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Original Article

Effect of Smoking on Membrane Integrity of Sperms – Study by Hypo-osmotic Swelling Test

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ABSTRACT

Aim: Routine semen analysis does not give information about the fertilizing ability of the sperm; therefore sperm function tests are essential to explore fertility potential of the sperm. Sperm capacitation, acrosomal reaction, and penetration of egg are also dependent upon membrane integrity of spermatozoa. Therefore, assessment of membrane function is a useful indicator of the fertilizing ability of spermatozoa. Hypo-osmotic swelling test (HOS) is a laboratory index of the functional integrity of the sperm plasma membrane. Cigarette smoke has deleterious effect on membrane integrity of sperm and as the HOS test can assess it. The present study was aimed to compare the membrane integrity of sperms by using hypo-osmotic swelling test in smokers and non-smokers. Methods: The present cross-sectional study included 60 subjects (30 smokers and 30 non-smokers) in age group of 20-40 years. Smokers were having the history of smoking for at least 3 years or more and non-smokers were the subjects who were not having any history of active smoking. The subjects were referred to Reproductive Biology Lab as a part of investigation for infertility. In all the subjects routine semen analysis was done and Hypo-osmotic swelling test was performed. Result: In smoker group, out of 30 subjects, 20 subjects have HOS test result <60% and 10 subjects have the HOS test result >60%. In non-smoker group, out of the 30 subjects, 10 subjects have HOS test result <60% and 20 subjects have HOS test result >60%. There was statistically significant ($P < 0.05$) association between smoking and the abnormal HOS test (coiling of sperms <60%) observed. Conclusion: We conclude that smoking increases oxidative stress in seminal fluid of male smokers which has deleterious effect on membrane integrity of human sperms which can be detected by Hypo osmotic swelling test.

Keywords: Hypo osmotic swelling test, smoking, semen analysis, membrane integrity of human sperms

1. Introduction

Smoking has become a serious health and social problem today. Cigarette smoke contains many mutagens and carcinogens, which have unfavorable effects on male reproduction1. Several studies from different parts of the world have observed that cigarette smoke has an adverse effect on semen quality parameters especially in those who are heavy smokers or who have been smoking for many years [1]. Different parameters of semen quality have been used as an indicator of male fertility in clinical Andrology. Over the years, however, among different parameters, sperm count has been given undue importance. Many a times sperm count is normal but still the person is infertile. Sperm count is supposed to be meaningless without the required motility or normal sperm morphology. Moreover, routine semen analysis does not give information about the fertilizing ability of the sperm; therefore sperm function tests are essential to explore fertility potential of the sperm. It is the known fact that for successful union

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of the spermatozoa with female gametes, integrity of sperm membrane is very essential [2]. Sperm capacitation, acrosomal reaction, and penetration of egg are also dependent upon membrane integrity of spermatozoa. Therefore, assessment of membrane function is a useful indicator of the fertilizing ability of spermatozoa.

There are various tests of sperm function directed towards different regions of sperm viz, plasma membrane, acrosome, nuclear chromatin, and mitochondrial sheath which indicate the activity of each sperm organelle. Accordingly four commonly used tests are - Hypo-osmotic swelling test, test for acrosomal intactness, sperm nuclear chromatin decondensation test and sperm mitochondrial activity index test [3]. Hypo-osmotic swelling test (HOS) was introduced as a laboratory index of the functional integrity of the sperm plasma membrane. This test measures the ability of the sperm plasma membrane to transport water when exposed to hypo-osmotic solutions, thus inducing cell swelling and plasma membrane stretching [2].

This sperm function test assesses the sperms ability to fertilize ovum and allow the accurate diagnosis of many infertile patients not diagnosed by the routine semen analysis. Naturally it helps to improve the treatment modalities [4]. Cigarette smoke has deleterious effect on membrane integrity of sperm [5] and as the HOS test can assess it [2], the present study was undertaken to compare the membrane integrity of sperms by using hypo-osmotic swelling test in smokers and non-smokers.

2. Materials and Methods

The present cross-sectional study was carried out in 60 subjects (30 smokers, 30 non-smokers) in adult age group of 20-40 years after getting their informed written consent in Reproductive Biology Unit, Department of Physiology, MGIMS, Sevagram. Smokers were having the history of smoking for at least 3 years or more and non-smokers were the subjects who were not having any history of active smoking. The subjects were referred to Reproductive Biology Lab as a part of investigation for infertility. A detailed history was asked from all the subjects and thorough clinical examination was done. Subjects below 20 years and above 40 years; subjects having the history of major diseases like hypertension, diabetes, any endocrinal diseases; and the subjects who have not given the written consent were excluded from the study. An approval from Institutional Ethics Committee was obtained.

2.1.Methods

Routine semen parameters like sperm concentration, sperm morphology, functional sperm concentration, sperm motility index, motile sperm concentration were assessed by using SQA II C-P in all the subjects. Hypo-osmotic swelling test was performed on semen sample of all subjects.

