

*Research Article***Tobacco chewing (Madgha) and semen quality**

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Abstract

Introduction: Lifestyle factors like tobacco smoking or chewing, obesity, heat, radiation, and drugs have negative impacts on male reproduction. The considerable prevalence of male cigarette smoke, the numerous adverse health effects caused by smoking and the fact that cigarette smoke contains more than thirty agents which is carcinogens or mutagens add to the significance of this issue. Smoking has been a direct cause of many cancers and other health conditions and there is concern about the possible negative effects of smoking on semen parameters and male reproduction. **Aim of the study:** Our study assesses the hazards of tobacco chewing on semen quality of infertile male. **Patients and methods:** Two equal groups were included in this cross-sectional study, 1st group 50 infertile tobacco chewer patients and the 2nd group 50 infertile nontobacco chewer patients, all patients were subjected to semen evaluation (count, motility, and morphology of sperm), seminal total antioxidant capacity (TAC) and Malon dialdehyde (MDA). **Results:** The means of sperm concentration, percentage sperm progressive motility and seminal TAC are significantly lower in the tobacco chewing group than control and the means of percentage sperm abnormal morphology and seminal MDA are significantly higher in the tobacco chewers group. With significant correlations between patients age, sperm concentration, progressive motility, abnormal sperm morphology, TAC, MDA, and tobacco chewing index. In heavy tobacco chewers, sperm morphology, sperm concentration, and motility were affected more than those with a mild and moderate habit. **Conclusion:** These results showed that Tobacco chewing affects sperm parameters and oxidative stress. Also, sperm concentration, morphology and oxidative stress were affected in a dose-dependent manner.

Keywords: Male infertility, Smokeless tobacco, Tobacco chewing, Toombak, Madgha

Introduction

Lifestyle factors like tobacco smoking or chewing, obesity, heat, radiation, and drugs have negative impacts on male reproduction. (Kumar, 2009). These factors may be worsen the male fertility (Pramanik 2012).

Tobacco is a plant growing in India, China, Japan, Southeast Asia, the Middle East, and West Africa (Ali, 2017) with green foliage and tubular flowers (Taymour, 2010). However, it has different forms for use (burning, snuff, chewing etc...). Smokeless tobacco (ST) has many different names in different countries (Madgha in Egypt, Shamma in KSA, Saot in Sudan, tombak in Yamane) (Ali, 2017). In general, ST is substantially less harmful than

smoking (Bates, 2003). Tobacco smoking causes increased oxidative stress (Bruno, 2006). Lipid peroxidation is one of the unhealthy effects of reactive oxygen which can associated with oxidation of membrane polyunsaturated fatty acid (Fraczek et al., 2001; Alvarez et al., 1987; Alvarez & Storey, 1995). It attacks the fluidity of sperm plasma membrane, resulting in loss of the ability for adhesive to oocyte and fertilization (Mammoto et al., 1996). Malon dialdehyde (MDA) is a stable peroxidation product of polyunsaturated fatty acids and a diagnostic tool for lipid peroxidation (Laudat et al., 2002)

Chewing tobacco used in Egypt, mainly in upper Egypt. Our study trying to evaluates the

effects of tobacco chewing on semen parameters and oxidative stress state in Al-Azhar University Hospital, Assiut branch as a model for upper Egypt.

Aim of the study

Assesses the hazards of tobacco chewing on semen quality of infertile male.

Patients and Methods

- **Study design:** Cross-sectional analysis study.

- **Place and duration of study:** Infertility unit of Al-Azhar University Hospital, Assiut branch, Egypt from April 2017 to April 2018 for fertility evaluation.

- **Study population:** Patients attending the Infertility unit, 50 infertile tobacco chewing patients (Group I) were included and compared with another 50 infertile patients not consumed any types of smoking (Group II).

- **Study method and data collection:** Data were collected through a questionnaire that included the name, phone number, age, residence, marital status and tobacco habits in details. Semen analyses were done for all subjects. Patients age 20-50 years and should have 2-5 sexual abstinence days, exclusion criteria were males with an infectious disease, patient undergoing an antibiotic or antioxidant treatment in last 3 months, patients using other types of tobacco or alcohol, diabetes mellitus, varicocele, pyospermia, prostatitis or orchitis.

