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Obesity with High Body Mass Index Does Not Influence Sperm Retrieval in Males with Azoospermia

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Background: The aim of this study was to evaluate the influence of male body mass index (BMI) on the retrieval of sperm from azoospermic patients who were undergoing testicular sperm extraction (TESE).

Material/Methods: The study included retrospective data of male patients suffering from non-obstructive azoospermia (NOA). Age, BMI, testicular volumes, the serum concentration of the follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, and prolactin were investigated and collected.

Results: A total of 75 azoospermic males were evaluated between 2014 and 2019, including 35 patients (46.7%) with positive sperm retrieval. The majority of patients (57.3%) had normal BMI (between 20 kg/m^2 and 25 kg/m^2) or first degree obesity (from 25 kg/m^2 to 30 kg/m^2). No statistically significant correlation between BMI and positive sperm retrieval or hormone levels (LH, FSH, SHBG, prolactin) were found. However, lower serum testosterone levels were observed in patients with higher BMI (P=0.035). Receiver operating characteristic curve analysis showed that none of the hormones could potentially predict the positive outcome of TESE.

Conclusions: The hormonal levels or patient's BMI could not predict positive sperm retrieval outcome, however a negative correlation between serum testosterone and BMI levels was calculated implicating influence on fertility.

MeSH Keywords: **Azoospermia • Body Mass Index • Obesity • Sperm Retrieval • Testosterone**

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Background

Azoospermia is identified as an absence of spermatozoa in the sperm of men and is found in approximately 15% of all men suffering from infertility. Azoospermia might be diagnosed as obstructive (OA) or non-obstructive azoospermia (NOA), namely they differ in the ability of the testis to produce spermatozoa [1]. During screening for azoospermia, patients often suffer from Klinefelter syndrome (KS). Karyotype of KS patients contains 1 or more of Y chromosomes and 2 or more of X chromosomes [2].

Surgical procedure such as testicular sperm extraction (TESE) has been developed for positive sperm retrieval from azoospermic men [3]. But not every retrieval is successful. Various parameters can influence the sperm profile, including basal follicle-stimulating hormone (FSH) levels, body-mass index (BMI), smoking, and patient's age. The influence of these parameters on sperm retrieval is controversial and not clear yet [4].

Obesity has become a significant factor on the health of humankind [5] and has been identified to negatively influence the male reproductive and endocrine system by depreciation of the quality of semen. A meta-analysis from 2012 showed that obesity elevates the probability for male infertility [6]. Moreover, with the effect on the endocrine system, it can lead to hormonal imbalances such as hypoandrogenemia or hypogonadism [7]. Therefore, higher BMI might be a harmful factor on positive treatment outcomes in infertile men and simultaneously could decrease male fertility. Several reports have identified negative correlations between BMI and sperm concentrations [8,9], but these findings have not been confirmed by others [10]. Thus, the accurate association between BMI and sperm quality remains unknown.

A previous report detected an association between BMI and outcome of TESE in infertile NOA males and showed that males with lower BMI had a higher probability for successful sperm retrieval [11]. In our retrospective study, the effect of body BMI on sperm retrieval was investigated. Moreover, the associations between BMI and levels of hormones in men was assessed as the secondary endpoint to add to the current knowledge about the influence of obesity on positivity of TESE outcomes.

Material and Methods

Patients

Our study was conducted as a retrospective analysis of our database of male patients with infertility conditions, such as non-obstructive azoospermia (NOA), who underwent TESE at the Urology Department of the Merkur Clinical Hospital, Zagreb, Croatia between 2014 and 2019. Clinical assessment of infertile men was evaluated according to the description by Esteves et al. [12]. Finally, 75 patients were retrospectively analyzed. Seventy patients (93.3%) had a 46,XY karyotype with an average age of 35.6±3.5 years, and 5 patients (6.7%) had a Klinefelter (46,XXY) karyotype with an average age of 34.3±4.7 years. The study followed the Declaration of Helsinki guidelines and the study design was approved by our Departmental Committee. The data were obtained from our existing database; therefore, patients' consent of participation was not necessary.

Patient and laboratory measurements

Patient's body weight and height were detected routinely, and patient's BMI was calculated accordingly. Different levels of obesity were identified and patients were divided into lean (BMI ≤20 kg/m^2), normal (BMI from 20 kg/m^2 to 25 kg/m^2), first degree of obesity (from 25 kg/m^2 to 30 kg/m^2), second degree (from 30 kg/m^2 to 35 kg/m^2), third degree (from 35 kg/m^2 to 40 kg/m^2) and forth degree (BMI >40 kg/m^2). The size of male testis, length, width and height, were measured at room temperature, and the volume of testis was calculated according to these measurements. Average testicular volume was calculated with both testical sides. Levels of luteinizing hormone (LH), FSH, sex hormone-binding globulin (SHBG), and testosterone (T) were measured from serum samples from all patients. Endocrinologic evaluations were done 1–2 months prior to the TESE procedure. Blood was drawn from the forearm of all patients and hormone concentrations were measured using the immune assay method (Access 2, Beckman Coulter, CA, USA).

