Original Article

Influence of Bacteriospermia, Host and Lifestyle Factors on Sperm DNA Integrity: A Cross-Sectional Study Based on a Fertility Center of Nepal

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Abstract

Objective: To determine the sperm DNA fragmentation in the semen of suspected sub-fertile men of Nepal and find its association with bacteriospermia demographic and lifestyle factors.

Materials and methods: A cross-sectional study was conducted with the subjects, males among sub-fertile couples visiting a fertility center in Kathmandu Valley, Nepal for consultation. Information on demography and exposure factors was obtained with a structured questionnaire, and bacteriospermia and sperm DNA fragmentation was determined from the semen samples collected from the study subjects. The data obtained were used to assess sperm DNA fragmentation and its association with various risk factors in sub-fertile men of Nepal.

Results: Out of 186 samples analyzed, 41.4% had low DNA fragmentation (<15%), 38.7% had moderate DNA fragmentation (≥15% and <30%), and 19.9% had high DNA fragmentation (≥30%). Among the risk factors analyzed, sperm DNA fragmentation was found to be significantly associated with the age of the patients ($p<0.05$). Other factors analyzed body mass index, smoking, alcohol consumption, physical activity, and bacteriospermia were not found to be associated with sperm DNA fragmentation in our study.

Conclusion: Sperm DNA integrity may be distorted with the increasing age of men, leading to decreasing fertility potential.

Keywords: Age; Bacteria; Male Infertility; Sperm DNA Fragmentation

Introduction

The male-factor, alone or in combination with the female factor, is responsible for the sub-fertility of married couples in about 50% of cases worldwide (1).

An initial screening of male partners of sub-fertile couples starts with the physical semen characteristics (liquefaction time, semen viscosity, color, ejaculate volume), and conventional semen analysis (sperm concentration, motility, morphology, and vitality). It is considered the only diagnostic male infertility test. However, the discriminatory capability of conventional semen analysis for the fertility potential

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is lacking as about 40% of infertile men have normal semen parameters. Conventional semen assessment does not predict infertility in men sensitively and specifically (2). With an understanding of the role of intact and unfragmented DNA in spermatozoa, a sperm DNA fragmentation test can be an important diagnostic tool to diagnose male infertility cases (3). Sperm DNA integrity plays a crucial role in normal fertilization and development and implantation of embryos, pregnancy as well as in fetal development (2, 4). Several lifestyle factors contribute to sperm DNA fragmentation, including alcohol consumption, tobacco, obesity, diet, exposure to high temperatures, and psychological stress (5). Advanced age, genital tract infection, and diseases like diabetes, cancer, and varicocele may have a potential impact on sperm DNA integrity (2).

The sperm DNA fragmentation index (DFI) is thus considered a promising biomarker for the assessment of the quality of sperm. It has been included as an extended semen examination in the World Health Organization (WHO) Manual for the Laboratory Examination and Processing of Human Semen (6). Several studies on the different aspects of sperm DNA integrity have been done and are ongoing all over the world (4, 7, 8). However, the studies on male infertility from Nepal are limited to conventional semen assessment (9-14). The problem of sperm DNA fragmentation has not been studied in Nepal to date. Since the data on sperm DNA fragmentation in men with fertility issues is still unknown in Nepal, this needs to be studied and explained.

The integrity of sperm DNA in the semen with the concentration of sperms in the normal reference range (≥16 million/mL of ejaculate) according to WHO (2021) was thus assessed in this study. The sperm DNA integrity was evaluated by the sperm chromatin dispersion test that helps us to differentiate sperms with intact and non-fragmented DNA from sperms with fragmented DNA. This gives the basic data on the sperm DFI of Nepalese men under fertility treatment. The role of host and lifestyle factors and the presence of bacteria, termed bacteriospermia, in the semen may have a potential impact on the fragmentation of sperm DNA, directly or indirectly. Therefore, the correlations between these factors (host and lifestyle factors and bacteriospermia) and sperm DFI were also studied, which might be helpful in the prevention or reduction of sperm DNA fragmentation in men dealing with fertility issues.

Materials and methods

A cross-sectional study based on a fertility center Creator's IVF Pvt. Ltd., Lalitpur with laboratory support of the Central Department of Microbiology, Tribhuvan University, Kathmandu, Nepal was carried out from June 2021 to July 2022. The source population was males among the couples attending Creator's IVF Pvt. Ltd., Lalitpur for fertility consultation during the period of study. The study population included the subjects who were ready to give consent. The subjects who were under antibiotic therapy within the past week and not willing to give consent were excluded from the study. A total of 217 subjects were recruited irrespective of their age, and type of infertility. The study was approved by the ethical review board of the Nepal Health Research Council (NHRC), Kathmandu, Nepal (Approval No. 874/2019).

