Role of Lifestyle Risk Factors for Azoospermia: Insights from Ebenezer Clinical Laboratory - Kampala Capital City, Uganda

Abstract

Background: Azoospermia is one of the social problems affecting families/countries today in the whole world, which has resulted in an involuntary declining birth rate [1]. Worldwide, more than 70 million couples suffer from infertility, and it is estimated that azoospermia is found in up to 10 to 20 percent of the men who present to an infertility clinic [2]. Uganda is among the countries where male infertility is assumed to be a big challenge, with an estimated 5,000,000 people facing infertility, where 10 to 15% of the couples are unable to have children. Hence the current study aimed at establishing the risk factors associated with azoospermia among patients attending Ebenezer Clinical Laboratory, Kampala, Uganda.

Methods: A retrospective case-control study design was conducted on men who had visited the Microbiology Department for semen analysis from 1st January to 31st December 2015. Cases were azoospermic participants in the ECL database with no sperm cells in the ejaculate whereas controls were normozoospermic participants in the ECL database with normal sperm cells in the ejaculate. Systematic sampling was employed in the selection of respondents using their clinical records. The sample size was 204 (102 cases and 102 controls) clients, determined using a formula from the OpenEpi software package for Kelsey. The sample involved 102 cases and 102 with a ratio of cases to controls being 1:1. The study used a data abstraction structured questionnaire for data collection. Data was analyzed using descriptive statistics to generate frequencies and percentages. Chi-square test and logistic regression analyses were used to determine whether there was a significant association between risk factors and azoospermia with about 0.05 statistical significance.

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Results: The study found out that tobacco use [AOR = 11.245, (CI 95% = 3.913 – 32.312), p = 0.000], marijuana use [AOR = 21.975, (CI 95% = 5.710 -44.571), p = 0.000], type of underwear [AOR = 9.366, (CI 95% = 4.360 – 19.9471), p = 0.000] were statistically significant factors associated with azoospermia among patients

Conclusions: Interventions should be focused on improving these factors, such as minimizing tobacco and marijuana usage among the populations, as well as sensitizing the males on the dangers of wearing tight underwear.

Keywords: Azoospermia, Normozoospermia, Sperm Count, lifestyle-related determinants, Ebenezer Clinical Laboratory, Kampala Capital City Authority.

Introduction

Globally one of the social problems affecting families/countries today is the involuntary declining birth rate, although the number of infertile couples is not well documented [1]. Worldwide, it is believed that more than 70 million couples suffer from infertility [3]. Approximately 1% of all men in the general population suffer from azoospermia, and azoospermic men constitute approximately 10 to 15% of all infertile men. Male infertility accounts for 40-50% of infertility, affecting approximately 7% of all men. Yet approximately 10% of infertile men are azoospermic [4]. Also, azoospermia is found in up to 10 to 20 percent of the men who present to an infertility clinic [2]. Thus, this group of patients represents a significant population in the field of male infertility [5].

The highest numbers of male infertility have been recorded in the "African Infertility Belt" of which 43% of the infertility cases are due to men. Among the developing countries, the exact number of male infertilities is unknown due to a lack of proper registration and well-performed studies [6]. According to WHO, one in every four couples in developing countries is affected by infertility. There has been a notable difference in the prevalence according to geographical locations, and environmental, cultural, and socioeconomic influences. E.g., in

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Nigeria, male infertility is at 11% [7]. In some parts of sub-Saharan Africa including the Republic of Sudan and Cameroon, the infertility rate could exceed 30% [8]. In Kenya, Sharma [9] in a national study concluded that azoospermia (no sperm) and Oligospermia (low sperm) were the major causes of infertility among men; responsible for 41 percent of all male infertility.

In Uganda, infertility is a major challenge, with an estimated 5,000,000 people facing infertility, mainly handled by the private centers in urban areas; and about 10-15% of the couples cannot have children due to infertility. According to the MOH-Uganda, 75% of people are affected by infertility, the problem is due to Sexually Transmitted Infections (STIs) which often lead to blockage of sperm ducts among men [10]. However, the actual percentages showing infertility rates in Uganda are hard to come because people are not open about it and we do not have data, but medical consultants noted there are more Ugandans seeking help for fertility problems [11]. Social and environmental factors have been cited to be behind the increase in the number of patients with infertility, among men contributing to almost half of all cases in advanced nations [12].

