

## REPRODUCTIVE HEALTH IN RICKSHAW DRIVERS: Occupational Exposure to Environmental Stressor

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**Background:** In urban environment, exposure to the emission of motor vehicles is common. In urban peoples it is a very difficult task to distinguish among peoples with different grades of momentous period exposure to such pollutants. **Objective:** The objective of this study was to determine the effects of diesel exhaust, gasoline emission, Particulate Matter (PM) noise and heat on the reproductive health of rickshaw drivers. **Methods:** Adult married male individuals were recruited randomly in the study from Btkhella, Malakand agency, Khyber Pakhtunkhwa, Pakistan. Two groups were made, control (n=45) and rickshaw drivers (n=50). A special questionnaire was designed about occupational activities, socio-demographic and clinical characteristics. From both groups 5 mL of the blood was collected and was analyze for serum total testosterone and cortisol using Biocheck (USA) and Antibodies-online GmbH (Germany) kits. **Results:** In control group the Mean±SEM of total serum testosterone was 657.6±16.84 ng/dl and cortisol was 443.8±14.67 mU/L. In rickshaw drivers the Mean±SEM of total serum testosterone was 577.1±11.42 ng/dl and cortisol was 595.1±8.879mU/L. In rickshaw drivers there was a significant reduction in total serum testosterone ( $P < 0.0002$ ) but a significant increase in serum cortisol level ( $P < 0.0001$ ) at 95% confidence interval. **Conclusions:** Reproductive health problems like decreased libido, erectile dysfunction, absent morning and nocturnal erection, ejaculatory problems, primary infertility and secondary infertility were prevalent in rickshaw drivers but, no such problems were found in control group. Chronic exposure to pollutants such as diesel exhaust, gasoline emission, Particulate Matter (PM) noise and heat negatively regulate Hypothalmo-Pituitary Gonadal axis (HPG) leading to reproductive problems.

**Keywords:** Testosterone, Cortisol, Ejaculation, Infertility, Pollutants

### INTRODUCTION

In our era one of the most important environmental health problems is pollution from vehicles.<sup>1,2</sup> In urban environment, exposure to the emission of motor vehicles is common. In urban peoples it is a very difficult task to distinguish among peoples with different grades of momentous period exposure to such pollutants. To estimate the effects of different emission sources, professional contact can offer an alternative background.<sup>3</sup>

Urban peoples are exposed to chemical pollutants such as gasoline, diesel exhaust and noise. Gasoline contains very complex, inflammable and volatile substances. It comprises

of more than 500 saturated or unsaturated hydrocarbons having 3 to 12 carbons and is mainly used for internal combustion of engine. The various characteristics of gasoline depends on blends, additives, seasons to season's changes, differences in processing techniques and crude oil origin.<sup>4</sup> At self-service gasoline stations about 110 million people are exposed to gasoline constituents.<sup>5</sup> Exposure to both gasoline and smoking causes hepatotoxicity, haematotoxicity, nephrotoxicity and alters lipids metabolisms and some biochemical activities.<sup>6-11</sup> Gasoline exposure induces male reproductive toxicities by decreasing testosterone and increasing testicular IL-6 by induction of oxidative stress and inhibition of steroidogenesis.<sup>12,13</sup> Gasoline exposure increases IL-6 can cause infertility because; it was found that its concentration is greater in the seminal plasma of infertile men as compared to fertile men.<sup>14</sup> Gasoline components such as n-pentane, n-hexane, toluene and benzene decreases luteinizing and testosterone

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hormone.<sup>15</sup> Diesel exhaust emission contains thousands of chemical constituents such as, dioxin like compounds, polycyclic aromatic hydrocarbons and nitrogen oxide affecting the reproductive tract directly or indirectly.<sup>16-21</sup>

Nitrogen oxide is involved in the regulation of hypothalamic-pituitary-gonadal and -adrenal axis functions.<sup>22-28</sup> In diesel exposed rat testicular functions were reduced because, sperm production and hyaluronidase activity were lowered.<sup>29-31</sup> In neonatal and gestation life if diesel exhaust were inhaled for a long time, will disrupt reproductive functions in males by disrupting the development of sex organs.<sup>32-35</sup>

Urban workers are exposed to psycho-social stressor, noise and chemical pollutants affecting hypothalamic-pituitary-adrenocortical (HPA) axis and related systems.<sup>36-43</sup> ACTH induces an increase in adrenal corticosteroids that suppresses the episodic secretion pattern of LH and testosterone.<sup>44,45</sup> Long-lasting contact with noise can leads to alteration in testosterone concentration.<sup>46,47</sup> Milder form of stress decreases testosterone level in male but, chronic stress leads to disturbances in spermatogenesis including arrest of spermatogenesis.<sup>48,49</sup>

In the present study blood samples were taken from professional rickshaw drivers and shepherd. Specific questions were asked from them via a standard format questionnaire. These drivers are exposed to gasoline emission, diesel exhaust and noise for more than 12 hours. Similarly while driving their scrotum and so testes are directly exposed to extra heat generated by engine. The goal of this study was to determine the effects of gasoline emission, diesel exhaust, noise and heat on the reproductive system of rickshaw drivers.

