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The Effect of Environmental Tobacco Smoke on the Testicular Functions of 7-10 Years Old Children

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Purpose: To investigate the effect of environmental tobacco smoke (ETS) on the testicle functions of 7-10 years old children.
Materials and Methods: The study included healthy male children aging between 7-10 years. Based on the questionnaire data, 50 passive smoking (PS) cases and 50 passive smoking free controls were determined. Serum levels of cotinine, and biochemical parameters of the testicle functions; testosterone, inhibin B, anti-mullerian hormone (AMH) along with FSH and LH levels were evaluated.
Results: Serum cotinine levels in PS group were found higher than the controls ($p<0,05$). Testosterone, inhibin-B, FSH, and LH levels of the control group was found higher than the PS group. The AMH levels were found lower in the controls in comparison with the PS group ($p<0,05$).
Conclusions: Our results showed that ETS leads to testicular dysfuntions. We think that preventing exposure to cigarette smoke in childhood is very important for their reproductive system.
Key Words: Testis; Cigarette; Environmental Tobacco Smoke; Cotinine; Inhibin B; Anti-Mullerian Hormone; Testosterone; FSH; LH.

Çevresel Sigara Dumanının 7-10 Yaş Grubu Çocuklarda Testis Fonksiyonlarına Etkisi

Amaç: Pasif içicilik olarak tanımlanan çevresel sigara dumanına maruz kalma sık karşılaşılan bir durumdur. Bu çalışma ile pasif sigara içicisi durumunda olan 7–10 yaş grubu çocuklarda çevresel sigara dumanının testis fonksiyonları üzerindeki etkisini incelemeyi amaçladık.
Gereç ve Yöntem: Çalışmaya ilköğretim okullarında okuyan, 7-10 yaş grubunda ek bir sağlık problemi olmayan erkek çocuklar dahil edildi. Pasif sigara içicisi durumunda olan (Pİ) 50 olgu ve olmayan (K) 50 olgu anket çalışmasıyla belirlendi. Belirlenen olguların serumlarından kotinin düzeyleri ile testis fonksiyonunun biyokimyasal göstergeleri olan testosteron, inhibin-B, anti-müllerian hormon (AMH) ile beraber FSH ve LH düzeyleri çalışıldı.
Bulgular: Pİ grubunda serum kotinin düzeyleri K grubuna göre belirgin artmış bulundu ($p<0,05$). Testosteron, inhibin-B, FSH, LH düzeyleri K grubunda Pİ grubuna göre belirgin olarak yüksek bulunurken; bu yaş grubunda testosteron ile ters orantılı hareket eden AMH seviyelerinin K grubunda Pİ grubuna göre düşük olduğu saptandı ($p<0,05$).
Sonuç: Elde ettiğimiz sonuçlar çevresel sigara dumanının testislerde fonksiyon bozukluğuna yol açtığını göstermektedir. Bunun da erişkin yaşlarda ortaya çıkabilecek infertiltenin nedenlerinden birisi olabileceği düşünülmektedir.
Anahtar Kelimeler: Testis; Sigara; Çevresel Sigara Dumanı; Kotinin; İnhibin B; Anti-Müllerian Hormon; Testosteron; FSH; LH.

Introduction

Children's exposure to other people's cigarette smoke (environmental tobacco smoke, ETS) is probably one of the most important public health hazards in our country. Exposure to ETS prevalence among Turkish adolescents is alarmingly high (89.0%).[1-4] Children are exposed to tobacco smoke not only in their homes, but also in cars, child care settings, and other public places. However, exposure at home during parental smoking is likely the commonest source of ETS exposure to children.[5,6]

Tobacco smoke contains more than 4000 compounds.[7,8] ETS has been found to be causally

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associated with a large number of diseases in various organs. There have been many investigations regarding passive smoking exposure and health status during childhood. These investigations show that ETS or passive smoking, increases the risk of sudden infant death syndrome, lower respiratory tract illness, prevalence of wheeze and cough, and exacerbates asthma.[1,9,10]