Methods

Study design

The study adopted a retrospective case-control study design descriptive survey design with a cross-sectional design to establish the risk factors associated with azoospermia among men who had visited the Microbiology Department for semen analysis from 1st January to 31st December 2015 [13]. Cases were azoospermic participants in the ECL database with no sperm cells in the ejaculate whereas controls were normozoospermic participants in the ECL database with normal sperm cells in the ejaculate. Quantitative research approaches were used, where data from the patient files was analyzed using descriptive and inferential statistics.

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Setting

The study was carried out at Ebenezer Limited Clinical Laboratory, Kampala, Uganda. It's found on the First Floor of Sure House Building Plot 1, Bombo Road within the central area of Kampala Capital City Authority (KCCA), Uganda. This laboratory was chosen because it's accredited to ISO 15189 Testing Laboratory No. M0221, by SANAS from South Africa [14] serves a larger number of people for infertile investigations than any other Laboratory in Kampala and Uganda in general. The laboratory participates in external and internal quality assurance schemes and runs control daily. Over the past two years since 2013, there has been a rapidly increasing number of clients visiting the Ebenezer laboratory, testing positive for azoospermia among those seeking semen analysis tests, where an average of five azoospermic patients were reported per day from 2013 to date. This raised a concern to conduct the current study at Ebenezer laboratory.

Sample

A sample size of was 204 (102 cases and 102 controls) clients, determined using a formula from the OpenEpi software package for Kelsey [15] which is suitable for determining sample size in unmatched case-control study design. This sample size was determined with an assumption of a proportion of controls with exposure being 10 % at a 95% confidence interval with 5% marginal error and power of 80% as well as an odds ratio of three detected. The sample involved 102 cases and 102 with a ratio of cases to controls being 1:1.

Sampling

A systematic sampling technique was employed to select 102 cases and 102 controls. According to the records at ECL, a total of 2,880 clients visited the Microbiology Department for semen analysis tests in the period of 1st January to 31st December 2015 [14]. Since daily half of the clients were turning out to have azoospermia; this implies that out of the total population of clients, half of them (1,440 clients) were the estimated population of

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azoospermia clients (cases). Hence from this population, 102 azoospermia clients were picked using a systematic sampling technique, and these were the cases. Whereas, according to the daily trend of normozoospermia (controls), 2 out of 10, were normozoospermia and hence the study population for controls was 576 clients, and the sample size for controls was 102.

Data was captured from a pre-arranged laboratory register book in the microbiology department which is usually filled whenever one comes for semen examination. Systematic sampling was employed to select the study participants. Since the sample size for cases was 102, the sampling interval was 1,440/102 = 14. Therefore, hence every 14th element in succession was chosen from the sampling frame to be a part of the case group. Using the rotary method, the first case participant between 1 and 14 was selected randomly in the register which was 1. Then, 1, and every 14th element to follow was picked from the target population of 1,440.

The sample size for controls was 102, therefore the sampling interval was 576/102 = 6. Therefore every 6th element in succession was chosen from the sampling frame to be a part of the control group. Using the rotary method, a first-case participant between 1 and 6 was selected randomly in the register which was 4. Then, 4, and every 6th element to follow was picked from the target population of 576. The collection of the data took 2 months to be completed.

For inclusion criteria, all the azoospermic and normozoospermic patients in the data register aged 18 years and above, who visited the ECL Microbiology Department for semen analysis from 1st January to 31st December 2015 were considered in the study. Whereas, for exclusion criteria, the following categories of patients were not included in the study; Oligozoospermic (Sperm concentration less than the reference values); Asthenozoospermic

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(Less than the reference value for motility); Teratozoospermic (Less than the reference value for morphology); Oligoasthenoteratozoospermic (Signifies disturbance of all three variables); Aspermic (No ejaculate); Incomplete responses in the data register; Men that were below 18 years of age; and non-Ugandans.

Data Collection Methods

The study used a questionnaire survey method for data collection. This is because the data to be gathered is quantitative. The questionnaire survey method was used to collect data from the laboratory records.

Instruments

A data abstraction questionnaire captured information from cases and controls on clients who had visited the facility in 2015. The data abstraction semi-structured questionnaire had closed-ended questions developed to address the objectives of the study. The questionnaire was used because it is the most appropriate instrument given the nature of the topic of the study.

