare worldwide. The World Health Organization reported that 20% of girls and 5% to 10% of boys experience sexual abuse worldwide.³ The victims of sexual abuse may suffer from physical and psychological disorders after trauma. More than one of 3 victims of sexually assault may suffer from psychiatric disorders including post traumatic stress disorder (PTSD), depression or panic attack.⁴ A forensic medicine specialist performs the medical examination of sexually abused children (SAC) in order to help justice. The practitioner looks for a traumatic finding including ecchymosis, bruising, laceration, avulsion, hematoma, edema, and so forth, during the physical and genital examination. Many of the findings are not associated with the claims. The factors that cause difficulty in diagnosing include the ability of children' tissue to heal up faster, the prolonged time between the incident and the medical examination, presence of a parasitic disease in the child, itching of anal area, diarrhea, constipation, anal fissure, internal and external hemorrhoids, lack of experience of examining physician, and variations of anatomic structure of perineum. The examination of SAC should be supported by objective biomarkers due to the causes mentioned above. In the literature, there are many vital findings and biomarkers which were performed and recorded for this purpose. Stress influences central nervous system in 2 common ways. 1) Activating physical receptors (for instance heat, cold, and injury of receptors). The stimulus is activated directly from the brain stem to the hypothalamus. 2) Determining the physiological stressors (real or imagined) in higher regions of the brain, and to activate the hypothalamus. The paraventricular nucleus of hypothalamus wherein the stressful data is assessed activates the HPA axis. The HPA axis makes up the immune system through controlling the hemostasis and behavioral adaptation.⁵ Inflammatory response of our body is performed to the source of stress through regulation of hormone and cytokines in peripheral blood. When a person encounters a condition that causes stress, sympathetic nervous system (SNS) becomes activated thus HPA

works. The HPA promotes secretion of cortisol, a peptide hormone, and cytokines including interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF- α) mainly. These 3 cytokines are called pro-inflammatory cytokines. If inflammation should be reduced, levels of anti-inflammatory cytokines including interleukin-10 (IL-10) and interleukin-4 (IL-4) mainly increases due to harming potentials of pro-inflammatory process. Cytokines have an important role in the brain function through influencing neurocircuitry and neurotransmitter systems to produce behavior alterations. The long-term exposure of elevated serum levels of inflammatory cytokines may cause depression and other neuropsychiatric disorders.⁶

We decided to determinate IL-6 as a pro-inflammatory cytokine, IL-10 as an anti-inflammatory cytokine and cortisol as a hormone that is secreted when SNS is activated in peripheral blood. The objective of this study was also to prevent relapsing examination of SAC. A study that included 120 victims of sexual assault reported that all the victims were examined more than once.⁷ The aim of this study was to investigate whether serum levels of cortisol, IL-6, and IL-10 differed between children with or without a sexual trauma.

Methods. The study was designed as a descriptive study and conducted between May 2016 and July 2016. The data collection was performed in the Department of Child Protective Service (CPS), Bursa Yuksek Ihtisas Training and Research Hospital, Bursa, Turkey. This study included 17 children who were under the age 18 and sexually abused, and 10 children who were not abused as a control group. The inclusion criteria were age (under 18), having examined within 72 hours following a sexual abuse, and giving consent to the study. Exclusion criteria were obesity, autoimmune and chronic diseases including any metabolic or inflammatory diseases, and usage of drugs for both groups. Comorbidity of mental disorders and additional anti-inflammatory medications were mentioned as an exclusion criteria. A few participants agitated were refused. The participants were divided into 2 groups SAC and healthy children. The race, age, weight, and the height did not differ between the groups. Blood sample was collected through antecubital venipuncture. The samples were collected from all participants to measure cortisol and cytokines. Forensic examinations were performed only by bi-manual retraction of genitalia and inspection. No instruments were used for the examination and no camera was used during the examination. Children and their parents provide their consent to the study after they read the informed consent form and oral presentation. No PTSD or psychologic disorder symptoms were

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observed in the participants and all subjects were anti-inflammatory medication free.

An approval was obtained from the ethics committee of University of Health Sciences, Bursa Yuksek Ihtisas Training and Research Hospital for the study (25.05.2016-2011-KAEK-25 2016/10-11).

Data collection. The participants' blood samples were obtained at 05:00 a.m. and 10:00 a.m. due to diurnal rhythm of cortisol. Control subjects arrived at the clinic of pediatrics at 08:30 a.m. to 10:00 a.m. Thus, the results were not affected by the time of the day. After the genital examination of SAC, 5 ml blood sample was obtained from cubital venipuncture. The blood samples were collected into an untreated vacutainer tube by standard venipuncture. After a clot was seen (approximately in 30 minutes), the tube was centrifuged at ambient temperature for 15 minutes at 3500 rpm. Serum was obtained by a micropipette and transferred to 3 microtubes. Each portion was added into a small tube, which had a label of the participant's name on it. The small tubes were stored frozen in -80°C until assayed by enzyme-linked immunosorbent assay (ELISA). The study was performed within 45 days of collection on frozen serum samples. At the same time, 3 markers were analyzed by the ELISA method.