Data on demography and lifestyle factors (independent variables) were collected by asking the subjects based on the questionnaire approved by NHRC, Nepal. DNA fragmentation of sperms (dependent variable) and bacteriospermia (independent variable) were examined by the laboratory analysis of semen samples of the subjects. Semen samples were collected in a sterile leak-proof container by masturbation with an abstinence period of 2-5 days, following the criteria of the World Health Organization (WHO) for semen sample collection for microbiological analysis (6).

Semen samples were processed after liquefaction of samples at 37 ^{o}C for routine semen analysis at the andrology laboratory of the fertility center. Semen samples were processed for analysis of sperm concentration, motility, vitality and morphology following WHO guidelines (WHO 2021). The samples only with normal sperm concentration (≥16 million/mL of ejaculate) were further assessed for sperm DFI test.

Sperm concentration in the sample was assessed by using a Neubauer hemocytometer, and total sperm count was obtained by multiplying sperm concentration by the volume of ejaculate. Motility was assessed by microscopic observation of the sample placed in Makler's chamber. Sperm morphology was investigated by the Diff-Quik method using a Morphology-D kit (Sperm Processor Pvt. Ltd., India) following the manufacturer's instructions. The vitality of sperm was assessed by the Eosin method. Sperm DNA fragmentation was evaluated by halosperm test, a sperm chromatin

dispersion assay using a Sperm chroma kit (Cryolab International, India) following the manufacturer's instructions.

Semen culture was done in blood agar, Mac-Conkey agar, and chocolate agar media by inoculating 0.01 mL of the sample to isolate bacteria in the sample. It was considered bacteriospermia if the growth of bacteria in an agar plate was greater than 10^3bacteria/mL (15).

Statistical analysis: The use of statistical software IBM SPSS version 21.0 analyzed data. Demographic and semen characteristics are expressed as mean/median. Data on lifestyle factors of study subjects are expressed as percentages. Pearson's correlation test at a 95% confidence interval was used to correlate sperm DFI with bacteriospermia, age and BMI of the study subjects. The data on lifestyle factors are categorical, non-parametric tests for the significance of association should be used. Therefore, the associations between the lifestyle factors (independent variables) and sperm DFI (dependent variable) were analyzed by a significance test, a chi-square test at a 95% confidence level. Results were considered significant if the p-value was less than 0.05.

Results

The study population consisted of 186 men among couples trying to conceive for more than 12 months and attending the fertility center for consultation. The mean age of study participants was 36.08 ± 5.88 years. Most participants were overweight (BMI: 25-29.9 kg/m^2) and the mean BMI was 25.87 ± 3.06 kg/m^2. Regarding lifestyle factors, most of the participants are non-smokers (38.7%), alcohol non-consumers (27.4%), and physically active (72.6%) (Table 1).

The sperm DFI of the samples ranged from 4.35% to 76.15% with the mean (SD) DFI % of 20.54 (12.99) and median DFI of 20.54%. The values obtained from sperm DFI were categorized into three groups: low, moderate, and high. Most of the samples under study (41.4%) had good sperm DNA integrity with a mean DFI of 10.20% (Table 2).

Bacteria in significant count (>10^3 cfu/mL of ejaculate) was detected in 24.7% of samples. Although bacteria present in semen due to the infection in the reproductive tract and male accessory gland could be one of the factors responsible for the fragmentation of sperm DNA, we could not observe the influence of seminal bacteria on sperm DNA integrity (Table 3).

Table 1: Characteristics of participants

Characteristics	Frequency	Percentage
Age (years)		
20-24	1	0.5
25-29	23	12.4
30-34	51	27.4
35-39	63	33.9
40-44	29	15.6
45-49	15	8.1
50 and above	4	2.2
BMI (kg/m^2)		
Underweight (<18.5)	1	0.5
Normal (18.5-22.99)	34	18.3
Overweight (23-24.99)	41	22.1
Obese (≥25)	110	59.1
Smoking		
Non-smoker	72	38.7
Ex-smoker	55	29.6
Occasional	20	10.7
Regular	39	21.0
Alcohol consumption		
Never	50	26.9
In the past	16	8.6
Occasional	96	51.6
Regular	24	12.9
Physical activity		
Yes	135	72.6
No	51	27.4

BMI: Body mass index according to WHO-Asian BMI classification

Pearson's correlation test was done to correlate sperm DFI with the age and BMI of participants. We observed that both age and BMI positively correlated with sperm DFI. However, the correlation between BMI and sperm DFI was insignificant. There was a significant correlation between age and sperm DFI ($p<0.001$) (Table 4).

