Tobacco on Kidnev

Original Article Effects of Smokeless Tobacco Alters the Histology of Kidney of Offspring's in Swiss Albino Mice

Shoukat Ali Memon¹, Abdul Hafeez Dall², Qadir Bux Memon³ and Zaheer Ahmed Memon¹

ABSTRACT

Objective: To study the micro structural changes in the kidney of the offspring's of Swiss albino mice exposed to smokeless tobacco during pregnancy.

Study Design: Observational / descriptive study.

Place and Duration of Study: This study was conduct at the Animal House of the Department of Animal Husbandry and Veterinary Sciences (AHVS) Sindh Agriculture University, (SAU) Tandojam from July 2015 to December 2015.

Materials and Methods: Healthy adult female mice were mated. After confirmation of pregnancy, 20 pregnant mice were categorized into two categories, experimental group A and control group B. Group-A was provided Tobacco 5% mixed with standard diet along with clean water ad libitum, whereas group B, the control was provided standard diet and clean water ad-libitum throughout their pregnancy. After birth 20 offspring (10 male & 10 female) were selected randomly from each group. At 15 days after birth, the offspring were sacroned by cervical dislocation and their kidneys were dissected out for histological analysis.

Results: The histological marked changes were seen in the kidney of offspring's obmice. In the experimental group of offspring there were very few glomeruli and also more immature glomerul, were observed. Glomerular degenerative changes, micro calcifications were noticed in both female and hele offspring's of experimental group. Fatty change was observed in the renal parenchyma of the experimental group in 14 animals 9 male and 5 female offspring's showed edematous change and fatty infiltration. Glomerulus a chitectural distortion and displacement were also seen in kidneys of both offspring's.

Conclusion: Consumption of smokeless tobacco having significant effects on structure of kidney of offspring of mice that presented with the cellular injury to kidney pareachyma especially fatty infiltration as well asglomerular distortion and degenerative changes.

Key Words: Smokeless Tobacco, Offspring, Kidney

Citation of article: Memon SA, Dall AH, Memon B, Memon ZA. Effects of Smokeless Tobacco Alters the Histology of Kidney of Offspring's in Swiss, Albino Dice. Med Forum 2016;27(12):36-40.

INTRODUCTION

Tobacco is being used since centuries in variety of ways and forms like smoking and snokeless tobacco.¹ The smokeless tobacco (ST)usege c raing day by day because of indoor smoking bans unproven awareness of safety, as well as reported "positive" physiological outcomes, for example remeation, increased alertness, raised concentration, halucination and produce anorexia.² The commonest types of smokeless tobacco existing and utilized in Pakistan includes: Betel/pan with tobacco, Naswar, snuff, Chaallia/supari with tobacco, Gutka and pan masala.³

Correspondence: Dr. Zaheer Ahmed Memon, Assoc. Prof. of Anatomy, Isra University Hyderabad Sindh Contact No: 0313-2851728 Email: dr.sajidarain@gmail, comzaheermemon32@yahoo.com

Received: September 17, 2016; Accepted: October 29, 2016

The utilization of ST can lead to cancer amongpeopleas well as have greater risk of gum &cheek cancer. ST utilization may be addictive, causing gingival recession, (oral mucosal lesions)oral leukoplakias, and can possibly contribute significant occurrence of peripheral vascular disorder, cardiovascular disorder, peptic ulcers, hypertension, and fetal comorbidity & mortality.4.5 The utilization of Smokeless Tobacco imbalances the electrolytes in kidney hemodialysis patients as well as alters the renal antioxidant mechanism and renal microstructure in rodents.⁶⁻⁸ Health experts have long reflected interaction to tobacco smoke injurious to reproduction, distressing features from fertility as well as pregnancy consequence to fetus and its development. The Smoke of tobacco comprises thousands of compounds, a few of them are identified to impose toxic outcomes on reproductive health, for example nicotine, carbon monoxide (CO), and metals.⁹ Several surveys have reported the relationship amid maternal tobacco chewing and long-term health effects within the offspring, together with obesity, cardiovascular and respiratory disorders.¹⁰ Maternal use of smokeless tobacco is correlated with intrauterine growth

^{1.} Department of Anatomy Isra University Hyderabad

² Department of Anatomy Peoples University of medical &

Health Sciences Nawabshah (SBA)