Assays. Serum IL-6, IL-10 and cortisol were measured by ELISA using Rel Assay Diagnostic kits reagents (Rel Assay Diagnostics, Turkey) specific for human according to the manufacturer's instructions. Assay sensitivity was (1.03 ng/L, 2.59 pg./ml, and 0.25ng/ml, respectively). Each sample was tested once. To minimize and control for interassay variability, analysis of the samples was deferred until a minimum of plasma and supernatants from 27 participants could be run together. Intra-assay coefficients of variation for the IL-6 ELISA was $<10\%$ and interassay was $<12\%$. The minimum detection threshold was 0.3 pg./mL. Then the results were statistically analyzed.

Statistical analysis. Statistical analysis was performed using the IBM SPSS Statistics for Windows, Version 20.0 (Armonk, NY: IBM Corp). Statistics Version 20 program was used for the statistical analysis. Because the scores in the table were not consistent with the normal distribution, we performed the Shapiro Wilk test. In between-groups comparisons, independent sample T (Levene) or Mann-Whitney U tests were used because the number of independent groups was 2. A p -value of <0.05 was considered statistically significant. Interluken-6 and cortisol did not show a normal distribution and Mann-Whitney U tests were computed to compare SAC to control subjects. Interluken-10

showed a normal distribution and independent sample T (Levene) test was computed to compare SAC to control subjects.

Results. The mean age of the victims was 15 years (range 3-17 years) and healthy children was 10.4 years (range 6-16 years). We recruited 17 SAC and 10 children without trauma for this study (Figure 1). Member of the control group provides normal values of the markers that we analyzed in this study. Most victims were examined within 72 hours following the abuse. All of the participants' blood pressure was in the normotensive range, and BMI was normal weight range. The findings indicate that serum levels of interleukin-6 were higher in the SAC than those of children no abused. Neither serum levels of IL-10 nor cortisol were significantly associated with sexual abuse history ($p=0.835$, $p=0.604$, respectively) (Table 1).

Discussion. Examination of SAC is a significant issue that is very important. All physicians as well as forensic medicine specialists should meet the patient or victim. The practitioner prepares the report after the medical examination of the victim for justice help. When the sufferer was a child, the examination became harder due to some physical limitations. For instance, physical symptoms of the victim of sexual assault can be healed quickly and sometimes it can be illusive.⁸ To evaluate the findings of examination more accurately, some markers or tools are needed. From this point of view, a biochemical marker is required as an evident of sexual assault. The aim of the current study was to evaluate feasibility of determination of cortisol as a stress hormone, IL-6 as a pro-inflammatory cytokine and IL-10 as an anti-inflammatory cytokine in cases of SAC. In further studies, a diagnostic marker can be found. In the light of our findings, it can be said that serum levels of IL-6 were correlated with sexual abuse in children within 72 hours following the assault.

In the literature, many of the biochemical markers have been studied to achieve a diagnostic marker of the rape examination. A study revealed that no significant correlation was seen between the serum levels of CSF corticotropin-releasing hormone and trauma in combat veteran adults with PTSD.⁹ Another study reported that significant correlation was observed between the childhood trauma and the decreased serum levels of TNF- α .¹⁰ Nicola et al¹⁰ revealed that the significant correlation was found between the childhood trauma and the higher serum levels of IL-6, IL-1 β , and TNF- α in adults. Gouin et al¹¹ suggested that elevated serum levels of IL-6 as a response to stressful events were seen

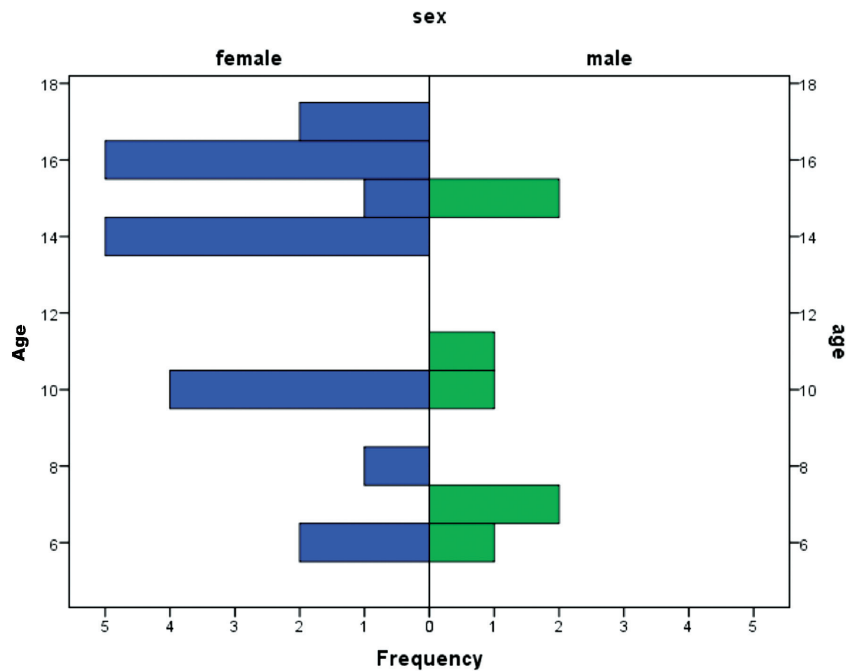


Figure 1 - Age distribution of 17 children, age less than 18 years old and sexually abused and 10 children who were not abused (control group), according to gender.

