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Prevalence of abnormal semen parameter among the male partner of infertile couple and factors associated with abnormal semen parameter in a tertiary centre Northeast India

[1]Subrat Panda, [2]Manika Agarwal, [3]Anusmita Saha, [4]Nalini Sharma, [*5]Ananya Das, [6]Wansalan Shullai, [7]Ritu Parna Das, [8]Dimple Kharkongor, [9]Pratitee Baruah, [10]Ruksana Makakmayum, [11]Namitha Gowda (NEIGRIHMS)

[*5] CORRESPONDING AUTHOR: DR ANANYA DAS

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Abstract: **This is a hospital-based observational study to estimate the prevalence of abnormal semen parameters in the northeast part of India and the effect of aging, lifestyle, and habits associated with it.**

Methodology: This study was conducted among the male partner of the infertile couple who attended our infertility clinic from Jan 2019 to July 2021. After written consent semen samples were collected and analyzed in our andrology lab based on the 2010 WHO recommendation. For each male partner two consecutive sample collected one month apart and the study result was mean of two sample analysis. Result of seminal analysis,.age, lifestyle, habits, and occupational exposure associated with different semen parameters were collected and analyzed by using SPSS Software version 22, with unpaired T test and Logistic regression analysis.

Result: In this study 14.8% males had oligospermia, 14.2% had Semen volume <1.5ml, and 51.8% males had total motility less than 40% ,(62.2%) had progressive motility less than 32% and 23.3.% had abnormal morphology <4% and 25.6% had vitality <58%. Semen volume was reduced in the2017478 age group more than 40 years (P value 0.000 ExpB 2.6) and with smoking (ExpB1.99). Sperm count was significantly less in the age group > 40years and with smokers(P VALUE-0.000,.0.012). Smoking affects the total motility of sperms (ExpB 1.873). Progressive motility was affected by smoking and alcohol (P value 0.000,0.000 ExpB 2.19,2.02). Vitality was affected by smoking, alcohol, and in underweight BMI (p-value 0.010,0.041, 0.001,). Morphology was affected by smoking, alcohol, (p-value –0.000, 0.000,)

Conclusion: Increased age affect semen volume, and sperm count. Alcohol intake affects progressive motility, vitality and morphology. Smoking affect all parameter of semen, low sperm vitality was associated with low BMI.

Keywords: **Semen Quality Analysis; Aging; Smoking; Alcohol; Body Mass Index; Pesticides.**

1. INTRODUCTION

Semen analysis is a basic infertility investigation of public health importance for several reasons, and it is, the first step to identifying male factor infertility(1) Study shows an overall 57% diminution in mean sperm concentration over the past 35 years, which, when analyzed for each geographical region, identified a significant decline in North America, Europe, Asia, and Africa. The association of male age with such a trend is reported (2). No consensus exists, and results are conflicting or inconclusive with methodological flaws. More prospective, large-scale, population-based studies are to be considered to have sound evidence of possible global trends in sperm count. (3) According to a 10-year comparison study

conducted on sperm quality and quantity (2000–2001 to 2010–2011), the percentage of semen volume, which is considered less than normal (below 4 ml), increased from 34% to 65% and the most suitable ejaculation volume (more than 4 ml) went down from 15% to 3%.[4] Over 37 years Semen parameters in Indian men have declined with time and the deterioration is quantitatively higher in the infertile group.[5] In this modern era, there has been a rise in sedentary lifestyles, obesity, and increased use of recreational substances such as alcohol, smoking, etc. Stress, alcohol abuse, and smoking have deleterious effects on sperm parameters and sperm DNA fragmentation.[,6,7] . In men, obesity is associated with abnormal semen parameters and can adversely affect fertility.[8] With the increase in the age of marriage in other countries as well as in India, the effect of demographic factors such as the age of males and females, and fertility have also become an important arena for further research. Hence, the study of the association of these environmental and lifestyle factors on semen quality becomes important. Evidence shows that semen quality varies with geographical and regional differences.[9] In Northeast India , epidemiological studies on semen parameters have been limited.