All patients were subjected to semen evaluation (count, motility, and morphology of sperm), seminal total antioxidant capacity (TAC) and Malon dialdehyde (MDA). By using (CASA, MIRALAB, ISO9001, ISO 13485), semen analysis was performed manually according to the World Health Organization (WHO) standard guideline (WHO, 1999), volume ≥ 2 ml, concentration ≥ 20 million/ml, total count ≥ 40 million, progressive motility $\geq 50\%$, vitality $\geq 75\%$ and normal morphology $>15\%$ (Kruger's criteria).

- **Madgha** it is a chewable tobacco leaves mixed with a stony salty material called (Atron) used by patient in Egypt, the Weight of tobacco packet is 30 grams, one pinch = about 2 grams put between the gum and lower lip for about 15 minutes for single use, spitting according to each patient habits. Patients used from 1 to 3 packets per day. There is no clear index for Madgha use so we create an index in the form of (number of packets per day x number of years of use). And consider mild (≤ 20), moderate (21- 40), and heavy (>40). All the patients subjected to complete history taking, general and local examination.

- **Measurement of TAC and MDA:** Seminal TAC was done by colorimetric method (Koracevic, 2001; Satoh, 1978). Using (Sigma- Aldrich, lot: MAK187, USA). MDA levels were estimated using (Sigma- Aldrich, lot: MAK085, USA).

- **Ethical considerations:** Permission was taken from the ethics committee of the university and an informed consent obtained from all participants in this study.

- **Statistical Analysis:** Using SPSS version 22 software (Chicago, IL. USA); Results were expressed as mean \pm standard deviation (SD) and percentage. The unpaired t-test was applied to test the difference between means, one-way ANOVA test was applied to test the difference between subgroups of tobacco chewers, Chi-Square were applied to nominal data and Pearson' correlation coefficient was determined for the intensity of tobacco chewing and other parameters.

Results

In this study, patient's age ranged from 25 to 45 years, duration of tobacco chewing ranged from 2 to 26 years and the number of packets/day ranged from 1 to 3 packets.

Table 1: Characteristics of patients and control groups, infertility unit of Al-Azhar University Hospital (Assiut), from April 2017 to April 2018.

	Group I (n = 50)	Group II (n = 50)	P value
Age	33.84±4.7	33.12± 3.7	0.4
Semen volume (ml)	2.9± 1.1	2.9± 1.4	0.7
Sperm count (million/ml) *	30.4± 20.5	47.5± 27.9	0.001
Total sperm count*	85.1± 54.7	115.4± 62.8	0.01
Sperm Progressive motility %	30.6± 13.04	32.2± 7.2	0.4
Sperm Abnormal Morphology*	86.4± 2.2	84.3± 2.7	0.000
FSH mIU/ml	4.59± 1.49	4.6± 1.6	0.9
Total testosterone ng/ml	4.12±1.4	4.29±1.3	0.5
TAC nmole/µL*	2.27 ± 0.5	2.54 ± 0.6	0.01
MDA nmole/µL *	2.26±0.78	1.61±0.6	0.000

* = P<0.05

Table (1) showing there was no significant difference between the two groups as regard age, and semen volume. As regard sperm

count, morphology, TAC, and MDA there is significant statistically difference between the two groups.

Table 2: Percentage of seminal parameters (both groups), infertility unit of Al-Azhar University Hospital (Assiut), from April 2017 to April 2018.