Data Analysis

Data from the respondents was edited to detect errors, cleaned daily, and sorted and questionnaires were given numbers for identification. Filled questionnaires were reviewed at the end of the day for completeness and accuracy. Data was entered into a datasheet and analyzed in the computer using the Statistical Package for Social Scientists (SPSS) version 25.0 while ensuring the accuracy and consistency of data. Different statistical methods were used for instance descriptive statistics and inferential statistics.

Data was analyzed using descriptive statistics to generate frequencies, percentages, means, standard deviations, and ranges. To establish the significant associations between the predictor variables and azoospermia, a chi-square test was used for bivariate analysis. The x^2

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test was used to determine whether there was a significant association between risk factors and azoospermia about 0.05 statistical significance. Variables that were significant under chi-square analysis were further subjected to binary logistic regression to obtain crude odds ratios (COR) and adjusted odds ratios (AOR) and their corresponding 95% confidence intervals. To test the hypotheses the researcher used the p-value and a significance level of (α=0.05) and the researcher rejected the null hypothesis (Ho) when the p-value was less than (≤ 0.05) when the findings were statistically significant and then accepted the null hypotheses (Ho) when the p-value is greater than (≥ 0.05).

Age category as a variable was included in the final logistic model despite its lack of significance in bivariate analysis to control for potential confounding, as it is a confounder in Azoospermia analysis. Considering interaction effects is crucial, and incorporating seemingly non-significant variables, like age, may reveal important insights. This approach ensures a more accurate assessment and a robust interpretation of factors associated with azoospermia in the studied population.

Results

About Table 1 below, age category (18-49), azoospermia cases accounted for 27 (26.5%), while Normozoospermia controls constituted 72 (70.6%). For the age group 50 and above, azoospermia cases represented 75 (73.5%), contrasting with 30 (29.4%) for Normozoospermia controls, indicating a higher prevalence of azoospermia cases in the older age group. Regarding tobacco use, 57 (65.9%) of azoospermia cases reported positive, compared to 18 (17.6%) among controls. Similarly, alcohol use was more prevalent among azoospermia cases (69, 67.6%) compared to controls (45, 44.1%). Physical exercise showed a balanced distribution between cases and controls. Marijuana/bhangi use was higher among azoospermia cases (96, 94.1%) compared to controls (30, 29.4%). The choice of tight

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underwear was more prevalent among azoospermia cases (60, 58.8%) than controls (15, 14.7%).

Table 1: Lifestyle Attributes of Study Participants

		Sperm Count		
	Cases: (Azoospermia) n (%)		Controls: (Normozoospermia) n (%)	
	Frequency (n)	Percent (%)	Frequency (n)	Percent (%)
Age Category				
18 - 49	27	26.5	72	70.6
50 >	75	73.5	30	29.4
Tobacco use				
Yes	57	65.9	18	17.6
No	45	44.1	84	82.4
Alcohol use				
Yes	69	67.6	45	44.1
No	33	32.4	57	55.9
Physical Exercise				
Yes	45	44.1	54	52.9
No	57	55.9	48	47.1
Marijuana/bhangi use				
Yes	96	94.1	30	29.4
No	6	5.9	72	70.6
Type of Underwear				
Loose	42	41.2	87	85.3
Tight	60	58.8	15	14.7

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Lifestyle Risk Factors for Azoospermia among Men Attending Ebenezer Clinical Laboratory

Chi-square and bivariate logistic regression analyses were performed at a 95% significance level to assess the association of individual factors with azoospermia among patients at Ebenezer Clinical Laboratory, with study findings detailed in Tables 2 and 3.

Table 2: Association between Lifestyle Attributes and Azoospermia at Ebenezer Clinical Laboratory

Factors	**Azoospermia Status**		**χ^2**	**df**	**p-value**
	Cases (Azoospermia) n (%)	**Controls (Normozoospermia) n (%)**			
Age category					
18 - 49	27 (26.5)	72 (70.6)	0.219	1	0.640
50 >	75 (73.5)	30 (29.4)			
Tobacco exposure					
Yes	57 (65.9)	18 (17.6)	16.742	1	0.000***
No	45 (44.1)	84 (82.4)			
Alcohol use					
Yes	69 (67.6)	45 (44.1)	11.453	1	0.001***
No	33 (32.4)	57 (55.9)			
Physical exercise					
Yes	45 (44.1)	54 (52.9)	1.590	1	0.207
No	57 (55.9)	48 (47.1)			
Marijuana/bhangi use					
Yes	96 (94.1)	30 (29.4)	19.429	1	0.000***
No	6 (5.9)	72 (70.6)			
Type of underwear					
Loose	42 (41.2)	87 (85.3)	42.698	1	0.000***
Tight	60 (58.8)	15 (14.7)			

