Effects of Smoking on Seminal Cytokine Network

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ABSTRACT

Introduction: The seminal cytokine network is involved in priming female reproductive tract reception for embryo implantation and may have a role in infertility and affected by smoking.

Objective of the study: To evaluate the association between smoking and sperm parameters, and effect on Seminal Cytokine Network.

Subjects and Methods: Eighty men who presented with infertility were evaluated. Clinical evaluation including smoking habits were noted and semen analysis was carried out according to WHO guidelines. A panel of ----cytokines TNF-α, IL-12, IL-6, IL-13, IL-10 and IL-4 were estimated in smokers and non-smokers. Using Enzyme-linked immune sorbent assay (ELISA). After a program of cessation of smoking, the seminal cytokines were repeated in those men with signification cessation of smoking through low carbon monoxide level in the breath using Smokerlyzer (Bedfont inc Uk)

Results:
Of the 80 men, 29 (36%) were addictive smokers and 51 (64%) were non-smokers. The T helper proinflammatory cytokines expression were significantly higher in smokers IL-6; 36 ± 8 versus 26 ± 4, p<0.05, IL-12, 44 ± 6 versus 27 ± 6, p <0.01, TNF-α =12 ±3 versus 7 ±1.3, p<0.05. On the other hand, T helper 2 expression was higher in non-smokers than smokers. IL-13; 12±3 versus 6±2, p<0.01, IL-10 : 9±4 versus 4±1.2, p<0.01, IL-4: 6± 1.2, versus 3±1.2, p<0.05. Semen analysis revealed Normozoospermia, Oligozoospermia 14, Asthenozoospermia and leucocytospermia, had higher expression of proinflammatory cytokines IL-12, IL-6 and TNF-α.

Conclusion: Smoking is associated with proinflammatory cytokine (T helper 1) expression. This may explain the detrimental effect of smoking on sperm parameters and function.

Key words: Seminal Cytokines, Smoking, Impairment, Fertilization, Implantation

INTRODUCTION

According to epidemiology trends, 10-15 percent of couples are infertile. Male contribution to infertility is about 50 percent. There is increasing incidence of male infertility as a result of environmental factors such as environmental and outcome of modern life habit such as obesity, smoking, obesity, ageing, exposure to gonadotoxins and endocrine disruptors (Omu et al 1998).
Smoking prevalence; males (% of adults) in Kuwait was last measured at 35.1% in 2009, according to the World Bank. Prevalence of smoking, male is the percentage of men ages 15 and over who smoke any form of tobacco, including cigarettes, cigars, and pipes, and excluding smokeless tobacco. Smoking is now recognized as a major public health problem (Omu F). It is noticed that the majority of regular smokers begin their smoking at an early age (Ali et al 2007). Tobacco smoke contains more than 4000 chemicals and around 40 carcinogens, including nicotine, tar, carbon monoxide (CO), methoprene, propylene glycol, benzopyrene, butane, cadmium, acetone, ammonia, lead, benzene, formaldehyde, etc. The CO in the blood then re-enters the alveoli because of concentration gradient at the alveoli. The CO present in expired air can be measured using a portable CO analyzer. The breath CO concentration has been found to be a reliable indicator of COHb level in the blood5. Therefore, indirect measurement of COHb through breath analysis is preferred over direct measurement of blood COHb levels because of its non-invasive nature, easy procedure and better compliance (Kumar et al 2010)

The substantial harmful effects of tobacco smoking on fertility and reproduction have become apparent (Omu et al.1998). Because of the complexity of tobacco smoke components, the toxicological mechanism is notably complicated. Most studies have reported reduced semen quality, reproductive hormone system dysfunction and impaired spermatogenesis, sperm maturation, and spermatozoa function in smokers compared with nonsmokers.
Studies in rodents, livestock species and humans show that the introduction of semen into the female tract orchestrates striking molecular and cellular changes that facilitate conception and pregnancy. Seminal plasma contains oestrogen and testosterone, several prostaglandins and glycoprotein signaling substances, including several cytokines and growth factors (Aumuller and Riva 1992; Maegawa et al. 2002).
Several lines of evidence indicate that cytokines (IL-1β, IL-2, IL-6) are involved and/or of their soluble receptors (SR IL-2), in male fertility (Dousset et al 1997).