Seminal parameters		Group I (n = 50)	Group II (n = 50)	P value
Sperm volume	Normal volume	42 (84%)	40 (80%)	0.3
	Hypospermia	8 (16%)	10 (20%)	
Sperm count	Normal Count	28 (56%)	38 (76%)	0.02*
	Oligozoospermia	22 (44%)	12 (24%)	
Sperm progressive motility	Normal sperm motility	19 (38%)	29 (58%)	0.03*
	Asthenozoospermia	31 (62%)	21 (42%)	
Sperm abnormal morphology	Normal morphology	19 (38%)	30 (60%)	0.02*
	Teratozoospermia	31 (62%)	20 (40%)	

* = Fisher's Exact Test

Chi square significant < 0.05

In Table (2) we able to detect a significant relationship between tobacco chewing habit and

semen parameters (Count, Motility and Sperm morphology) as dichotomized value.

Table 3: Percentage of seminal parameters in tobacco chewing group, infertility unit of Al-Azhar University Hospital (Assiut), from April 2017 to April 2018.

Seminal parameters		Group I (n = 50)			Group II (n = 50)	P value
		Mild (n = 23)	Moderate (n = 20)	Heavy (n = 7)		
Semen volume	Normal volume	21	17	4	40	0.2
	Hypospermia	2	3	3	10	
Sperm count	Normozoospermia	15	11	2	38	0.05*
	Oligozoospermia	8	9	15	12	
Sperm progressive motility	Normal sperm motility	12	6	1	29	0.04*
	Asthenozoospermia	11	14	6	21	
Sperm abnormal morphology	Normal morphology	11	8	0	30	0.02*
	Teratozoospermia	12	12	7	20	

* = Fisher's Exact Test

Chi square significant < 0.05

Further, the effect of intensity of tobacco chewing on semen parameters and oxidative stress state was analyzed by dividing the Group

I patients, according to tobacco chewing index into three sub-groups mild (n= 23), moderate (n= 20) and sever (n= 7) (Table 3 and 4).

Table (4): Patients characters of different subgroups of tobacco chewers group and nontobacco chewer group, infertility unit of Al-Azhar University Hospital (Assiut), from April 2017 to April 2018.

Parameter	Nontobacco chewer (n = 50)	Tobacco chewer (n = 50)			Post hoc test P**	
		Mild (n = 23)	Moderate (n = 20)	Heavy (n = 7)		
Age of patients(years)	33.1±3.7	31.9±3.8	34.7±4.5	37.7± 5.6	a vs d b vs d	0.34
Seminal volume(ml)	2.9±1.4	3.2±1.1	2.9±1.1	2.2±0.9	-----	-----
Sperm count (10 ⁶ /ml)	47.5±27.9	36.1±22.8	27.8±17.6	18.5±15.07	a VS c,d	0.01
Total sperm count	115.4±62.8	106 ±60.3	75.4±38.9	44.7±49.5	a VS c , d	-----
Motility (%)	32.2±7.2	32.1 ±14	31.8 ±12.5	22.1±8.2	-----	-----
Morphology (%)	84.3±2.7	85.6±1.5	86.2±2.1	89.5±1.7	a VS c ,d b.c VS d	0.005 0.02
FSH mIU/MI	4.6±1.6	4.3±1.3	4.5±1.5	5.3±1.8	-----	-----
Total Testosterone ng/ml	4.2±1.3	4.6±1.4	3.7±1.4	3.4±0.9	-----	-----
TAC nmole/μL	2.5±0.6	2.4±0.5	2.1±0.4	1.9±0.6	a VS c ,d	0.000
MDA nmole/μL	1.6±0.6	1.9±0.7	2.5±0.7	2.3±0.9	a VS c ,d	0.03

* Post hoc multiple comparison of continuous variables was performed by Tukey’s test. Values are expressed as mean ± standard deviation. P<0.05 was considered significant using one-way ANOVA compared to: ^a Nontobacco chewer; ^b Mild group; ^c Moderate group; ^d Severe group.

Decrease in the mean of sperm concentration was shown in a heavy tobacco chewing patients compared to patients with mild habit (P 0.01), with an increase in the mean of sperm abnormal morphology in patients with a heavy tobacco chewing habit compared to patients with mild and moderate habit (P 0.005 and 0.02) respectively. As regards the OS state, there were significant low level in the mean of seminal TAC in heavy tobacco chewer (P

0.000) compared to patients with mild and moderate habit subgroups and low level in the mean of seminal TAC in patients with a moderate chewer habit compared to those with mild habit (P 0.003) with an increase in the mean of seminal MDA inpatients with a heavy and moderate tobacco chewer habit compared to those with mild habit (P 0.01 and 0.03) (Table 4).