It is surprising that studies on the effects of ETS on the testis function are rather rare in children. However, a majority of investigations about the relationship between male reproductive system and tobacco smoke are about the effect of active smoking in adults or ETS exposure in rats.[11-14] The main objective of the current study was to evaluate the effects of ETS exposure on testicular function in children.To our knowledge, it is the first study conducted in the literature. For assessing ETS exposure in children a parent-completed questionnaire and the measurement of serum cotinine level were used. Cotinine is a breakdown product of nicotine with a half-life of 20 h; it is stable with temperature change, or current infection, and has high specifity and sensitivity. Cotinine can be detected in several body fluids, such as serum, urine and saliva.[15]

For evaluation of the testicular function, serum anti-Müllerian hormone (AMH), inhibin B, testosterone, follicle stimulating hormone (FSH) and luteinising hormone (LH) levels were measured in ETS exposed and non-exposed children.

Materials and Methods

This study was approved by the Ethical Committee of Inonu University. After a complete and detailed explanation about the nature of the research, its aims, methods, and the inconvenience may the methodology cause, a written informed consent form was requested from the parents to allow their child's participation into the study.

Healthy primary schoolchildren, whose socioeconomic status and vegetable–fruit consumption were similar, were enrolled in this study. Parental cigarette smoking was assessed using a self-completed parental questionnaire. The questionnaire consisted of 9 questions regarding demographic data (sex, age), exposure status (number of smoking household members, number of daily exposed cigarettes, duration of exposure, outside exposure) and parents' educational status. Children were classified as exposed to ETS if ≥10 cigarettes per day were smoked for two years. According to previous validation studies in which parental self-reported smoking of ≥10 cigarettes per day was associated with a significant increase of children's urinary cotinine levels.[16]

All the children were divided into two groups; first group exposed to ETS (50 children) and the second group nonexposed (50 children) to ETS. Thus, the study group included 100 subjects for the biochemical evaluation. The median age of the participants was 8,32±1,07 years (range 7-10). For hormone and cotinine analyses, blood samples were drawn between 8:00 and 10:00 a.m. from the cubital vein. The serum was frozen at -80 ^{o}C and analysed within 1 month.

Serum concentration of cotinine was measured as the parameter of ETS exposure in children using the ELISA Kit (DRG Diagnostics Marburg, Germany). Serum levels of Inhibin B and anti-Müllerian hormone were measured as indicators of testicular function using the ELISA Kits (Diagnostics Systems Laboratories Inc. Texas, USA). Serum concentrations of testosterone, FSH and LH were measured by an autoanalyser (Immulite 2000, California, USA) using chemiluminescent immunometric analys kits (Diagnostic Products Corparation -DPC- Los Angeles, California, USA),

All data were analysed by using the Microsoft Office Excel 2003 software program. Results are expressed as mean ± SD or proportions, as indicated. Comparisons of quantitative variables among groups were made using unpaired t-test. A P value of <0.05 was considered significant.

Results

As seen on Figure 1, first group (exposed to ETS) had a significantly higher cotinine level in serum when compared to the second group (nonexposed to ETS) (6,70±2,60 ng/mL; and 3,16±0,91 ng/mL, respectively, p<0,05)

Figure 2 shows the serum inhibin B levels of first group and second group. The mean inhibin B level of the first group was significantly lower compared to the second group (3,66±2,75 pg/mL; and 11,70±8,94 pg/mL,

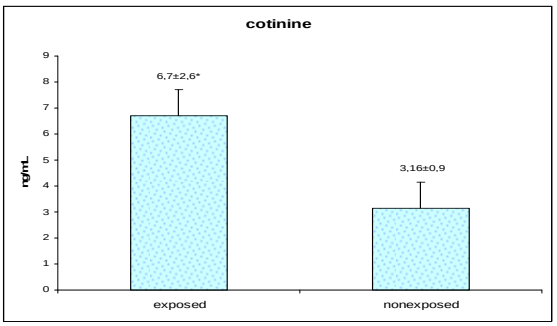

Figure 1. Serum levels of cotinine in groups. Values expressed as mean± SEM, *p<0.05 in comparison with nonexposed group.