^{3.} Department of Anatomy, AL-Tibri Medical College Karachi

retardation (IUGR); whereas intrauterine growth retardation in turn, is correlated with diminished quantity of nephron within the off spring.¹¹ Undoubtedly, human research has exhibited that either use of smoking and smokeless tobacco is strongly associated with lower fetal renal volumes in the course of 2nd&3rd trimester, and lesser the birth weight.^{12,13} In human beings, nephrogenesis initiates at gestational week¹²⁻¹⁴ and terminates at gestational week 36.¹⁶ Majority of nephrons are created during 3rd trimester,¹⁵ as well as the definitive quantity of nephrons in every kidney is formed at birth. Though, among rodents, nephrogenesis remains following birth for a littletime period till weaning.¹⁷ Modification of the growth factors at any point of renal development may result in underdevelopment of kidney as well as potential kidneys dysfunction.^{18,19} Although there are many studies about the hazards of usage of smokeless tobacco in the course of pregnancy and its effects in literature, but due to the structure and scope of these studies, the utero-placental mechanism of smokeless tobacco and its effects on fetal and infant organs is not clearly understood and histological experiments are also limited. The purpose of this study was to examine the modifications of renal micro structure in mice offspring due to consumption of ST during pregnancy.

MATERIALS AND METHODS

Female & male Swiss albino rates were acquiredvia the animal house of the Department of Animal Husbandrs and Veterinary Sciences (AHVS) Sindh Agriculture University, (SAU) Tandojam. Healthy female Swis albino mice were mated. The animals were rearectin a hygienic as well as well-aired setting. Milet were given diet (lab chow) as well as tap water at lib. The dark/light cycle was looked after at 11 hours intervals. 20 non-pregnant Swiss albino mice aged from 10 to 12 weeks with 28gm of average weigh were selected randomly, as well as were integorized into two categories; each category with ten mice. Each female mated with male adult mice reserved for sex for four to ten days to increase their uge of sex for female mice. One male mouse was mated with two females. Pregnancy was confirmed by presence of mucus vaginal plug between 1-10 days of pairing. On pregnancy confirmation the male mice were isolated.

After confirmation of pregnancy 20 pregnant female mice were categorized into two categories, Group A was given Tobacco 5% mixed with usual food along with clean water ad-libitum, whereas group B, the control was provided regular diet and clean water ad libitum throughout their pregnancy. After birth 20 offspring (10 male & 10 female) were selected randomly from each group.

Offspring of both A and B categories were allocated sub categories as follows:

Group A-1 (Experimental group) 10 male offspring

Group A-2(Experimental group)10female offspringGroup B-1 (Control group)10male offspringGroup B-2 (Control group)10female offspring

15 days after birth, the offspring were sacrificed by cervical dislocation and their kidneys were dissected out for histological analysis.

The kidneys were removed and set in 10% formaldehyde later dehydration was performed in leading qualities of alcohol. The tissues then were freed from xylene quickly to eliminate the alcohol. Impregnation/infiltration was performed for two alterations of soft molten paraffin wax; each at the temperature of 58°c upto 30 minutes. Implanting & dipping in paraffin wax with two L- formed pieces of metal was performed as well as sectioning was done with a microtome. Four micron (u) thick sections were done on rotary microtome then dipped in hot water container.

The sections were fixed in sides by a thin layer of egg albumen coated on every slide. De-waxing was performed by hot pate a 37° afterwards clearing in two alterations of x len

Isolation of Xyane vas oone through absolute alcohol and at last prior to staining, hydration was carried out. The sections were stained with hematoxylin & Eosin and fixed in canada balsam. The slides were assessed for instopathological variations under light microscope. Al dat, was recorded in the proforma.

RESULTS

The Histological Profile of the Kidney Section: The marked histological changes in the kidney were seen in smokeless tobacco exposed offspring.



Figure No.1: A section of kidney of offspring of mice showing normal architecture of kidney

In the experimental group of offspring there were lessglomeruli & further immature glomeruli were observed as contrasted to Control offspring. Glomerular size was also significantly decreased that there was





Figure No.2: A section of kidney of male offspring of mice showing shrinkage of glomeruli, necrosis and disruption of renal tubules



Figure No.3: A section of kidney of offspring of mice showing Distortionand Edematuschange of Glomerulus and Tubular Necrosis



Figure No.4: A section of kidney of offspring of mice showing micro calcifications

shrinkage and distortions of glomerular architecture seen in the experimental male and female offspring. The animals of treated group subjected to smokeless tobacco exhibited significant degenerative variations, fatty infiltration and edematous changes in kidney parenchyma. The more potent destructive structural changes of glomerulus as well as tubules were seen in male offspring as compared to female offspring. In which 14 male offspring and 9 female offspring showed edematous change and fatty infiltration. Glomerulus shrinkage and distortion were seen in both offspring in which 11 male offspring and 5 female offspring showed these findings in their kidney architecture.