Table (5): Correlation coefficient between age, semen parameters, seminal TAC, MDA, and Tobacco chewing index, infertility unit of Al-Azhar University Hospital (Assiut), from April 2017 to April 2018

	Tobacco chewing index	
	r-value	P-value
Age	0.33	0.01*
Semen volume	-0.14	0.3
Sperm count	-0.4	0.001*
Sperm total	-0.4	0.001*
Sperm progressive motility	-0.29	0.03*
Sperm abnormal forms	0.4	0.001*
FSH mIU/ml	0.19	0.1
Total testosterone ng/ml	-0.21	0.1
TAC nmole/μL	-0.63	0.000*
MDA nmole/μL	0.26	0.07

* Significant < 0.05

A fair association was noticed in tobacco chewer patients between age and sperm abnormal forms ($r = 0.33$ and 0.37) respectively with increasing chewing tobacco index. Regarding sperm concentration, sperm progressive motility and TAC show fair negative correlation of sperm concentration ($r = -0.43$, -0.35 and -0.63) respectively, with increasing chewing tobacco index ($P < 0.005$) (Table 5).

Discussion

Our cross-sectional study reported a reduction in the quality of semen among the tobacco chewers. Regardless of how tobacco is consumed, its adverse effects are clear (Nelson, 1996). Furthermore, ST is highly addictive (Spangler, 1995). In our study, we reported that semen parameters were significantly lower in a tobacco chewer group than the control; these results are in matching with findings of others like (Banerjee et al., 1993; Said et al., 2005; Sunanda, et al., 2014; Parmar et al., 2016).

Our study demonstrates a decrease in the mean of sperm concentration and sperm morphology with the increase in consumption of tobacco chewing. These observations are in agreement with the findings of Said et al., 2005.

By comparing patients according to their degree of consumption, we noticed that; the mean of sperm concentration and normal sperm morphology were significantly declined with increasing intensity of tobacco consumption. These observations are matched with the findings of other studies such as Parmar et al., 2016; Sunanda, et al., 2014; 1993; Said et al., 2005 and Banerjee et al., 1993. Rather than we noticed a significant correlation between sperm concentration, motility, sperm abnormal morphology and tobacco chewing index. The effect of ST on semen parameters may be due to nicotine, lead or arsenic as ST contains Nicotine as a major component, TSNA, pesticides, and metals (IARC, 2007). Nicotine reduces the percentage of viable sperm and promotes spermatozoa apoptosis with DNA fragmentation or alters the chromatin compactness (Condorelli et al., 2013).

Another possible explanation by which ST can affect semen parameters is oxidative stress. Our study reported that the mean of TAC is significantly lower in the tobacco chewing group than tobacco non-chewing group with a significant decrease in the mean of TAC level in patients with heavy tobacco chewer habits. Rather than tobacco chewing induce more lipid peroxidation leading to an increase in the mean of seminal MDA; high level of MDA in seminal plasma of tobacco chewing men was a sign of increasing oxidative stress associated with decrease in sperm quality and the risk of idiopathic infertility in our study the mean of seminal MDA in tobacco chewing group was significantly higher than tobacco non-chewing group, with a significant increase in MDA level in patients with increase the severity of tobacco consumption. Supriya et al., 2017 compared serum MDA of control, active smokers, passive smokers, tobacco chewers and active smokers, plus tobacco chewers and reported that serum MDA level was less significant in tobacco chewers compared to control.

Conclusion

Tobacco chewing has a bad effect on male fertility by decrease the sperm concentration, motility, normal sperm morphology and antioxidant capacity. As the consumption of tobacco chewing is increasing; it is associated with more declines on semen parameters and antioxidant capacity.

Conflict of interest

All authors declare no competing financial interests.

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