respectively, p<0,05). Their serum anti-müllerian hormone levels were, however, significantly higher. The mean serum anti-müllerian hormone levels were 88,99±26,72 ng/mL and 69,64±25,12 ng/mL in the passive smokers and control groups, respectively (p<0,05; Figure 3)

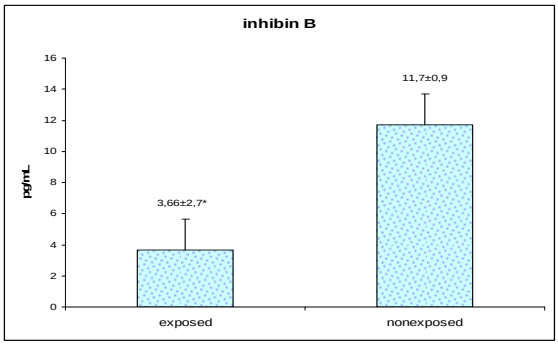

Figure 2. Serum levels of inhibin B in groups. Values expressed as mean± SEM, *p<0.05 in comparison with nonexposed group.

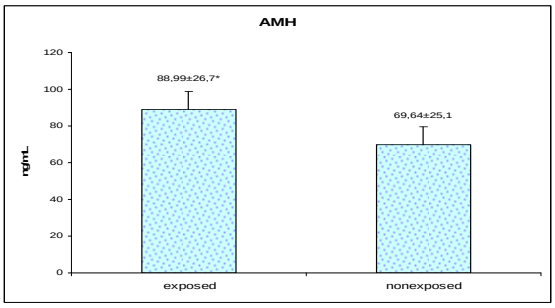

Figure 3. Serum levels of AMH in groups. Values expressed as mean± SEM, *p<0.05 in comparison with nonexposed group.

Serum testosterone, FSH and LH levels of first and second groups are shown on Table 1. Serum mean testosterone level of children exposed to passive smoking was found to be significantly lower than those of not exposed (p<0,05). Passive smoking decreased the level of serum FSH in the first group compared to second group (p<0,05). Similarly, the serum LH level in the exposed group was decreased by ETS (p<0.05).

Table 1. Serum testosterone, FSH and LH levels in groups.

Groups	Testosterone (ng/dL)	FSH IU/L	LH IU/L
Exposed	29,93±9,1*	0.93±0.61*	0,21±0,2*
Non-exposed	180,95±183,6*	1,47±0.92	0,56±0,5

Values expressed as mean± SEM, *p<0.05 in comparison with nonexposed group.

Discussion

This study demonstrates for the first time that ETS exposure of young children via smoking by their caregivers and other house-hold members is associated with increases in serum cotinine and AMH levels and decreases in serum inhibin B, testosterone, FSH and LH levels. As we know that ETS has been regarded as one of the most important public health issues. It has been estimated that approximately 75-89% of Turkish children are exposed to ETS.[3,4] It was demonstrated that young age of the child was significantly associated with higher ETS exposure. Because they spend more time in closer proximity to their smoking parents, and they can not choose whether or not to be in a smoky environment.[6]

The subject of possible detrimental effects of cigarette smoking or passive smoking on reproductive system in the male is of great interest. In literature, there are several cross-sectional studies about the effects of active or passive smoking on semen quality or testicular hormone levels, but most of which have included adolescent or adult patients or have been done in rats.[12,14,17,18] Some studies have shown a negative effect of smoking on semen quality in terms of the conventional semen characteristics whereas others could not demonstrate such an effect.[12,17] Furthermore, it has not been clarified whether smoking has an impact on levels of male reproductive hormones. Significantly increased,[12] decreased and unchanged levels of total testosterone in male smokers have been reported in various studies.[12,13,17] In a study semen parameters and hormone concentrations of infertile smokers had been compared with infertile non- and ex-smokers. In this study Trummer et al reported that smoking does not affect conventional semen parameters, but increases serum free and total testosterone.[12] Similarly, Chohan et al found that there are no differences between smokers and non-smokers for ejaculate volume, motility, concentration and sperm morphology.[18] However, in an another study it is reported that smokers had significantly less spermatozoa.[19] In a study, Yardımcı et al reported that exposure to cigarette smoke decreases the testosterone level and Leydig cell number in rats.[13] In another study by Yamamoto and associates, secretory dysfunction of the Leydig cells, deficiency in sperm maturation and spermatogenesis, decreased testosterone response to hCG stimulation were shown after exposure of rats to cigarette smoke.[20]