It is a tribal region border area of India where many tribes live. Here food habit lifestyle also varies from tribe to tribe. In Northeast India use of tobacoo and alcohol is more than in other part of India.(10). Use of tobacco and alcohol consumption was highest amongst males, 25−44 years of age and 62 % of participants rarely or never engaged in any physical recreational activity(11). Hence, the purpose of this study is to evaluate the prevalence of abnormal semen parameters in couples presenting with infertility in a tertiary care hospital in North East India This study also aims at evaluating the effect of various lifestyle factors and its relationship to semen quality

2. METHODOLOGY

The study was started after ethics committee approval. The ethical approval number was NEIGR/IEC/M7/T9/19

This was a Prospective observational cross-sectional study conducted in NEIGRIHMS from January 2019 to July 2021. Male partners of infertile couples attending infertility clinic at NEIGRIHMS were the study group.. The subjects were selected by consecutive sampling. - Male partners of couples failing to conceive for $\geq$ 12 months of regular unprotected intercourse (both primary and secondary infertility). The subjects who fulfilled the inclusion criteria & were willing to participate in the study were selected & written informed consent was taken from them.

The sample size of 379 was calculated expecting 56% incidence of abnormal semen parameters on the basis of prevous study(12) and 10% extra samples collected because of loss of follow-up for the second sample, with using open epi software was 417. Men on antidepressant and recent history of illness ,on testosterone medication ,cases of diabetes and other endocrinopathy were excluded from the study.Cases of azospermia were excluded from the study 15 cases, so total number of sample size was 402

A thorough history was obtained to know patients' socio-demographic variables to identify any high-risk factors associated with infertility.

Special instruction to the selected subjects- The subjects were advised to abstain from intercourse for at least three days. Semen samples were collected after masturbation in sterile wide-mouthed bottles within the hospital premises & submitted to the laboratory. We analyzed two consecutive sample of each man 1month apart and the study finding was the mean of two samples.The specimens were evaluated within one hour of the collection of samples. Data collection with history, clinical findings, and laboratory findings of semen analysis were recorded in Microsoft excel . The height and weight of individual subjects were recorded and BMI was calculated using the formula. Subjects consuming alcohol occasionally not every month was not considered as exposed to alcohol. We considered alcohol exposure when the men consumed 14 pegs or more per week. (one peg is equivalent to 30 ml) Similarly, the person with occasional smoking was not considered as exposure. but who has smoked at least 100 cigarettes in his lifetime, and who now smokes every day considered as exposed. Pestiside exposure cases were farmers who used pesticide in farms without using any protctive measure . All samples were kept at 37 plus/minus two degrees Centigrade (37 o ± 2 o C) temperature and processed immediately after complete liquefaction. All semen samples were analyzed for the following primary semen parameters: Volume, pH, Sperm concentration, Total sperm count, Total Motility, Progressive motility, Vitality, Normal morphology, and Fructose was seen. The semen parameters were analyzed by trained lab technicians according to the WHO Manual, 2010. Cases of azoospermia were excluded from the study. Datas were stored in an MS Excel spreadsheet. and analyzed by using the SPSS software version to see the factors associated with abnormal sperm parameters by using logistic regression and the mean of sperm parameters of different factors compared by using an unpaired T-test.

OBSERVATION- In our study, we had 402 cases of semen samples that were analyzed. Out of these cases, 198 males had regular alcohol intake and 153 cases were smokers. As per BMI 9 were underweight, 205 had normal BMI,188 cases were obese and overweight. (Table no-1) On seminal analysis 58 (14.8%) had oligospermia , 52 (14.2%) had volume <1.5ml , 208(51..8%) cases had total motility less than 40% and 250 (62.2%) cases had progressive motility less than 32%. 94 (23.3.%) had abnormal morphology <4% and

103 (25.6%) had vitality <58% (table no 2)In this study there was a significant difference in mean semen volume in the age group less than 40 and more than forty.(P-value 0.000) (table no 3) On logistic regression analysis age and smoking affects the semen volume with ExpB ratio 2.66 and 1.99(table no 4). Mean sperm count was significantly less in the age group of more than 40 (pvalue0.0194). There was a significant difference in sperm count in age group more than 40(0.000) and smokers. (0.012) (Table no 3) Increase in BMI did not affect sperm concentration and total sperm count (table no 4). Mean non-motile sperm concentration was less in the smoker. Non-motile sperm increases with smoking with an ExpB1.873 (table no 4). The mean number of progressive motility was less with smokes(p value0.000) and males having regular alcohol intake (pvalue0.000). Non-progressive motility increases with increases with smoking and alcohol with ExpB (2.19,2.02) respectively. (table no4) The mean no of abnormal sperm and vitality was significantly different among smokers and non-smokers (0.010) and males having alcohol and without alcohol.(0.041)(table no 3). Males exposed to pesticides do not get abnormal sperm morphology(Table no 4). Males who are underweight had low vitality of sperms.