Table 2: Sperm DFI of samples

Category	Sperm DFI	Frequency (%)	Mean ± SD	Median	Range
Low	<15%	77 (41.4)	10.20 ± 2.97	10.13	4.35–14.97
Moderate	≥15% and <30%	72 (38.7%)	20.76 ± 4.29	20.40	15.02–29.77
High	≥30%	37 (19.9%)	41.63 ± 11.23	38.93	30.08–76.15

DFI: DNA fragmentation index

Table 3: Correlation between sperm DFI and bacteriospermia

Bacteriospermia	Sperm DFI			Correlation coefficient	P value
	Low	Moderate	High		
No	51	62	27	-1.01	0.170
Yes	26	10	10		

DFI: DNA fragmentation index

Table 4: Correlation of sperm DFI with age and BMI of participants

Dependent variable	Independent variables	Correlation coefficient	P value
Sperm DFI	Age	0.229	0.002
	BMI	0.040	0.590

No significant association between the DNA integrity of spermatozoa and lifestyle factors under study – smoking, alcohol consumption, and physical activity of the study population was determined in this study (Table 5).

Discussion

Sperm DNA integrity is an important prerequisite for success in natural and assisted fertilization and embryo development (16). Sperm DNA damage also increases the risk of miscarriage (2), and might decrease the birth weight of a child (8). World Health Organization has considered sperm DNA fragmentation test to detect the degree of damage in the single or double strands of DNA packed in the head of spermatozoa (6). Various lifestyle, environmental, and health factors might be associated with the increased risk of sperm DNA fragmentation (17).

The presence of bacteria in semen may be due to the infection of the genitourinary tract or male accessory gland infection. Bacteria present in semen may affect the semen characteristics along with sperm DNA integrity (18, 19). However, we could not show a positive association between the presence of bacteria and sperm DFI in our study. Besides bacteriospermia, sperm DNA can be fragmented by various other factors.

Aging causes various physiological changes in the human body, including changes in the reproductive potential in males and females. Specifically, a change in the sperm DNA with the age of men was observed in this study. The age of the study population was positively correlated to sperm DNA fragmentation and was statistically significant. A similar result with a strong correlation between age and the percentage of DFI was observed by Deendayal Mettler and coworkers (20). The risk of sperm DNA fragmentation in men above 50 years was 4.58 times more likely than in those aged 21-30 years (21). Men over 40 years exhibited higher sperm DNA fragmentation than men of younger age (22). A large study including 16945 semen samples also resulted in a linear trend of DFI and oxidative stress (OS) with the age of men (23). The body's antioxidant capacity decreases with aging, which leads to oxidative stress in the spermatozoa. The oxidative stress might be responsible for the DNA damage of sperm cells, resulting in high sperm DNA fragmentation. Sperm DNA fragmentation is also associated with mitochondrial damage (24) that can occur due to age-related oxidative stress.

Table 5: Association between sperm DFI and lifestyle factors

Independent variables	Sperm DFI			Chi-square	P value
	Low n (%)	Moderate n (%)	High n (%)		
Smoking				6.076	0.415
Non-smoker	28 (38.9%)	30 (41.7%)	14 (19.4%)		
Ex-smoker	23 (41.8%)	19 (34.6%)	13 (23.6%)		
Occasional	5 (25.0%)	11 (55%)	4 (20.0%)		
Regular	21 (53.8%)	12 (30.8%)	6 (15.4%)		
Alcohol				0.511	0.203
Non-consumer	20 (40.0%)	18 (36.0%)	12 (24.0%)		
Ex-consumer	5 (31.3%)	4 (25%)	7 (43.7%)		
Occasional	40 (41.7%)	40 (41.7%)	16 (16.6%)		
Regular	12 (50.0%)	10 (41.7%)	2 (8.3%)		
Physical activity					
No	24 (47.0%)	17 (33.3%)	10 (19.6%)	1.071	0.585
Yes	53 (39.3%)	55 (40.7%)	27 (20.0%)		

Obesity is considered a potential hazard to human health, which is also responsible for increasing oxidative stress and causing DNA damage in the cells (25). In the present study, we could not observe a significant positive correlation between BMI and sperm DFI. A meta-analysis done in 2019 could not conclude the association between the BMI of men and SDF (17, 25). Similarly, another recent meta-analysis of the studies of a decade also could not associate BMI with DFI percentage (26). However, the probable influence of obesity on sperm DNA damage could not be neglected. Various studies showed that SDF was significantly higher in overweight infertile men than in those with normal body weights (27-29). A study done to find the association between BMI and sperm apoptosis found that inflammation inducer adipokines are upregulated with greater BMI, which leads to the increased formation of ROS resulting in sperm damage (28). Obesity may also increase the intestinal permeability, and endotoxin levels, leading to oxidative mediated sperm DNA damage (30).