Despite all this intensive work, the study number about negative effects of ETS on testicular functions is quite rare in children. Evaluation of testicular function can be done several methods. Testicular biopsy for histopathological examination is not an appropriate method for children. Therefore we preferred hormonal

and specific markers analysis in serum samples. In this investigation cotinine serves as a marker of tobacco smoke exposure. It is the major degradation product of nicotine metabolism; it is stable with temperature change, or current infection, and has high specificity and sensitivity.[15] In our study, the higher levels of serum cotinine found in children of smoking parents, compared to children of non-smoking parents. The presence of slight elevated cotinine levels in children without known household exposure suggests that ETS should be considered as an urban toxicant as well as an individual residential exposure.[21]

We found decreased levels of serum FSH and LH in the exposed group. But in these children gonadotropins may be unreliable predictors of gonadal damage because the hypothalamic–pituitary–gonadal axis is relatively quiescent.[22] Earlier detection of gonadal damage has been hampered by the lack of a sensitive marker of gonadal function in prepubertal children. The Sertoli cell plays a central role in development of a functional testis.[23] AMH and inhibin B, are testicular hormones, secreted by Sertoli cells and responsible for the regression of Müllerian ducts in male fetuses. Both hormone is normally detectable throughout childhood where it is a direct marker of the presence and function of Sertoli cells.[23,24] Leydig cells secrete androgens.[25] We preferred AMH, inhibin B and testosteron as biochemical markers of testicular function. We found that ETS exposure increased the AMH level and decreased the inhibin B and testosteron levels. As we know that AMH is synthesized during fetal and post-natal life and decreases progressively thereafter until puberty. Therefore, during male puberty, serum AMH and testosterone levels show a significant negative correlation.[23,25,26] In our study, increased AMH and decreased inhibin B levels are suggested that ETS exposure has a inhibitor role on maturation of Sertoli cells. This inference is parallel with similar studies. In a study reduced mean diameter of the seminiferous tubules and reduced number of the Sertoli cells were shown after exposure of rats to cigarette smoke by Ahmadnia and associates.[14] In a another study Guven et al reported that evident degeneration of the spermatogenetic cells and ultrastructural changing of the Sertoli cells were common findings in testicles from smoke exposed rats.[26] In addition to the Sertoli cells reduced testosterone levels may be related to the toxic effects of smoke on Leydig cells. In a previous study Yardimci et al investigated the effect of ETS exposure on the histology of Leydig cells in the testes of rats. In this study, histological examination of the testes showed fewer Leydig cells and degeneration of the remaining cells in testes of ETS exposed rats.[13]

The mechanism behind the the gonadotoxic effect of ETS exposure is not yet understood. The harmful effects of ETS on testicular function might be mediated through oxidative damage. The toxicity of tobacco smoke is due to nicotine, oxidants, and inducers of reactive oxygen species (ROS) like NO, NO2, and peroxynitrite, that initiate oxidative damage.[27] Kosecik at al demonstrated that increased ROS production was associated with increased cigarette smoke exposure in children.[28] In another study it was reported that exposure to cigarette smoke causes changes in the oxidative enzyme levels in rat testis and these harmfull effects can be reversed by caffeic acid phenethyl ester.[29] The other mechanism of negative effect of ETS may also be an inflammatory effect of polycyclic aromatic hydrocarbons (PAH) which is one of the compounds of cigarette smoke.[17] In a study about effects of PAH on cultured rat Sertoli cells, Raychoudhury at al presented that PAH have a direct cytotoxic effects on Sertoli cells and isolated cells of the mammalian seminiferous epithelium.[30]