Table 1: Descriptive statistics of percentage of independent factors i.e. pesticide, smoking, alcohol, age, BMI

Pesticide		Smoking		Alcohol		Age		BMI		
Exposed	Non-exposed	Exposed	Non-exposed	Exposed	Non-exposed	>40years	≤40years	Low BMI	Normal	Overwt and obese
73	329	153	249	198	204	36	366	9	205	188

Table 2: Percentage of normal and abnormal values of sperm parameters

Volume		Total Sperm Count		Sperm count per ml		Vitality		Total Motility		Progressive Motility		Morphology	
abnormal	normal	abnormal	normal	abnormal	normal	abnormal	normal	abnormal	normal	abnormal	normal	abnormal	normal
57 (14.2%)	345 (85.8%)	247(61.4%)	155(38.6%)	58 (14.4%)	344(85.6%)	103(25.6%)	299(74.$%)	208 (51.8%)	194(48.2%)	250 (62.2%)	152(37.8%)	94(23.7%)	308(76.3%)

Table 3: Difference Of Mean Of Semen Parameters Inrelation To Age, Alcohol Consumption, Smoking, Pesticide Exposure And Bmi

	VOLUME		SPERM CONC		TOTAL SPERM COUNT		Total motility		Progressive motility		Morphology		Vitality	Vitality
no	Mean	PVALUE UNPAIRED T TESTsig 2 tailed/oneway annova	Mean	PVALUE UNPAIRED T TESTsig 2 tailed/oneway annova	Mean	PVALUE UNPAIRED T TESTsig 2 tailed/oneway annova	Mean	PVALUE UNPAIRED T TESTsig 2 tailed/oneway annova	Mean	PVALUE UNPAIRED T TESTsig 2 tailed/oneway annova	Mean	PVALUE UNPAIRED T TESTsig 2 tailed/oneway annova	Mean	PVALUE UNPAIRED T TESTsig 2 tailed/oneway annova
AGE,<40/>40	1.99, 1.65	0.000 (S	40.65, 31.85	0.0194(S)	88.41, 52.47	0.000(S)	51.55, 50.22	0.387(NS)	25.73, 25.53	0.86(NS)	4.36, 4.33	0.894(NS)	58.42, 59.15	0.546(NS)
AlcoholY/N	1.90, 2.00	0.066(NS)	41.25 40.94	0.934	82.56, 87.05	0.382(NS)	50.58, 52.11	0.095(NS)	24.39, 26.91	0.000(S)	4.13, 4.58	0.000(S)	58.39, 59.72,	0.041 (S)
SMOKING Y/N	1.87, 2.01	0.70(NS)	43,173,46.430	0.374(NS)	76.12, 90.36	0.012(S)	50.35, 51.98	0.083(NS)	23.89, 26.79	0.000(S)	4.01, 4.59	0.000(S)	58.08, 59.73	0.010 (S)
PESTICIDE,Y/N	1.82, 1.99	0.051(NS)	46.22,44.91	0.692(NS)	79.81, 85.83	0.41(NS)	52.20, 51.23	0.24(NS)	25.16, 25.84	0.447(NS)	4.24, 4.39	0.357(NS)	58.54, 59.20	0.454 (NS)
BMI UW, NWT/OERVERWT,OBESE	1.60, 2.00, 1.92,	0.486(NS)	57.00, 41.53, 38.62,	0.771 (ns)	102, 89.73, 79.38,	0.80(NS)	42.33, 51.7, 51.34,	0.80(NS)	19.67, 25.59, 26.06,	0.128 (NS)	3.67, 4.33, 4.438	0.478 (NS)	39.67, 59.4,59.8,	0.001 (S)

Table 4: Logistic Regression to see the Odd's ratio and significance of association of each independent factor on dependent factor

NO	VOLUME			SPERM CONC			TOALSPERM			TOTAL MOTILITY		
	B	SIG	ExpB	B	SIG	Exp B	B	SIG	ExpB	B	SIG	ExpB
Age	.968	.016	2.66	-.012	.980	.988	.282	.517	1.326	-.634	.311	.531
BMI	.080	.738	1.084	-.777	.001	.460	-.827	.000	.437	-.092	.707	.912
ALCOHOL	.384	.179	1.469	-.911	.003	.402	-.599	.034	.549	-.292	.329	.747
SMOKING	.691	.016	1.996	.467	.120	1.596	.566	.048	1.760	.627	.038	1.873
PESTISIDE	.148	.668	1.160	-.395	.343	.674	-.574	.164	.563	-.898	.068	.407
CONSTANT	-.009	.0.987	.991	3.505	.000	33.287	3.074	.000	21.639	3.130	.000	22.875

