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Impact of Altitude Variation and Lifestyle variables on the Sperm Parameters among Indian Population residing in Northeast India particularly North Bengal and surrounding areas: A Cross Sectional Study

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Abstract
Background: *Several lifestyle factors have been proven to have a significant impact on male fertility; however, the effects of geographic location, such as continuous living at high altitudes, on fertility in the male population of India have not yet been identified. The purpose of this study is to ascertain how lifestyle variables coupled with altitude impacts the quantity and quality of sperm in male Indian inhabitants who have been long term residents from the northeastern region of India, specifically hailing from North Bengal and the surrounding areas.*
Materials & Methods: *In this cross-sectional study, 243 male participants were divided into two groups: the High-altitude group (HAG, n=105) and the Low-altitude group (LAG, n=138). Participants' demographic information was gathered using a set questionnaire, the laboratory semen analysis was done in compliance with WHO-2021 recommendations. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) Version 20.0 from SPSS Inc. in Chicago, Illinois, USA.*
Results: *On semen analysis, HAG showed significant negative correlation on all parameters. The analytical findings indicated that 11.9% of the total patients had oligozoospermia, of which 19.8% belonged to the HAG and 6.16% to the LAG. Among lifestyle variables, increase in BMI and age showed a significant difference on the overall parameters ($p<.05$), smoking showed a negative impact on all parameters ($p<.01$) with a strongly negative corelation with sperm count, motility and progressiveness, and alcohol and occupation had no significant difference only on the sperm volume ($p>.05$), with urbanization showing no significant difference on all parameters ($p>.05$).*
Conclusions: *Sperm count, motility, and progressiveness have all been found to be severely negatively impacted by high altitude, whereas sperm volume has been found to be less negatively impacted. Additionally, it was shown that the three lifestyle factors that had the greatest detrimental effects on sperm quality were smoking, obesity, and advancing age, followed by sedentary employment and alcohol use, while urbanization had no discernible effect.*
Keywords: *altitude, male fertility, semen analysis.*

Introduction

Male factor infertility accounts for over 40% of instances of infertility, which has long been recognized as a serious clinical problem for 8%–12% of couples globally[1,2]. About 8% of instances have male factor infertility as the sole known cause, whereas 35% of afflicted couples have both male factor origin and female factor infertility, according to the Centres for Disease Control and Prevention (CDC).(1) In accordance with standards from the World Health Organization (WHO), the following measurements are universally standardized for assessing overall male infertility indicators, the most prevalent indicators being aberrant sperm shape and motility, low or absent sperm counts, and issues with sperm ejection.(2) Despite current developments, semen analysis—which enables the examination of sperm count, concentration, motility, and morphology—remains the most reliable gauge of a man's overall reproductive status.(1)

Infertility in the male reproductive system may result from: abnormalities in the ejection of semen due to blockage of the reproductive tract. The tubes that convey semen, such as the seminal vesicles and ejaculatory ducts, may become blocked. Genital tract infections or traumas are frequently the cause of blockages; hormone anomalies resulting from pituitary, hypothalamic, and testicular production of hormones. Sperm production is controlled by hormones like testosterone. Testicular or pituitary tumours are two examples of conditions that cause an imbalance in hormones; failure of the testicles to generate sperm, for instance as a result of varicoceles or medical interventions that damage the cells that create sperm (such as chemotherapy); aberrant sperm quality and function. Fertility is adversely affected by circumstances or events that result in aberrant sperm morphology and motility. Anabolic steroid usage, for instance, might result in aberrant sperm characteristics including count and morphology.(3) There is a need to assess the effects of altitude on male fertility health among the Indian population. This study aims to assess the effect of factors like age, lifestyle and habits coupled along altitude change on male fertility health.

Materials and Methods

Subjects, Design and Data Collection

The Study population consisted of 243 male participants. They were divided into two groups namely: High Altitude group (HAG) n=105 and low altitude group (LAG) n= 138. Every individual who was enrolled for the study, provided informed consent. The study was carried out Implementing the guidelines of the Helsinki Declaration (2013). A brief, organized questionnaire was provided to each person in order to gather data on the demographic characteristics. *(Table 1)* Age, body mass index (BMI), personal addictions (such as alcohol consumption and smoking), region of residence and type of occupation were among the demographic factors that were evaluated.