Figure No.5: A section of kidney of offspring of mice showing glomerulusdegenerativechanges, loss of architecture & tubular inflammation

DISCUSSION

This study showed that smokeless tobacco having significant effects on the microstructure of kidney of offspring mice. The most important finding of this study exhibits that maternal smokeless tobacco exposure in gestational period is evidently correlated with loss of kidney architecture causing kidney dysfunction as well as raised inflammatory indicators. Structurally, subtle variations were noticed in glomeruli and tubules within the kidneys of ST exposure progenies. Inflammation associates with kidney impairment and plays a vital role in the development of chronic renal disorder, which was well exhibited in current study. Consequently, the progenies of SE dams can possibly be inclined to more kidney impairment with the progression of adulthood. Varying forms of cellular degeneration were noticed in the proximal convoluted tubules that can possibly settle the functional reliability of proximal convoluted tubules. This attribute can possibly cause the retention of metabolic waste products as well as endurance of such abnormalities can possibly lead to loss of delicate homeostatic systems of the kidney.^{20,21} as well as

histological cardiac, hepatic lungs and renal surveys, as well as testes were conducted in terms of procedures defined by Disbrey & Rack²² and Drury & Wellington.²³

Usage of smokeless tobacco is relatively prevalent in the Middle East, Far East as well as European nations⁵. Chewing tobacco of different brands is available in most part of our country. The commonest types of smokeless tobacco present and utilized in Pakistan comprise: catechu (Acacia catechu), tobacco, Betel/pan with tobacco a chewed areca nut mixture (Areca catechu), slaked lime [calcium hydroxide (CaOH)₂ and calcium oxide (CaO)], wrapped in a (Piper betel)betel leaf with sweetening agents.²⁸

Some studies^{24,25} have been conducted to determine the outcome of nicotine on fetal growth as well as whether this could be correlated with the activities of this drug over the metabolism of maternal adipose tissue. It has though been speculated that nicotine existing in tobacco smoke can possibly result in reduce maternal appetite, uterine vasoconstriction, or somehow produce metabolic variations within the mother and/or fetus reported by Nakamoto T, et al²⁶.

The placenta contributes significantly in prenatal development through carrying nutrients as well as waste products amid fetal & maternal circulation and by offering hormones required for typical development. Human placental explants can be developed as well as • examined experimentally in vitro reported by Jauniaux E, et al^{24} . In one such study, it was shown that nicoting unaccompanied was capable of inhibiting variation and thus inhibited to trophoblast invasion within an in view test. These authors more over exhibited that no otin retarded synthesis as well as activation of type-IV collagenase, which is essential for cytotrop oblast invasion. A few placental surveys have included an fascinating association of in-vivo and in-vivo experimentation^{24,25}. This study showed in the tubular structure of the cortex of kidney or animal's nicotine exhibited disturbance the hist ogical structure of kidney.Nicotine is a fundamental component of tobacco that retards the development and variation of cytotrophoblasts within human placenta. Nicotine can possibly caused decreased blood flow and vasoconstriction reported by Kazi AS et al.²⁸ Nicotine can as well escalate maternal blood pressure (BP) and cardiac rate, dropping uterine blood flow²⁶. In our study the necrotic and inflammatory changes were noticed in the ST dams, which are in relation to the study of Agarwal R, et al.²⁷ similarly in the study of Jagadapillai R, et al²⁹ reported that renal proteins expression was affected by CSE belonged to inflammatory diseases, as well as indicated that CSE altered kidney proteome.

CONCLUSION

From these interpretations, it can be concluded that exposure of the smokeless extract may be associated with structural damage of kidney in the offspring of mice.