Table 1 - Serum levels of cortisol, interleukin-6 and interleukin-10 differed between children with or without a sexual trauma.

Group	Mean	Standard deviation
<i>Interleukin-6 levels</i>		
SAC (n=17)	0.839076	0.1186400
Healthy (n=10)	0.361880	0.3254211
Total (n=27)	0.662337	0.3169608
<i>Interleukin-10 levels</i>		
SAC (n=17)	0.137282	0.0183691
Healthy (n=10)	0.135780	0.0171771
Total (n=27)	0.136726	0.0176160
<i>Cortisol levels</i>		
SAC (n=17)	0.181759	0.0392435
Healthy (n=10)	0.184300	0.0302384
Total (n=27)	0.182700	0.0355780

SAC - sexually abused children

increased levels of C-reactive protein; an inflammatory biomarker and decreased levels of IL-6 than those without.¹³ On the other hand, a study suggested that twins had a childhood trauma showed elevated levels of CRP but not IL-6 to those in controls.¹⁴ In another study, reported that greater levels of plasma IL-6 and CRP was observed in women exposed to sexual abuse in childhood relative to the control group.¹⁵ A meta-analysis revealed that significant correlation was seen between adults with history of childhood trauma and serum levels of CRP, TNF- α , and IL-6.¹⁶

The cortisol is the most studied hormone among the hormones of HPA axis.¹⁷ Cortisol is a member of glucocorticoid family that is also secreted in circadian rhythm and reaches maximum levels in 15 minutes after the stressful event.⁵

Study limitation. The time of day for collection of blood samples. On the other hand, cortisol levels can be changed according to the genetic polymorphism.² A study revealed that decreasing biphasic serum cortisol level was found in school-aged children with history of sexual or physical abuse in first 5 years of their life.¹⁸ Schalinski et al¹⁷ reported that although decreased levels of cortisol were found in women with history of sexual assault, lower serum phasic cortisol could be associated

in adults with history of sexual assault to compare control group in similar conditions. Our findings are consistent with the literature. A study evaluated that IL-6 has a biphasic pattern in young adults, including 2 nadirs at almost 08:00 and 21:00, and 2 zeniths at almost 19:00 and 05:00 hours.¹² In our study, circadian pattern of IL-6 was not observed. A study reported that women suffered from partner stalking history showed

with higher tonic levels after trauma.¹⁷ Another study revealed that physical abuse caused lower levels of cortisol, and physical neglect caused higher levels of cortisol in adults with history of child abuse.¹⁹ A study indicated that sexual abuse in childhood resulted in increased levels of cortisol, and lower serum levels of cortisol were seen in SAC who exposed to enset behavior.²⁰ Another study revealed that women suffered from sexual abuse in childhood showed elevated levels of cortisol awakening response during pregnancy compared to the women with the history of abuse and no abuse.²¹ A meta-analysis reported that decreased levels of cortisol were found in adults with PTSD.²² De bellis et al²³ suggested that sexually abused girls showed total and free basal and cCRH stimulated plasma levels of cortisol in peripheral blood, and 24-hours urinary free cortisol measures were not different to those in controls.²³ A study revealed that elevated hair cortisol levels were varied by history of child abuse.²⁴

A study revealed that levels of IL-10 and IFN-gamma in peripheral blood were increased in victims of rape.⁴ Another study suggested that serum levels of IL-6 and IL-10 were not statistically different between the groups.²⁵ A meta-analysis revealed that people with trauma history showed increased levels of CRP, IL-1b, IL-6, TNF- α compared to the people without trauma.²⁶ The same meta-analysis reported that no correlation was found between people with trauma history and serum levels of fibrinogen, IL-2, IL-4, IL-8, and IL-10.²⁶ The genetic polymorphism of cortisol levels and the circadian pattern of IL-6, IL-10, cortisol was not observed. These were the limitations of our study.

In conclusion, our study revealed the increased inflammation marker after a sexual assault in human body. Diagnosis of such cases is of vital importance. No significant differences were seen between the patients with or without sexual assault in terms of cortisol and IL-10 levels. It is found that compared to age-matched controls, SAC had higher IL-6 levels. Increased IL-6 levels are evidence for dysregulation of the HPA-axis in SAC. Further studies are recommended with large patient groups for the purpose of enlarging generalizations.

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