Table 1. Questionnaire for the collection of demographic data

Questions	Option 1	Option 2
1. How old are you?	Below 40 years	Above 40 years
2. BMI	Normal	Obese/ overweight
3. Place of residence	Urban	Rural
4. Do you smoke	Yes	No
5. Do you consume alcohol	Yes	No
6. How would you describe your occupation?	Office worker	Ground worker

Semen collection and analysis

The sample Collection Method: Which entailed masturbating into sterile containers containing transportation medium (mHTF; Vitrolife, Gothenburg, Sweden) after at least two days, but no more than five days of abstention from sexual activity, done in accordance with the WHO-2021 recommendations. In order to minimize the time between collection and analysis, the collection was done in close proximity to the lab. Following thirty minutes of liquidation at 37˚C, the samples were analysed within one hour after they were collected. Following liquefaction, 6–10 µL of the semen were placed in a Makler counting chamber along with the necessary dilutions.[4] The sperm count and the motility test was conducted at x400 under a bright field microscope. A minimum of 200 sperm were tallied. The volume multiplied by the sperm concentration yielded the total count of sperm. The volume multiplied by the concentration and motility yielded the total quantity of motile sperm.[5]

Sperm Morphology: On a slide, a thin smear of well-mixed semen was made, and it was fixed in 95% ethanol for five to ten minutes while it was still wet. After that, it was left to air dry. To get rid of any remaining mucus, the smear was cleaned using a sodium bicarbonate formalin solution. After that, it was washed in several water changes. After applying diluted (1 in 20) carbol fuchsin (Blulux Laboratories Limited), the smear was left to stain for three minutes. Water was used to wash away the discoloration. The smear was covered for two minutes with a counter stain of diluted (1 in 20) methylene blue (Avondale Laboratories Limited), and then it was rinsed with water. After letting the smear air dry, it was examined using an oil-smeared ×100 microscope.[6]

The data collected was subjected to statistical analysis utilising the (SPSS) Version 20.0 of the Statistical Package for the Social Sciences, SPSS Inc. in Chicago, Illinois, USA. The descriptive statistics for age among the HAG and LAG group was compared and the study variables (volume, count, motility and progressivness) among the two groups were compared using t-test. Chi- square test was utilised to study the prevalence of normozoospermia and oligozoospermia among HAG and LAG and binary logistic regression was used to compare the odds of oligozoospermia in HAG and LAG. The study variables were compared with each demographic parameter.

Results

Table 2 indicates descriptive statistics for the age of study subjects in two groups. The average age of study subjects in the High-altitude group was 38.543 (SD=5.783) and the average age of study subjects in the Low altitude area group was 38.572 (SD=6.365). No significant difference in the mean age of study subjects in the two groups was observed (t=-0.037, p=0.97)

Table 2. Descriptive statistics for age according to high altitude and low altitude area

Group	N	Mean	SD	SEM	t-stat	p-value
High altitude	105	38.543	5.783	0.564	-0.037	0.97
Low altitude	138	38.572	6.365	0.542		

Table 3 indicates a comparison of study variables in the two groups.

The Mean volume (ml) among subjects in high altitude group was 2.317 (SD=1.434) and in the low altitude group, it was 2.542 (SD=1.555). The t-test result indicates no significant difference in the mean volume (ml) in the two groups (t =-1.166, p=0.248).

The Mean count (m/ml) among subjects in high altitude group was 38.821 (SD=23.635) and in the low altitude group, it was 48.264 (SD=21.273). The result of the t-test indicates a significant

difference in the mean count (m/ml) in the two groups (t =-3.308, p<.001).

The Mean motility among subjects in high altitude group was 27.065 (SD=12.564); in the low altitude group, it was 31.266 (SD=12.217). The t-test result indicates a significant difference in the mean motility in the two groups (t =-2.538, p=0.012).

The Mean Progressive among subjects in high altitude group was 10.641 (SD=8.273); in the low altitude group, it was 13.814 (SD=8.153). The t-test result indicates a significant difference in the mean Progressive in the two groups (t =-3.002, p=0.003).