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

- 1. UTAH. Department Of Health. "History of Smokeless Tobacco" Utah Tobacco Prevention and Control Program, 2007; www.tobaccofreeutah.org/ hissmkls.pdf.
- 2. Centers For Disease Control And Prevention [CDC] (2014). Health united states with the special features of health of young adults. http://www.cdc.gov/nchs/data/hus/hus08.
- 3. Khawaja MRH. Chewing of betel, areca and tobacco: perceptions and knowledge regarding their role in head and neck cancers in an Urban squatter settlement in Pakistan. Asian Pacific J Cancer Preven 2006;7:5:99.
- 4. de Ávila ÉD, de aroon RS, de Almeida Lawall M, Bianco R, Consorro AC. Increased of Langerhans Cells vin Smorele's Tobacco-Associated Oral Mucosal Essions. Archives of Clinical and Experimental Surg ACES 2012;1(2):85-93.
- 5. US Department of Health and Human Services. The health consequences of using smokeless tobacco: Report of the Advisory Committee to the Surgeon General. NIH Publication No. 86-2874. Bethesda, MD: US Department of Health and Human Services, Public Health Service; 1986.
- 6. Iranloye BO, Bolarinwa AF. Effect of nicotine administration on weight and histology of some vital visceral organs in female albino rats. Nigerian J Physiol Sci 2009;24(1):7-12.
- 7. Avti PK, Kumar S, Pathak CM, Vaiphei K, Khanduja KL. Smokeless tobacco impairs the antioxidant defense in liver, lung, and kidney of rats. Toxicol Sci 2006;89(2):547-53.
- 8. Costantino J, Roberts C. Life-threatening hyperkalemia from chewing tobacco in a hemodialysis patient. J Renal Nutri 1997;7(2): 106-8.
- 9. Centers for Disease Control and Prevention. Cigarette smoking among adults—United States, 2007. Morbidity and Mortality Weekly Report 2008;57(45):1221–6.
- 10. Chen H, Saad S, Sandow S, Bertrand P. Cigarette smoking and brain regulation of energy homeostasis. Frontiers in Pharmacol 2012;3:147.
- 11. Chen H, Al-Odat I, Pollock C, Saad S. Fetal programming of renal development–influence of maternal smoking. J Diabetes & Metabolism 2013; 8:2-7.
- 12. Anblagan D, Jones NW, Costigan C, Parker AJ, Allcock K, Aleong R, et al. Maternal smoking

during pregnancy and fetal organ growth: a magnetic resonance imaging study. PloS one 2013; 8(7):e67223.

- 13. Gupta PC, Sreevidya S. Smokeless tobacco use, birth weight, and gestational age: population based, prospective cohort study of 1217 women in Mumbai, India. BMJ 2004;328(7455):1538.
- 14. Merkel CE, Karner CM, Carroll TJ. Molecular regulation of kidney development: is the answer blowing in the Wnt?. Pediatric Nephrol 2007; 22(11):1825-38.
- 15. Hinchliffe SA, Sargent PH, Howard CV, Chan YF, Van Velzen D. Human intrauterine renal growth expressed in absolute number of glomeruli assessed by the disector method and Cavalieri principle. Laboratory investigation. J Technical Methods Pathol 1991;64(6):777-84.
- Cass A, Cunningham J, Snelling P, Wang Z, Hoy W. Exploring the pathways leading from disadvantage to end-stage renal disease for Indigenous Australians. Social science & medicine. 2004 Feb 29;58(4):767-85.
- 17. Michos O. Kidney development: from ureteric bud formation to branching morphogenesis. Current opinion in genetics & development 2009; 19(5):484-90.
- Merlet-Benichou CL. Influence of fetal environment on kidney development. Int J Develop • Biol 2003;43(5):453-6.
- Cunha AR, Aguila MB, Mandarim-de-Lacerda CA Effects of early postnatal hyperglycaemia on rena cortex maturity, endothelial nitric oxide synthace expression and nephron deficit in mice. Int Exp Pathol 2008;89(4):284-91.
- 20. Stevens A, Lowe J. Human Histology. ded. Philadelphia: Elsevier Mosby; 2005.p.232.

- 21. Adedayo AD, Tijani AA, Musa AA, Adeniyi TD. Histological study of smoke extract of Tobacco nicotine on the heart, liver, lungs, kidney, and testes of male Sprague-Dawley rats. Nigerian medical journal. J Nigeria Med Assoc 2011;52(4): 217.
- 22. Disbrey BD, Rack JH. Histological Laboratory Methods. Edinburgh: Livingstone; 1970.p.56–128.
- 23. Drury RA, Wallington EA. Carleton's Histological Technique. 4th ed. London: Oxford University Press;1976.p.120–3.
- 24. Jauniaux E, Burton GJ. Morphological and biological effects of maternal exposure to tobacco smoke on the feto-placental unit. Early Hum Dev 2007; 699–706.
- 25. Genbacev O, Schubach SA, Miller RK. Villous culture of first trimester of human placenta placenta—model to study extravillous trophoblast (EVT) differentiation, Placenta 1992;439–461
- Nakamoto T, Yasuda Y Yasuhara M "Cigarette smoke extract engances oxytocin-induced rhythmic contractions of rat and human preterm myometrium. Reproduction 2006;343–353.
- Agarwal R. Po-inflammatory effects of iron success in chronic kidney disease. Kidney Int 2006 59(7):1259-63.
- 28. Kazi AS, Mughal F, Khan MA, Memon ZA. Effects of smokeless tobacco on the developing the mice. 2012;4(2):106-16.
- Jagadapillai R, Chen J, Canales L, Birtles T, Pisano MM, Neal RE. Developmental cigarette smoke exposure: Kidney proteome profile alterations in low birth weight pups. Toxicol 2012;299(2):80-9.