Surgical procedure

All procedures were performed by an experienced urological surgeon under local anesthesia with patients positioned in a supine position.

Statistical analyses

Statistical software SPSS 21.0 (IBM, New York, USA) was used to perform all statistical analyses. Normally distributed data were analyzed using ANOVA test, abnormally distributed variables were analyzed by Kruskal-Wallis test. Pearson's chi-square test was selected to calculate significance between classes of BMI as independent variable and sperm retrieval as a dependent variable. A univariate logistic regression with odds ratios was calculated to predict positive sperm retrieval. All tests were statistically significant if the *P*-value was less than 0.05.

Results

Overall, 75 male patients with NOA underwent TESE procedure at the Urology Department of our clinic between 2014

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Table 1. Basic characteristics and baseline findings of infertile male patients and comparison of factors between positive sperm retrieval and negative sperm retrieval after TESE.

	All patients N=75	Negative TESE (n=40)	Positive TESE (n=35)	*P*-value
Age [years]	35.7±4.0	35.6±4.2	35.7±3.8	0.944
BMI [kg/m^2]	27.0±4.1	26.4±3.0	27.6±5.1	0.319
T [nmol/L]	12.9±5.8	13.1±6.4	12.6±5.2	0.737
SHBG [nmol/L]	31.6±35.9	42.1±52.5	23.2±10.5	0.281
LH [IU/L]	7.7±5.7	8.5±5.6	6.7±5.8	0.281
FSH [IU/L]	18.7±12.5	20.3±13.1	16.9±11.9	0.342
Prolactin [mIU/L]	282.7±264.7	298.0±224.0	266.4±308.2	0.729
Testis volume right [mL]	11.6±9.3	11.9±10.3	11.2±8.2	0.822
Testis volume left [mL]	9.4±7.7	10.4±10.2	8.2±4.0	0.562

TESE – testicular sperm extraction; BMI – body mass index; T – testosterone; LH – luteinizing hormone; FSH – follicle-stimulating hormone; SHBG – sex hormone-binding globulin.

Table 2. BMI status of patients with positive and negative sperm retrieval.

BMI [kg/m2]	Negative TESE (n=40)	Positive TESE (n=35)	Sperm retrieval rate	*P*-value
Lean (BMI ≤20)	0	0	0	0.169
Normal (20 BMI ≤25)	8 (20.0%)	10 (38.6%)	55.6%	
First degree obesity (25< BMI ≤30)	17 (42.5%)	8 (22.9%)	32.0%	
Second degree obesity (30< BMI ≤35)	6 (15.0%)	5 (14.3%)	45.5%	
Third degree obesity (35< BMI ≤40)	4 (10.0%)	6 (17.1%)	60.0%	
Forth degree obesity (BMI >40)	5 (12.5%)	6 (17.1%)	54.4%	

TESE – testicular sperm extraction; BMI – body mass index.

and 2019. Basic characteristics and baseline hormonal levels of included patients are presented in Table 1. The average age and BMI of the male patients was 35.7±4.0 years and 27.0±4.1 kg/m^2, respectively. Sperm was successfully retrieved from 35 patients (46.7%). No statistical differences were observed in baseline levels of hormones between the patients with negative or positive TESE.

Most patients (57.3%) were classified with a normal BMI (from 20 kg/m^2 to 25 kg/m^2) or first-degree obesity (from 25 kg/m^2 to 30 kg/m^2 (Table 2). No patients were in a BMI ≤20 kg/m^2 group, so for further analyses this group was excluded from statistical calculations. Mean BMI among patients with positive TESE was higher than compared to patients with negative TESE procedure (Table 1), but the BMI difference was not statistically significant with *P*=0.319. Treatment outcome according to the obesity status was also not statistically significant with *P*=0.169 (Table 2). Majority of males in both groups were classified in the normal and first degree obesity group.

Testicular volume of both testis and hormone levels were evaluated separately according to the BMI (Table 3). The association between BMI and reproductive hormone levels of LH, FSH, SHBG, and prolactin were not found to be significantly different between BMI classes. On average, the testis volumes were not different among patients with different BMI classes. However, a statistically significant difference (*P*=0.035) in testosterone concentrations between the BMI groups was observed. Confirmed with linear regression found that the more obese patients with higher BMI were identified with lower serum levels of testosterone (Figure 1).