Conclusion

In conclusion, ETS exposure is associated with a range of adverse health outcomes for children. The current study, for first time in the international literature, reveals a harmful effect of ETS exposure on Sertoli cell secretory function in the children. This effect can remain after the cessation of ETS exposure. Preventing exposure to cigarette smoke in infancy and childhood is very important for their reproductive system. Children's exposure to passive smoking is usually involuntary, arising from smoking, in the places where children live and play. Parental smoking is a common source of children's exposure to ETS. It is most important that parents should be advised to give up smoking and prevent their children from exposure to ETS.

The authors declare that they have no conflict of interest.

References

1. World Health Organization. Tobacco Free Initiative. International Consolation on Environmental Tobacco Smoke and Child Health Report. Geneva: WHO; 1999.
2. Ersu R, Arman A, Save D, et al. Prevalence of Snoring and Symptoms of Sleep-Disordered Breathing in Primary School Children in Istanbul. Chest 2004; 126: 19-24 . DOI: 10.1378/chest.126.1.19
3. Erguder T, Soydal T, Uğurlu M, et al. Tobacco Use Among Youth and Related Characteristics, Turkey. Soz Preventiv Med 2006; 51: 91-8. DOI: 10.1007/s00038-005-0020-x
4. Avşar A, Darka O, Bodrumlu EH, et al. Evaluation of the Relationship Between Passive Smoking and Salivary Electrolytes, Protein, Secretory IgA, Sialic Acid and Amylase in Young Children. Arch Oral Biol 2009; 549; 457-63.
5. Henderson AJ. The Effects of Tobacco Smoke Exposure on Respiratory Health in School-Aged Children. Paediatr