2.2.Hypo-osmotic swelling test 2 : 500 micro litres of HOS solution was taken in a small tube and 50 to 100 micro litres of liquefied semen sample was added to it. It was mixed gently and incubated at room temperature for 5 minutes. At the end of the incubation time, 50 micro litres of colour stop solution was added and mixed gently. A small drop of the mixture on a clean glass slide was placed and it was covered with a cover slip. Thereafter it was observed under a microscope and the percentage of spermatozoa with coiled tail were counted. If the sample showed bent tail before the test, the number was deduced after the test to get the actual result. 60% or more coiled sperms were taken as normal6. Low positive reaction indicated loss of viability hence may be indication of degenerative changes occurring in the sperms.

2.3.Statistical Analysis:

All data was tabulated on a standardized data-collection form. Microsoft Excel 2007 software was used for data entry and analysis. Participants were categorized into two groups- smokers and non-smokers. Group differences were assessed using χ^2 (chi-square) and t tests. P value was taken as significant if found to be less than 0.05.

3.Results

Total 60 subjects were included in this study. 30 subjects (mean age 32.03 ± 4.03) with history of smoking for three years or more were recruited in smoker group and 30 subjects (Mean age 31.2 ± 3.68) who came to reproductive biology lab for semen analyses without any history of smoking were taken in non-smoker group. In smoker group, out of 30 subjects, 20 subjects have HOS test result <60% and 10 subjects have the HOS test result >60%. In non-smoker group, out of the 30 subjects, 10 subjects have HOS test result <60% and 20 subjects have HOS test result >60%. ((Table 1). There was statistically significant ($P < 0.05$) association between smoking and the abnormal HOS test (coiling of sperms <60%) observed. (Table 2 and 3).

Table1: Data depicting History of smoking and HOST result.

		HOS Test		
		Normal (>60%coiled sperms)	Abnormal (<60% coiled sperms)	Total
SMOKING	YES	10	20	30
	NO	20	10	30
	Total	30	30	60

HOS Test : Hypo-osmotic swelling test

Table 2: Table depicting Chi Square test, Degree of freedom and P value.

Chi Square Test	Degree of freedom	"P" Value(two tail)
6.6667	1	0.0098

Table 3: Odds based estimates and confidence limits

Type	Value	Lower	Upper
Odds ratio	4	1.367	11.7

4.Discussion

The present study was aimed at comparing the membrane integrity of sperms by using hypo-osmotic swelling test in smokers and non-smokers. We observed that in smoker group, out of 30 subjects, 20 subjects have HOS test result <60% and 10 subjects have the HOS test result >60% and in non-smoker group, out of the 30 subjects, 10 subjects have HOS test result <60% and 20 subjects have HOS test result >60%. We have also noted statistically significant association between smoking and the abnormal HOS test.

It is observed that many men who demonstrated normal parameter on standard semen analysis remain infertile. This suggests that the routine semen analysis (measurement of seminal volume, spermatozoa motility, density, viability and morphology) does not necessarily provide complete diagnostic information. As a result of active research in the area of evaluation of human semen, a series of sperm function assay have been developed such as Hypo osmotic Swelling test (HOS), Nuclear Decondensation Test (NCD), Acrosome Reaction (AR) and zona free hamster oocyte penetration test etc.

One of the tests is HOS test which determine the physiological integrity of plasma membrane. HOS test has been proved to be reliable test in predicting male fertility potential for identifying among sub fertile male those who have greater possibility of conceiving with time intercourse following ovulation induction [7]. Spermatozoa plasma membrane is a structure where processes such as motility, acrosomal exocytosis and sperm oocyte fusion occur. These processes are essential steps in successful fertilization. Membrane integrity is not only valuable for sperm metabolism but correct change in the properties of membrane is required for successful union of male and female gamete i.e. for sperm capacitaiton, acrosome reaction and binding of spermatozoa to egg surface.

In the present study we studied the association of smoking and sperm membrane integrity by using Hypo osmotic swelling test. Our observations go hand in hand with the findings by Chaudhari A et al [8] who reported negative correlation between oxidative stress and membrane integrity (HOS test). Several studies [9,10] also have shown that oxidative stress occurs in the seminal fluid of male smokers. The concentration of cadmium, lead, reactive oxygen species (ROS) and others are significantly higher and at the same time, the concentration of ascorbic acid and the activity of other components of the antioxidant defense are significantly reduced in smokers [11]. Oxidative stress has been linked to a number of physiological and structural abnormalities in human sperm. Fertilizing capacity is reduced due to failure to extrude residual spermatozoon cytoplasm, diminished membrane maturation and acrosin activity and an increased incidence of structural abnormalities in sperm tail [10]. We therefore conclude that smoking increases oxidative stress in seminal fluid of male smokers which has deleterious effect on membrane integrity of human sperms which can be detected by Hypo osmotic swelling test.

5.Conclusion

Based upon our observations, we conclude that smoking increases oxidative stress in seminal fluid of male smokers which has deleterious effect on membrane integrity of human sperms which can be detected by Hypo osmotic swelling test.

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