Lifestyle factors like smoking, alcohol consumption, physical inactivity, and excessive nutrition may affect the general health as well as reproductive health of humans (17). We could not observe the association between SDF and the lifestyle factors under study (smoking, alcohol consumption, and physical activity). Similar to our study, Shi et al. did not find a significant association of DFI with lifestyle factors including smoking and alcohol consumption (31). Our result on the association between smoking and SDF was contrary to many other studies. A meta-analysis of 190 studies showed increased DFI in smokers than in smokers (17). Moreover, a dose of smoking was associated with DFI; light smokers had a lower increase in DFI than heavy smokers (17). The correlation between cigarette smoking and sperm DNA fragmentation was also observed in a study that obtained data on smoking by quantifying a degree of intoxication using a CO-Tester which measures the rate of expired carbon monoxide (32). A study done in Saudi Arabia also correlated smoking and DFI significantly (33). Cigarette smoking along with many health problems may also harm DNA methylation causing sperm DNA fragmentation (34). Smoking can also cause the loss of antioxidation ability against free oxygen radicals in the seminal fluid, thus increasing the oxidative stress on spermatozoa (33). Cigarette smoking may also accumulate toxic chemicals like nicotine, cadmium, and others in the blood of smokers, which can accumulate in seminal plasma crossing the blood-testis barrier and eventually presenting a toxic effect on spermatozoa (32).

Drinking alcohol is not considered as beneficial to human health. However, the effect of alcohol consumption on reducing fertility potential is still controversial. Our study could not observe an association between alcohol consumption and sperm DNA fragmentation. A meta-analysis on sperm DNA fragmentation and lifestyle and other factors could not find a significant difference in the DFI of alcohol drinkers and non-drinkers (17). A similar observation was made in the study on the effect of alcohol use on sperm chromatin structure (35). However, in another study, sperm DFI in heavy drinkers was found to be significantly higher in comparison to that of non-drinkers (36). A survey of the relationship between alcohol on sperm DNA damage and pregnancy resulted in significant damage on sperm DNA damage in alcohol consumers and also in pregnancy achieved after infertility treatment (37). A recent study in Iran also observed a significant influence of alcohol consumption on sperm DNA fragmentation (38). An experimental animal study on mice proved the effect of alcohol abuse on the reduction of fertility potential by the production of spermatozoa with less condensed chromatin and also affecting the motility of sperms (39).

A sedentary lifestyle lacking physical activity is hazardous to human health, including reproductive health. Men lacking physical activity at work were found to have significantly higher DFI than men with physical activity at work, and also sedentary occupation increased the risk of sperm DNA fragmentation by two times (40). Moderate exercise helps to decrease testicular ROS, which finally helps to protect against the damage of oxidative stress to spermatozoa (41). However, we could not observe the association between physical exercise and sperm DFI in our study.

Conclusion

Bacteriospermia, host, and lifestyle factors may affect the characteristics of semen. Our study suggests that a host factor- age is associated with DNA damage in spermatozoa. However, we could not show a significant positive association of sperm DNA fragmentation with bacteriospermia, body mass index and lifestyle factors under study. DNA damage in sperm consequently leads to a decrease in the

possibility of conception either in a natural way or with the help of assisted reproductive technology, and also increases the chance of miscarriage after conception. The major mechanism of sperm DNA damage is the decrease in the antioxidant ability, production of ROS, and oxidative stress on spermatozoa. Aging, smoking, alcohol consumption, and infection are proven to be responsible for the oxidative stress that ultimately damages the sperm's nuclear material. Thus, the use of antioxidants may help reduce the oxidative damage to sperm. Moreover, avoidance of modifiable host factors such as obesity and lifestyle factors such as smoking, alcoholism, and physical inactivity is more beneficial to prevent sperm damage and improve reproductive health.

Conflict of Interests

Authors declare no conflict of interests.

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