Cytokines released by various cell subsets in the male urogenital tract are capable of markedly influencing sperm function and fertility (Gruschwitz et al 1996). Cytokine may be involved in reduced fertility. The seminal cytokine network is involved in priming female reproductive tract especially the endometrial preparation and reception for embryo implantation through the agency of specific factors in the seminal plasma (Robertson et al 2006). A combined determination of IL-6, IL-8, IL-11 in the Semen Plasma of men with genital infection and oligo-terato-asthenozoospermia may provide clinically useful information for the diagnosis of male accessory gland infection.

Objective of the study: To evaluate
(1)The association between smoking and sperm parameters
(2)The association between smoking and Seminal Cytokine Network.
(3)What is the mechanism of the association between Smoking and Sperm parameters?

SUBJECTS AND METHODS

Eighty men who presented with infertility were evaluated. This was a double blind study. Clinical evaluation including smoking habits were noted. Initially all the 80 men with infertility had clinical evaluation, Semen analysis according to (WHO Guidelines WHO 2010), estimation of seminal plasma Cytokines, serum hormone and lipid profiles.
Use of Smokelyzer (Bedfont, UK) according to the manufacturer's instructions
Counselling and use of Nicotine Replacement Therapy (NRT), Nicotine gum (most popular with them). Some used E-cigarette, while others stopped abruptly (cold turkey) after fully understanding the implications and effects of smoking on sperm parameters.
Ethical consideration and approval from the Institutional Review Board of the Maternity Hospital. Kuwait. Verbal informed consent was received from all men

Investigations.

Hormone Profile: Estimation of serum concentration of FSH, LH, Prolactin, Testosterone, Estradiol, TSH and FT4 was carried with radioimmunoassay.
Semen analysis was by WHO guidelines
Preparation of semen for ELISA determination of Cytokines
After 3 days sexual abstinence, fresh semen sample was obtained from 80 men and semen analysis was carried out according to WHO guidelines (WHO 2010). A repertoire of cytokines TNF-α, IL-12, IL-6, IL-13, IL-10 and IL-4 were estimated in smokers and non-smokers. Using Enzyme-linked immune sorbent assay (ELISA).

RESULTS

Table 1: Comparison of Characteristics of Smokers and non-smokers

	Variables	Smokers N=29	Non-smokers N=51	P Value
1	<30	10 (34.5)	18 (42.9)	NS
	30 – 39	14 (48.3)	25 (49.0)	NS
	≤ 40	5 (17.2)	8 (15.7)	NS
2	Marital status			
	Single	5 (17.2)	4 (15.7)	NS
	Married	20 (69.0)	45 (88.2)	NS
	Divorced	4 (13.8)	2 (3.9)	0.05
3	Education			
	Primary	3 (10.3)	6 (11.8)	NS
	Secondary	21 (72.4)	28 (54.9)	0.9
	University	5 (17.3)	17 (33.3)	0.05
4	Number of Children			
	0	6 (20.7)	35 (68.6)	0.05
	1 - 3	23 (79.3)	16 (31.4)	0.01
5	Sperm Parameters			
	Normozoospermia	9 (31)	30 (58.8)	0.05
	Oligozoospermia	12 (48)	11 (21.6)	0.05
	Asthenozoospermia	12 (48)	9 (17.6)	0.01
	Teratozoospermia	6 (20.7)	10 (19.6)	1.00
	Leucocytospermia	10 (34.5)	8 (15.7)	0.05

Figure 1: Comparison of T helper 1 Cytokines in Smokers and non-smokers

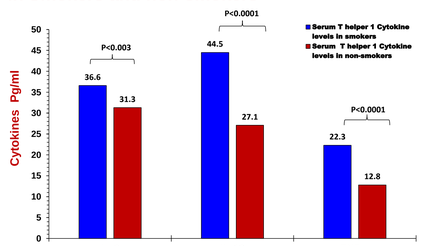


Table 2: Comparative expression of T helper 1 versus T helper 2 in seminal plasma in smokers and non-smokers.