Finally, receiver operating characteristic curve analysis for all hormonal levels to predict positive sperm retrieval were performed and found that none of the hormonal levels could potentially predict the positive outcome with TESE (Figure 2). Areas under the curve (AUC) for hormones in relation to positive sperm retrieval were calculated. Prediction accuracy was the highest for testosterone (AUC=0.648) and followed by LH (AUC=0.556), but with no statistical significance (Table 4).

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Table 3. Baseline serum hormonal levels in patients from different BMI classes.

BMI [kg/m²]	Normal (20≤ BMI ≤25)	1st degree (25< BMI ≤30)	2nd degree (30< BMI ≤35)	3rd degree (35< BMI ≤40)	4th degree (BMI >40)	*P*-value
T [nmol/L]	13.5±5.9	14.0±5.7	8.1±0.7	4.2±0.0	2.9±0.0	0.035
SHBG [nmol/L]	27.4±14.3	41.7±52.3	17.1±3.6	17.6±0.0	19.7±0.0	0.761
LH [IU/L]	7.8±6.1	8.0±5.9	5.5±3.4	3.8±0.0	17.5±0.0	0.349
FSH [IU/L]	16.4±12.2	20.6±12.7	17.5±12.9	5.3±0.0	33.7±0.0	0.455
Prolactin [mIU/L]	263.4±155.6	308.5±357.8	250.9±117.4	248.7±0.0	246.0±0.0	0.959
Testis volume right [mL]	9.4±6.7	12.5±10.9	13.4±8.9	14.4±0.0	3.0±0.0	0.758
Testis volume left [mL]	7.7±5.4	10.7±9.2	12.6±0.0	/	9.4±0.0	0.726

BMI – body mass index; T – testosterone; LH – luteinizing hormone; FSH – follicle-stimulating hormone; SHBG – sex hormone-binding globulin.

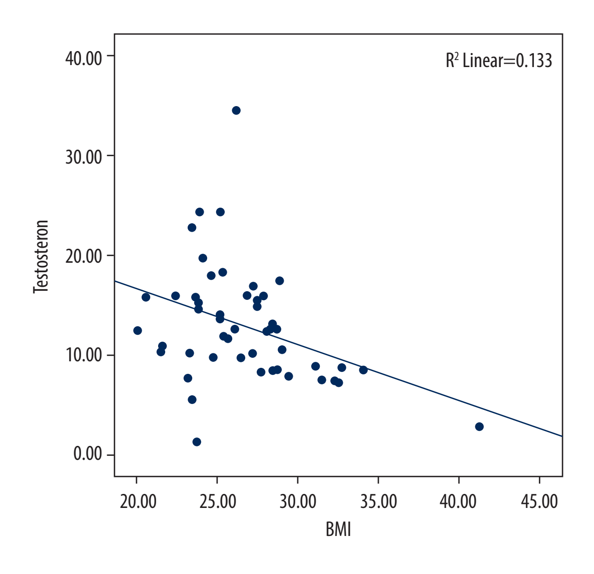

Figure 1. Correlation between body mass index (BMI) and serum testosterone levels in men with azoospermia. BMI demonstrated significant negative correlations with testosterone levels.

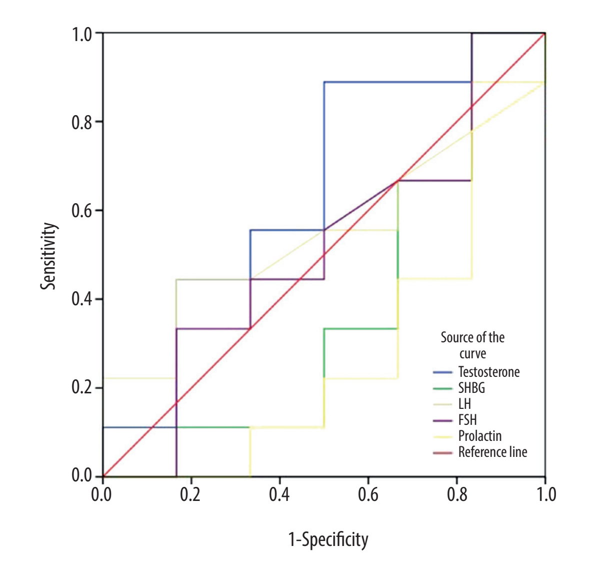

Figure 2. Receiver operating characteristic curve analysis for positive sperm retrieval.