Table 3. Comparison of study variables according to Race

Variable	Group	N	Mean	SD	SEM	t-stat	p-value
Volume (ml)	High altitude	105	2.317	1.434	0.140	-1.166	0.248
	Low altitude	145	2.542	1.555	0.129		
Count (m/ml)	High altitude	106	38.821	23.635	2.296	-3.308	<.001**
	Low altitude	144	48.264	21.273	1.773		
Motility	High altitude	93	27.065	12.564	1.303	-2.538	0.012*
	Low altitude	139	31.266	12.217	1.036		
Progressive	High altitude	103	10.641	8.273	0.815	-3.002	0.003**
	Low altitude	145	13.814	8.153	0.677		

Table 4 indicates the prevalence of oligozoospermia and normozoospermia in men from high altitude area and low altitude area region race. In high altitude area study subjects, out of 106 study subjects, 21 (19.81%) had Oligozoospermia, and in the low altitude area group, out of 146 study subjects, 9 (6.16%) were Oligozoospermia. The result of the Chi-square indicates a significantly higher prevalence of Oligozoospermia in the study subjects in the high-altitude area.

Table 4 Prevalence of oligozoospermia and normozoospermia in men from high altitude area and low altitude area

Group	Oligozoospermia		Normozoospermia		Total		Chi-square	p-value
	n	%	n	%	n	%		
High altitude	21	19.81%	85	80.19%	106	42.06%		
Low altitude	9	6.16%	137	93.84%	146	57.94%	10.91	<.001**
Total	30	11.90%	222	88.10%	252	100.00%		

Table 5 indicates the result of binary logistic regression shows that the odds of Oligozoospermia in the high-altitude area Population are 3.761 (95% CI: 1.646-8.594) times higher as compared to the study subjects in low altitude area.

Table 5. Odds Ratio using binary logistic regression equation

	B	S.E.	Wald	df	Sig.	Exp(B)	95% for Exp(B)
High altitude	1.325	0.422	9.869	1	0.002	3.761	1.646-8.594
Constant	1.398	0.244	32.917	1	0.000	4.048	

Table 6 indicates a comparison of study variables according to the habits and lifestyle. According to the age of study subjects (<40 and ≥40), a significant difference was seen in the Volume (ml), Count (m/ml), Motility, and Progressive (p<.05). According to the BMI of study subjects (Overweight & Obese versus Normal), a significant difference was seen in the Volume (ml), Count (m/ml), Motility, and Progressive (p<.05). According to the residence of study subjects (rural/urban). No significant difference was seen in the Volume (ml), Count (m/ml), Motility, and Progressive (p>.05). According to the Smoking habits of study subjects (Smokers/non-smokers), a significant difference was seen in the Volume (ml) (p<.01), Count (m/ml) (p<.01), Motility (p<.01), and Progressive (p<.01) According to the Alcohol drinking habits of study subjects (drinkers/non-drinkers), no significant difference was seen in the Volume (ml) (p>.05) and a significant difference in the Count (m/ml), Motility, and Progressive was observed (p<.05)

Table 6. Comparison of study parameters according to the Habits and lifestyle

Variable	Volume (ml)		Count (m/ml)		Motility		Progressive	
	Mean (SD)	p-value	Mean (SD)	p-value	Mean (SD)	p-value	Mean (SD)	p-value
Age								
<40	2.612 (1.02)	<.01	46.710 (20.12)	<.01	32.668 (11.24)	<.01	13.214 (5.642)	>.01
≥40	2.319 (1.38)		40.224 (22.69)		28.887 (10.32)		11.623 (7.214)	
BMI								
Normal	2.761 (2.10)	<.01	46.884 (15.71)	<.001	34.642 (12.41)	<.001	14.448 (11.04)	<.01
Overweight & Obese	2.019 (2.31)		37.514 (24.63)		27.087 (14.21)		10.037 (9.48)	
Residence								
Urban	2.514 (1.31)	>.05	42.541 (20.45)	>.05	29.914 (11.54)	>.05	11.356 (7.561)	>.05
Rural	2.526 (1.29)		44.483 (21.62)		31.614 (12.62)		12.471 (9.451)	
Smoking								
Yes	2.024 (1.24)	<.01	38.614 (26.43)	<.001	27.374 (13.61)	<.001	10.473 (8.754)	<.01
No	2.642 (1.30)		47.947 (18.24)		33.179 (11.73)		13.641 (10.471)	
Alcohol drinking								
Yes	2.521(1.32)	>.05	41.721 (21.13)	<.01	28.614 (12.24)	<.01	10.714 (8.426)	<.01
No	2.563 (1.29)		47.275 (24.24)		32.359 (11.34)		14.025 (8.175)	
Occupation								
Office worker	2.457 (1.19)	>.05	40.327 (23.41)	<.01	29.427 (13.54)	<.01	11.627 (9.414)	<.01
Ground worker	2.531(1.14)		44.642 (21.97)		33.476 (15.48)		15.618 (8.912)	