	Smokers N=29		Non-smokers N=51		P value
	T helper 1	T helper 2	T helper 1	T helper 2	
IL-6	36.3±7.9		31.3±6.4		0.003
IL-12	44.5±6.3		27.1±4.1		<0.0001
TNF-α	22.3±6.5		12.8±1.3		<0.0001
IL-13		5.7±2.4		11.5±2.7	<0.0001
IL-10		8.6±2.4		12.4±4.2	<0.0001
IL-4		4.8±1.2		8.6±3.2	<0.0001

Predominantly proinflammatory seminal cytokine; IL-6, IL-12 and TNF-α expression among smokers compared with non-smokers (P<0.001) and significantly higher Thelper 1 /T helper 2 ratios

Table 3. Comparision of T helper 1/T helper 2 ratios in smokers and non-smokers

	Smokers N=29	Non-smokers N=51	P value
Ratio	T helper 1	T helper 2	
IL-6 /IL-13	6.4	2.7	0.01
IL-6 / IL-10	4.2	2.5	0.05
IL-6 / IL-4	7.6	3.6	0.05
IL-12 / IL-13	7.8	2.4	0.01
IL-12 / IL-10	5.2	2.2	0.01
IL-12 / IL-4	9.2	3.2	0.001
TNF-α / IL-13	3.9	1.1	0.01
TNF-α / IL-10	2.6	1.0	0.05
TNF-α / IL-4	4.6	1.5	0.01

The seminal T helper 1/ T helper 2 ratios are compared between smokers and non-smokers. All the ratios were significantly higher in the smokers compared to non-smokers (p<0.05 to p<0.001)

Figure 2: Comparison of T helper 2 Cytokines in smokers and non-smokers

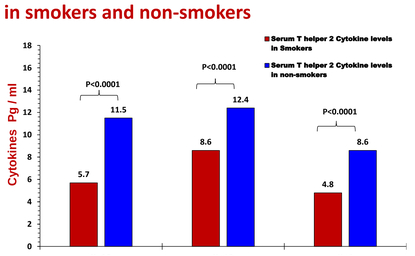
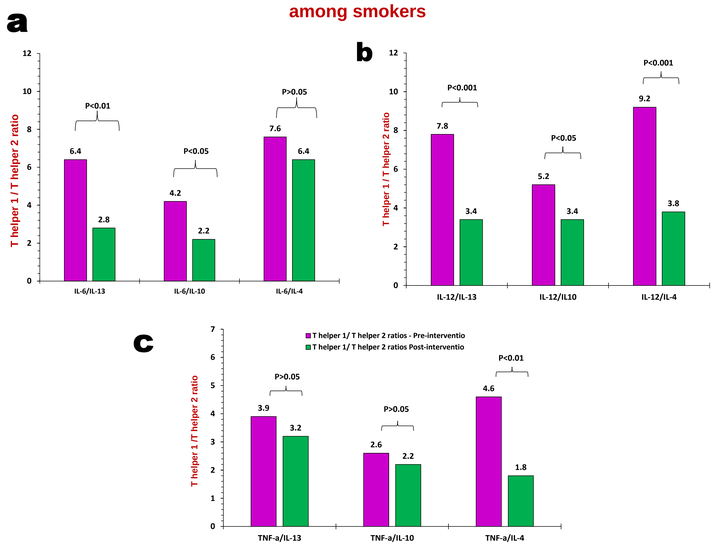


Figure 3: Comparison of Th 1 / Th 2 ratio before and after Cessation of intervention among smokers



Conclusion:

The present study has demonstrated that smoking is associated with poor sperm parameters. Exposure of spermatozoa from the non-Smoking Men to the Seminal Plasma from the Smoking Men yielded a significant reduction in the sperm motility. One of the mechanisms may be through expression of abundant proinflammatory cytokine (T helper 1 expression. This may explain the detrimental effect of smoking on sperm parameters and function. Cessation of smoking leads to improvement of sperm parameters through expression T helper 2 cytokines. This could could have implication in the role of cytokines in enhancing fertilization and implantation.

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