Chi-square results from Table 1 reveal that tobacco exposure, alcohol use, marijuana/bhangi use, and the type of underwear are significantly associated with azoospermia, while age category and physical exercise did not reveal significant associations in this study. Based on $\chi2 = 0.219$, df = 1, and p = 0.640, the age category did not exhibit a significant association with azoospermia in this study. Regarding tobacco use, the analysis yields $\chi2 = 16.74$, df = 1, p = 0.001**,** signifying a highly significant association between tobacco exposure and azoospermia. Similarly, in the context of alcohol use, $\chi2 = 11.453$, df = 1, p = 0.001,

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demonstrates a significant association between alcohol use and azoospermia. Conversely, for Physical Exercise, $\chi2$ = 1.590, df = 1, p = 0.207, indicating that physical exercise was not significantly associated with azoospermia in this study. In the case of marijuana/bhangi use, based on $\chi2$ = 19.429, df = 1, and p = 0.001, indicates a highly significant association between marijuana/bhangi use and azoospermia. Concerning the type of underwear, with $\chi2$ = 42.698, df = 1, and p = 0.001, there is a highly significant association between the type of underwear and azoospermia.

Table 3: Lifestyle Risk Factors for Azoospermia among Patients Attending ECL

Lifestyle Factors	**Azoospermia Status**		**COR (95%CI)**	**p**	**AOR (95%CI)**	**p**
	Cases (Azoospermia) n (%)	**Controls (Normozoospermia) n (%)**				
Age Category						
50 >	75 (73.5)	30 (29.4)	1.15[0.627 -2.135]	0.640	1.927[0.826 - 4.95]	0.0127
18 - 49	27 (26.5)	72 (70.6)	1		1	
Alcohol use						
Yes	69 (67.6)	45 (44.1)	2.648[1.498-4.683	0.001	1.331[0.615 – 2.881]	0.467
No	33 (32.4)	57 (55.9)	1		1	
Tobacco/cigarette use						
Yes	57 (65.9)	18 (17.6)	3.684[1.939-7.001]	0.000	11.24[3.913 – 32.312]	0.000**
No	45 (44.1)	84 (82.4)	1		1	
Marijuana use						
Yes	96 (94.1)	30 (29.4)	6.67[2.63 - 6.86]	0.000	21.975[5.710 - 44.571]	0.000**
No	6 (5.9)	72 (70.6)	1		1	
Type of Underwear						
Tight	60 (58.8)	15 (14.7)	8.28 [4.22-16.27]	0.000	9.366[4.360 – 19.947]	0.000***
Loose	42 (41.2)	87 (85.3)	1		1	

Findings from Table 3 show that tobacco use, marijuana use, and type of underwear were statistically significant individual lifestyle risk factors associated with azoospermia among patients attending Ebenezer clinical laboratory.

The crude odds ratios indicate that men who regularly consume alcohol face approximately three times higher odds of azoospermia compared to non-consumers. However, this association loses statistical significance when adjusted (adjusted odds ratio [AOR] = 1.331,

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95% CI: 0.615–2.881, p = 0.467). Tobacco/cigarette smokers exhibit a significant fourfold increase in the odds of azoospermia (crude odds ratio [COR] = 3.684, 95% CI: 1.939–7.001, p = 0.001), and this risk further escalates to eleven times when adjusted (AOR = 11.245, 95% CI: 3.913–32.312, p = 0.000). Similarly, marijuana smokers are at a sevenfold higher risk of azoospermia (COR = 6.67, 95% CI: 2.63–6.86, p = 0.000), with a significant correlation between marijuana exposure and increased likelihood even after adjustment (AOR = 21.975, 95% CI: 5.710–44.571, p = 0.000). The type of underwear also emerges as a significant factor, with those wearing tight undergarments having eight times higher odds of azoospermia (COR = 8.28, 95% CI: 4.22–16.27, p = 0.000), a risk that escalates to nine times when adjusted (AOR = 9.366, 95% CI: 4.360–19.947, p = 0.000). The study findings underscore the importance of considering lifestyle factors in understanding azoospermia risk, with tobacco use, marijuana exposure, and tight underwear being identified as significant contributors to the reproductive health concern among men.