## MATERIALS AND METHODS

### Inform consent

Before starting study, a written informed consent was signed from all the participants. Sufficient information was given to them about the purpose of study.

### Study design

Adult men (n= 90) were selected randomly from Batkhella city, Malakand agency, Khyber pakhtunkhwa, Pakistan. In 90 men, 45 were control (shepherd) and the remaining 45 were rickshaw drivers. Their age ranges from 35 to 40 years. Control group participant passes most of its time out of bazaar in greenery. They are exposed to fresh air and out of vehicles and industrial noise. Rickshaw drivers are exposed directly to gasoline emissions, diesel exhaust, engine generated heat and noise for more than 12 hours. A special format questionnaire was developed concerning their socio-demographic characteristics, addiction, occupational activities and clinical characteristics.

5 mL of the blood sample was obtained from each participant by a phlebotomist aseptically from 9:00AM to 10:00 AM in the laboratory. Blood samples were immediately transferred into vacutainer tubes containing no additives or anticoagulants. Blood sample was allowed to clot at room temperature then, centrifuged at 3200 rpm for 15 minutes at 4 C<sup>0</sup> to obtained serum. For later analysis all the serum samples were then stored at -20°C.

### Assays

Total serum testosterone and cortisol was measured in all the serum samples using testosterone enzyme immunoassay test kit, Bio-check (USA) and Antibodies-online GmbH (Germany) kit according to the company protocol and procedures.

### Statistics

Software Graph Pad Prism, *version 6.03*(Graph Pad Software Inc., San Diego, CA, USA), was used for data analysis. Impaired t-test was used to evaluate the significance of the difference between the mean values of the serum total testosterone in both groups. A significant difference was acknowledged at  $P < 0.05$ .

## RESULTS

### History

History of both control and rickshaw drivers are summarized in Table 1.

Table 1  
History of control and rickshaw drivers

Parameter	Group	
	Control	Rickshaw Drivers
N	45	45
Age range (years)	35-45	35-45
History of jobs (years)	≥ 10	≥ 10
Exposure time (h)	≤ 2	≥ 12
Use of protective clothing	mask	no
Addiction (%)	6 snuff 5 cigarette	9 snuff 8 cigarette
General health problems	01 DM*	05 HT* 01 A* 01 RA*
Reproductive health problems	none	5 PI* 3 SI* 07 DL* 03 EP* 06 AMN*

DL\* = Decreased Libido, EP\* = Erection Problems, AMN\* = Absent Morning and Nocturnal erection. DM\* = Diabetes Mellitus, HT\* = Hypertension, A\* = Asthma, RA\* = Rheumatoid Arthritis, PI\* = Primary Infertility, SI\* = Secondary Infertility.

### Hormonal profile in control and rickshaw drivers

The results showed that total serum testosterone is significantly reduced ( $p= 0.0002$ ) but serum cortisol is significantly increased ( $p < 0.0001$ ) in rickshaw drivers as compared to control group (Table 2).

Table 2  
Total serum testosterone and cortisol concentration in study population

Parameter	Group		p	95% CI
	Control	Rickshaw drivers		
Testosterone	657.6 ± 16.84	577.1 ± 11.4	0.0002	-121 to -40.09
Cortisol	443.8 ± 14.67	595.1 ± 8.879	0.0001	117.2 to 185.4