NO	PROGRESSIVE MOTILITY			MORPHOLOGY			VITALITY		
	B	SIG	EXPB	B	SIG	ExpB	B	SIG	ExpB
Age	0.192	.609	.825	-.826	0.134	.438	.348	.367	1.416
BMI	0..222	.242	.801	-.200	0.333	.819	.100	.617	1.106
ALCOHOL	0.784	.001	2.190	.129	0.607	1.138	.264	.267	1.302
SMOOKING	.706	.004	2.025	.457	0.075	1.579	.243	.319	1.275
PESTISIDE	.462	.131	1.587	-1.081	0.011	.339	241	.445	.786
CONSTANT	-1.517	.005	.219	2.961	0.000	19.326	.584	.271	1.792

3. DISCUSSION

In this study 14.8% Male had semen voume less than 1.5ml,14.2% males had sperm concentration <15million per ml, 62.2% male had progressive motility <32%, 25.6% male had vitality <58%, and 23.7% males had morphologically normal sperm <4%. Study by James Akpenpuun Ikyernum1 , Ayu Agbecha2,* , Stephen Terungwa Hwande had 39.3% males had oligospermia, and only 42.3% males had normal morphology(13). In our study most common abnormality is asthenozospermia. Other study has also same finding.(14) like ours. Our study had better semen parameter than this study ,it may be because of food habit and environment.and this region had no industrial pollution. .In our study age, of more than 40 and men with smoking habits affect the semen volume mean(p-value-0.000,0.016). Semen volume <1.5ml was seen more in smokers after adjusting all factors. On logistic regression analysis age and smoking affects the semen volume with an odds ratio of 2.66 and 1.99. Sperm concentration was affected by smoking and age. Men with age less than 40 have more mean sperm concentration than those aged more than 40yrs and total sperm count affected by smoking . Total motility and progressive motility were affected by smokers and men having regular alcohol intake. The mean no of abnormal sperm and vitality was significantly different among smokers and non-smokers (0.010) and males having alcohol and without alcohol. (0.041).Vitality was affected by smoking, alcohol and low BMI,but our sample size was 9 only for low BMI. Low BMI may be a harmful factor of male infertility.(15) Our study showed a fall in semen volume with age in males with more than 40years, The decrease in volume may be due to seminal vesicle insufficiency because seminal vesicle fluid composes most of the ejaculate volume (16,). Similar to our study results, a literature review comparing 30-year-old men to 50-year-old men found a decrease in semen volume (3-22%)(.14 ,19) In this study Semen volume was grossly affected by smoking. (17) In our study mean sperm concentration was affected age more than 40 and with a man with smoking.Study bySimon De Bruckere etal the only parameter affected with smoking was sperm concentration(18) . Similar to our study results, the risk of decreased sperm counts in semen analysis with increased with age(19,20) In a large sample size of males with known fertility, heavy cigarette smoking was associated with decreased semen volume((19) Cigarette smoking affects sperm concentration and sperm motility. Similar to our study a meta-analysis shows cigarette smoking is associated with reduced sperm count and motility. (22). In this study we had alcohol affects sperm motility,vitality and morpholology. Literature is quite conflicting on this issue. Some authors observed favourable effect of alcohol on semen parameter in moderate amount(23)). Alterations in morphology and decreased concentration, motility, and viability have been observed among smokers. (24,25). In our study exposure to pesticide number was less and it did not affect any parameter.. Study by Sánchez-Peña also had similar finding.(26)Our sample size for pesticide exposure was 73 so did not have any impact of pesticide on semen parameter but according to literature pesticide exposure negatively impact semen parameter.(27)

The weakness of our study is that RCT and cohort studies could not be done because of ethical problems,so cause relationship,and association can not be strongly established in this type of crosssectional studies.

4. CONCLUSION

Different abnormalities of semen parameter varies from 14% to 62%.The most affected parameter is progressive motility. Increased age which is a natural process affects semen volume and sperm concentration. Alchol affects progressive motility,vitality and morphology of sperms. Incresed Pesticide and BMI does not have significant impact on semen parameter.

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