Respir Rev 2008; 9: 21-7. DOI: 10.1016/j.prrv.2007.11.005
6. Delpisheh A, Kelly Y, Brabin BJ. Passive Cigarette Smoke Exposure in Primary School Children in Liverpool. Public Health 2006; 120(1): 65-9. DOI: 10.1016/j.puhe.2005.05.003
7. Zalata A, Yahia S, Ed-Bakary A, et al. Increased DNA Damage in Children Caused by Passive Smoking as Assessed by Comet Assay and Oxidative Stress. Mutat Res 2007; 629: 140-7.
8. Janson C. The Effect of Passive Smoking on Respiratory Health in Children and Adults. Int J Tuberc Lung Dis 2004; 8: 510-6.
9. Tang D, Warburton D, Tannenbaum SR, et al. Molecular and Genetic Damage from Environmental Tobacco Smoke in Young Children. Cancer Epidemiol Biomarkers Prev 1999; 8: 427-31.
10. Tutka P, Wielosz M, Zatoński W. Exposure to Environmental Tobacco Smoke and Children Health. Int J Occup Med Environ Health 2002; 159: 325-35.
11. Ramlau-Hansen CH, Thulstrup AM, Aggerholm AS, et al. Is Smoking a Risk Factor for Decreased Semen Quality? A Cross-Sectional Analysis. Hum Reprod 2007; 22: 188-96. DOI: 10.1093/humrep/del364
12. Trummer H, Habermann H, Haas J, et al. The Impact of Cigarette Smoking on Human Semen Parameters and Hormones. Hum Reprod 2002; 17: 1554-9. DOI: 10.1093/humrep/17.6.1554
13. Yardimci S, Atan A, Delibasi T, et al. Long- Term Effects of Cigarette-Smoke Exposure on Plasma Testosterone, Luteinizing Hormone and Follicle-stimulating Hormone Levels in Male Rats. Br J Urol 1997; 79: 66-9. DOI: 10.1046/j.1464-410X.1997.28314.x
14. Ahmadnia H, Ghanbari M, Moradi MR, et al. Effect of Cigarette Smoke on Spermatogenesis in Rats. Urol J 2007; 4: 159-63.
15. Jarvis MJ, Russell MA, Benowitz NL, et al. Elimination of Cotinine from Body Fluids: Implications for Noninvasive Measurement of Tobacco Smoke Exposure. Am J Public Health 1988; 78: 696-8. DOI: 10.2105/AJPH.78.6.696
16. Oddoze C, Dubus JC, Badier M, et al. Urinary Cotinine and Exposure to Parental Smoking in a Population of Children with Asthma. Clin Chem 1999; 45: 505-9.
17. Richthoff J, Elzanaty S, Rylander L, et al. Association Between Tobacco Exposure and Reproductive Parameters in Adolescent Males. Int J Androl 2008; 31: 31-9.
18. Chohan KR, Badawy SZ. Cigarette Smoking Impairs Sperm Bioenergetics. Int Braz J Urol 2010; 36: 60-5. DOI: 10.1590/S1677-55382010000100010
19. Sofikitis N, Miyagawa I, Dimitriadis D, et al. Effects of Smoking on Testicular Function, Semen Quality and Sperm Fertilizing Capacity. J Urol 1995; 154: 1030-4. DOI: 10.1016/S0022-5347(01)66968-4
20. Yamamoto Y, Isoyama E, Sofikitis N, et al. Effects of Smoking on Testicular Function and Fertilizing Potential in Rats. Urol Res 1998; 269: 45-8. DOI: 10.1007/s002400050022
21. Willers S, Skarping G, Dalene M, et al. Urinary Cotinine in Children and Adults During and After Semiexperimental Exposure to Environmental Tobacco Smoke. Arch Environ Health 1995; 50: 130-8. DOI: 10.1080/00039896.1995.9940890
22. Crofton PM, Thomson AB, Evans AE, et al. Is Inhibin B a Potential Marker of Gonadotoxicity in Prepubertal Children Treated for Cancer? Clin Endocrinol 2003; 58: 296-301. DOI: 10.1046/j.1365-2265.2003.01712.x
23. Young J, Rey R, Couzinet B, et al. Antimüllerian Hormone in Patients with Hypogonadotropic Hypogonadism. J Clin Endocrinol Metab 1999; 84: 2696-9. DOI: 10.1210/jc.84.8.2696
24. Andersson AM, Skakkebaek NE. Serum Inhibin B Levels During Male Childhood and Puberty. Mol Cell Endocrinol 2001; 30: 103-7. DOI: 10.1016/S0303-7207(01)00520-2
25. Sharpe RM, McKinnell C, Kivlin C, et al. Proliferation and Functional Maturation of Sertoli Cells, and Their Relevance to Disorders of Testis Function in Adulthood. Reproduction 2003; 125: 769-84. DOI: 10.1530/rep.0.1250769
26. Güven MC, Can B, Ergün A, et al. Ultrastructural Effects of Cigarette Smoke on Rat Testis. Eur Urol 1999; 36:645-9. DOI: 10.1159/000020061
27. Zhang J, Liu Y, Shi J, et al. Side-stream Cigarette Smoke Induces Dose-response in Systemic Inflammatory Cytokine Production and Oxidative Stress. Exp Biol Med 2002; 227: 823-9.
28. Kosecik M, Erel O, Sevinc E, et al. Increased Oxidative Stress in Children Exposed to Passive Smoking. Int J Cardiol 2005; 8: 61-4. DOI: 10.1016/j.ijcard.2004.05.069
29. Ozyurt H, Pekmez H, Parlaktas BS, et al. Oxidative Stress in Testicular Tissues of Rats Exposed to Cigarette Smoke and Protective Effects of Caffeic Acid Phenethyl Ester. Asian J Androl 2006; 8: 189-93. DOI: 10.1111/j.1745-7262.2006.00119.x
30. Raychoudhury SS, Kubinski D. Polycyclic Aromatic Hydrocarbon-Induced Cytotoxicity in Cultured Rat Sertoli Cells Involves Differential Apoptotic Response. Environ Health Perspect 2003; 111: 33-8. DOI: 10.1289/ehp.5458

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