### DISCUSSION

The PM in diesel exhaust contains substances having estrogenic, anti-estrogenic and anti-androgenic properties.<sup>50</sup> These PM enters into our bodies through skin, lungs and the intestinal tract affecting our reproductive functions.<sup>51-57</sup> Exposure to diesel exhaust in developing mice leads to degeneration of leydig cells, rise in the number of impaired seminiferous tubules, and decrease in 24 hours sperm production.<sup>55</sup> Diesel exhaust exposure during fetal life in rats decreases the number of sertoli cells and reduces sperm production in adult life.<sup>50</sup> Leydig cells take up diesel exhaust particles leading to cytotoxicity in cultured cells in vitro.<sup>58-60</sup> In leydig cells PM reduces testosterone production by repressing the expression of StAR (Steriodogenic Acute Regulatory protein).<sup>61,62</sup> Various factors like AP-1, C/EBPb, GATA4, and DAX-1 regulated StAR gene expression. In Leydig cells these factors may be associated with induction of StAR gene.<sup>63-65</sup> Polycyclic aromatic hydrocarbons (PAH) in diesel exhausts reduce expression of genes which encode enzymes responsible for androgenesis and affect gonadal development by reducing the expression of Ad4BP/SF-1 mRNA.<sup>50</sup> Persistently organic pollutants and lead exposure have been related with reduced intertubular tissue volume in the testes, significant reduction of plasma testosterone, altered rates of sexual maturation, decreased in measures of sperm quality and alterations in the character of mensens. Chemical pollutants such as toluene, PM, NO<sub>2</sub>, NO, Pb and Cd cause changes on production, secretion and/or action of testosterone.<sup>66,67</sup> Exposure to aromatic hydrocarbons, O<sub>3</sub>, CO, SO<sub>2</sub>, Pb, PM, and NO leads to hormonal reproductive problems.<sup>68,69</sup> In human male exposure to PAH decrease sperm count, sperm motility, and total plasma testosterone, increases plasma LH concentration but has no effect on FSH in exposed group.<sup>70</sup>

It has been found that gasoline exposure in male rat decreases testosterone level as compared to control group.<sup>71,72</sup> Offspring sex ratio at birth is also affected by gasoline exposure.<sup>73</sup> Workers in filling stations are chronically exposed to gasoline and are associated with decreased testosterone level and alter Offspring sex ratio at birth when compared with control group.<sup>71,72</sup> Toluene present in gasoline at a very low concentration of 0.9 ppm for 90 minutes per 24 hours decreases fetal plasma testosterone concentrations in male rat by decreasing 3b- hydroxysteroid dehydrogenase, the enzyme involved in testosterone synthesis.<sup>74</sup> Ethylene dibromide (EDB) in gasoline has testicular and post-testicular effects, reduces sperm velocity and semen volume after short exposure.<sup>75,76</sup> Workers exposed to ethylene dibromide (EDB) for a long time indicated decreased sperm concentration and increase in the number of abnormal sperm.<sup>77</sup>

Noise is a stressor and chronic exposure to noise causes hormonal alterations, cryptorchidism, varicocele and sperm abnormalities.<sup>78</sup> Decreased testosterone level was observed after chronic exposure to noise.<sup>41,47</sup> During acute or chronic stress the secretion of ACTH increases. At the same time the expression of RFRP-3(mammalian ortholog of GnIH) increases. Cortisol receptors are located on GnRH neurons, RFRP-3 neurons and gonadotrophs. High cortisol positively regulate RFRP-3 secretion that inhibit secretion of GnRH, LH and so testosterone but directly negatively regulate secretion of GnRH and LH by acting on GnRH and gonadotrops.<sup>79</sup>

Testes functions better below normal body temperature i.e. 3-4 C<sup>0</sup> less than 37 C<sup>0</sup>. Testes descent into scrotum during fetal life if, failed will lead to infertility because normal body temperature is unsuited for sperm production (Mieusset&Bujan 1995; Setchell 1998). Scrotal heat losses are affected by exposure to exogenous heat sources such as taking a hot bath or occupationally (welders, bakers, foundry workers).<sup>80,81</sup> Impairs spermatogenesis was observed in animal models soaked for 30 minutes in a moderately hot bath (40-428C).<sup>81</sup> Heat exposure causes sperm DNA damage and induces germ cell apoptosis.<sup>82-84</sup> Heat exposure induces hypoxia and oxidative stress in germ cells that pushes the germ cells towards apoptosis by increasing the expression of hypoxia inducible factor 1a, haemoxygenase 1, glutathione peroxidase 1 and glutathione-S-transferase-a.<sup>83</sup> In a sedentary position there is less efficient scrotal cooling so scrotal.

Temperature rises gradually with duration of sedentation, and this was associated with lower sperm counts.<sup>85-87</sup> Taxi and lorry drivers spend a lot of time seated, have also shaped evidence for negative effects on semen quality.<sup>88,89</sup> Scrotal

heating has been studied as a possible contraceptive choice in men and shown to be affective.<sup>80</sup>

## CONCLUSION

Reproductive health problems are common in rickshaw drivers because, they are exposed to environmental pollutant. These drivers are advised to use mask and other protective things to minimize the adverse effects of these pollutants.

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