Discussion

The current study found that tobacco use was a strong statistically significant factor associated with azoospermia among patients attending Ebenezer clinical laboratory, whereby the use of tobacco increases the likelihood of developing azoospermia. This is likely because tobacco has a nicotine component, which reduces the testosterone hormone that is responsible for sperm production. Therefore, interventions should be put in place to ensure that tobacco use is minimized in the country. This is also in agreement with Zhao [16] who also proved that men who smoke tend to have a decrease in total sperm count, density, motility, normal morphology, semen volume, and fertilizing capacity. In the same lane, Tang also proved that current smoking in adult life moderately impairs semen quality [17]. Whereas, according to findings of another study [12], confirmed that smokers with low prolactin levels demonstrated defects in sperm motility.

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The study also revealed that the use of marijuana increases the chance of an individual acquiring azoospermia, where the odds of developing azoospermia among patients who were using marijuana were higher compared to those who were not using marijuana. This is because marijuana contains chemicals that can cause sedation and decrease libido. This is in line with Salama [18] who obtained 9.2% of the case group that had a drug addiction to marijuana, and marijuana use was a significant factor associated with azoospermia. in the same way, Romo-Yáñez [1] also confirmed that marijuana use with low prolactin levels demonstrated defects in sperm motility.

The study findings also revealed that the type of underwear was a strong statistically significant factor associated with azoospermia, suggesting that putting on tight pants/underwear exposes the individual to higher risks of developing azoospermia. This is because tight underwears tend to pull the testes closer to the body, hence gaining a body temperature of 37^{o}C, which increases heat in the scrotum, and heat affects spermatogenesis, leading to azoospermia. Hence there is a need to disperse sensitization messages to the population on the dangers of wearing tight underwear and advising them on the right type of underwear to wear. Like the study findings above, Al-Kandari [19] found that the increase in scrotal temperature induced by polyester-lined athletic supports was insufficient to cause significant suppression of spermatogenesis or alteration of sperm function. Ali [20] also confirmed that wearing tight-fitting compared with loose-fitting underwear is associated with significantly higher scrotal temperatures. In the same way, Sapra [21], indicated that wearing tight-fitting trousers is associated with higher scrotal and consequently testicular temperatures than wearing loose-fitting trousers or none. Male underwear choice is associated with a few differences in semen parameters.

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Limitations

The study faced a recall bias as it was done among patients who visited the facility a year ago. This was overcome by proper explanation of the purpose of the study to the respondents and the importance of the study which could help formulate policies that would help the communities. Semen analysis studies and responses related to sexual activity resulted in the quality of data being very poor to very good depending on the individual being interviewed since reproductive information was private and couples may not be inclined to be truthful in surveys. Many men were not willing to participate in semen studies.

Conclusions

Identifying the lifestyle determinants influencing azoospermia helps to appropriately address the burden and consequences of azoospermia, and this would reduce such consequences as stigmatization, isolation, neglect, domestic violence, loss of social status, and failure to have children among the affected individuals, as well as improving the mental and social well-being of the affected. This implies that focus should be diverted to improving such factors as tobacco use, marijuana use, and type of underwear, as these were observed to have a significant reduction in the rates of azoospermia incidences among the male population in the country.

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Abbreviations

AIDS: Acquired Immune Deficiency Syndromme; AOR: Adjusted Odds Ratio; CI: Confidence Interval; COR: Crude Odds Ratio; CVI: Content Validity Index; DNA: DeoxyRibo Nucleic Acid; ECL: Ebenezer Clinical Laboratory; FSH: Follicle Stimulating Hormone; HBM: Health Belief Model; HIV: Human Immune Virus; IRB: Institutional Review Board; KCCA: Kampala Capital City Authority; LH: Luteinizing Hormone; MOH: Ministry of Health; NEMA: National Environment Management Authority; PCR: Polymerase

Chain Reaction; SANAS: South African National Accreditation System; SPSS: Statistical Package for Social Scientists; STDs: Sexually Transmitted Diseases; STIs: Sexually Transmitted Infections; TB: Tuberculosis; UBOS: Uganda Bureau of Statistics; UNRA: Uganda National Roads Authority; WHO: World Health Organization.

Ethical Approval

During the retrieval of respondents' information from their medical documents, the anonymity and privacy of the participants were observed. The participants remained anonymous during the whole process of the study. The participants' information was kept confidential and only used for the research, the local Research Ethics Committee (REC) and Uganda National Council for Science and Technology (UNCST) as entities that may have access to private information that identifies the research participants by name.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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