Discussion

Age has a significant impact on infertility as concluded from the study conducted by Kumar et al in 2017[7]. The increase in infertility has also been supported by other investigators which include Levitas et al 2007[8], Cardona Maya et al 2009[9], Harris et al 2011[10], Silva et al 2012[11], Stone et al 2013[12], Omran et al 2013[13] and Priyadarsini et al 2014[14]. Combined these authors have noted a sharp decline in semen volume, total

sperm count, motility, sperm concentration, increase in nuclear vacuole changes in increase in the DNA damage of the sperms with increase in age above 35 to 40 years onwards.[7]

Smokers and tobacco chewers have been reported to have a high concentration of free radicals in their seminal plasma, which damages sperm and decreases sperm motility, lowering the capacity to fertilize.[4] This could explain the result of low volume motility, count and progressiveness found among smokers. It has been found that the effect of smoking on sperm health is dose dependant.[15]

Men who drink excessive amounts of alcohol have shrinking of the testes, which results in aberrant testosterone production and impotence, decreased libido, and infertility which explains the significant difference found between those males that drink and do not drink excessive alcohol.[4,16]

Drinking alcohol and smoking cigarettes are linked to higher levels of oxidative stress as well as reduced activities of the accessory sex and epididymal glands.[17]

Significant difference was found in office workers which could be owing to the long sitting hours. Rural population had a slightly lower results while the difference between the obese and normal BMI individuals was very significant. This could be owing to the long sitting hours and sedentary lifestyle, which showed to have a impact by the study conducted by Blay et al, 2020,[6]. He also found from his study that individuals who spent more than four hours sitting had significantly reduced sperm motility.[6]

Longer stays at high altitudes have been linked to changes in the integrity of nuclear DNA in semen, sperm motility, sperm concentration, and testicular volume. These findings were made by Alcantara-Zapata et al. as they investigated the effects of hypobaric hypoxia on male reproductive health.[18] These findings are consistent with that obtained from this study among men in high altitudes. Oligozoospermia, that is low sperm count, was also found to be significantly higher in men in high altitude.

Ilacqua et al. concluded from his study that low physical activity, coffee consumption, stress, high fat diet intake, high intake of processed red meat, high temperatures and low intake of fruits and vegetables; all together leads to oxidative stress causing DNA damage in sperms affecting the number, motility and morphology impairment of the sperms.[19]

Conclusion

In conclusion, it has been discovered that altitude significantly affects the characteristics of sperm, such as volume, count, and motility and progressiveness.

High altitude was shown to have a detrimental effect on several male fertility parameters which include sperm count, motility and progressiveness conversely, it was discovered that sperm volume was also adversely affected, but less significantly. Furthermore, it has been shown that aging, obesity, consumption of substances like alcohol and tobacco, and sedentary lifestyle have a major, directly proportionate influence on the general health of male fertility. This research revealed that smoking, obesity, and aging were the three lifestyle variables that had the biggest negative effects on the sperm parameters. Sedentary work and alcohol use were the next two factors which showed lesser discernible effects. Urbanization was found to have no influence on the sperm parameters at all.

Limitations

- The research does not contain the DNA Fragmentation Index (DFI)
- Comorbidities and regular medication of subjects have not been taken into consideration.

Conflict of Interest:

There are no conflicts of interest.

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