Discussion

Although men with azoospermia are typically accompanied with metabolic syndrome and have increased body fat, the BMI range varies in such patients not only due to chromosomal abnormality but also because of environmental factors [13]. Increased BMI has been reported to be a measure and a factor for abdominal obesity, however it might also be elevated due to secondary issue such as androgen deficiency [13]. Obesity thus influences the endocrine system, it has an effect on the hypothalamus–pituitary–gonadal pathway, reduces a secretion of testosterone by increasing the secretion of cytokines [14], promotes insulin resistance [15], and reduces levels of gonadotropin [16]. According to our knowledge, only 2 reports have evaluated the association between obesity and positive TESE sperm retrieval in azoospermic male patients [11,17]. Both reports concluded that obesity did not influence sperm retrieval rate, suggesting that obesity in azoospermic men was not directly responsible for impaired spermatogenesis. Thus, with our study we tried to further assess the correlations between BMI and sperm retrieval with TESE and find correlation with reproductive hormone levels in men.

As mentioned, the results from our study confirmed the 2 previously published findings by Ramasamy et al. [11] and Iwatsuki et al. [17] showing that higher BMI patients do not

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Table 4. Values of ROC analysis to predict positive sperm retrieval with calculated AUC for measured hormones.

	AUC	95% CI	*P*-value
T [nmol/L]	0.648	0.345–0.951	0.346
SHBG [nmol/L]	0.370	0.049–0.692	0.409
LH [IU/L]	0.556	0.258–0.853	0.724
FSH [IU/L]	0.509	0.193–0.825	0.953
Prolactin [mIU/L]	0.269	0.000–0.547	0.141

ROC – receiver operating characteristic; AUC – area under curve; T – testosterone; LH – luteinizing hormone; FSH – follicle-stimulating hormone; SHBG – sex hormone-binding globulin.

have a lower probability for sperm retrieval. At this time, we need to mention that we did not differentiate between the patients with KS and patients without KS, because only 5 KS cases were included in our analysis. In this study, BMI of men evaluated with azoospermia only were investigated. It was demonstrated that the proportion of patients with positive retrieval rates ranges from 32% to 60%, irrespectively of BMI values (Table 2). Therefore, BMI could not predict the successfulness of sperm retrieval (P=0.169).

However, there is evidence that spermatozoa of obese males with higher BMI is of poorer quality compared to the semen in men with normal BMI [18,19]. For instance, in the research by Belloc et al. [20]. The authors found a correlation with the volume of retrieved semen and the influence of BMI on sperm count, concentration, and progressive motility. However, they did not find a correlation between sperm morphology and BMI. On the contrary, Relwani et al. [21] did not find any influence of BMI on sperm characteristics. There is a publication of a large meta-analysis including 5 studies and encompassing 4853 men by MacDonald et al. [22], who published the results on the obesity and male subfertility, and found no correlation with sperm characteristics. Sermondade et al. [23] published an updated study, including 13 077 patients from 21 studies, and their results demonstrated increased risks of azoospermia in overweight, obese, and morbidly obese males. In our study, different results were shown; thus, we propose to evaluate hormonal levels in such patients.

There are already quite a few reports on the relationship between BMI and hormonal levels in azoospermic patients. According to our study, males with higher BMI had a statistically lower testosterone levels than males with lower BMI (P=0.035). A similar result was observed by Iwatsuki et al. [17] and Fui et al. [7]. These findings point out the association between obesity with high BMI and androgen deficiency. A decrease in the secretion of testosterone is responsible for obesity. It was also shown that SHBG levels were measured as lower in more obese patients, but further analysis is necessary to confirm that. Thus, obesity with high BMI and androgen deficiency with lower testosterone levels form a self-perpetuating cycle [7]. It has been confirmed that in obese men, adipokines and estradiol (E2) reduce secretion of gonadotropin-releasing and gonadotropin hormones [24], which results in hypogonadotropic hypogonadism. Gonadotropin secretion is regulated by negative feedback factors such as inhibin B and testosterone that come from the hypothalamus–pituitary–gonadal axis. In azoospermic men, because of testicular dysfunction, decreased production of inhibin B and testosterone factors leads to hypergonadotropism. However so far, no reports have evaluated the correlation between gonadotropin secretion and obese males with elevated hormonal levels. In the current study, an association between BMI and gonadotropin levels from patients' serum were investigated, but interestingly did not prove any correlation. The reason for this is unknown. For instance, Iwatsuki et al. [17] found a negative correlation, suggesting that in obese men the hypothalamus–pituitary–gonadal pathway and feedback loop is impaired when comparing to lean men. This might be due to differences in levels of adipokine between obese and non-obese men or any other endocrine factors that might affect the secretion of gonadotropin. These suggestions contradict the findings of a large study by MacDonald et al. [22], which found a negative association for testosterone, free testosterone, and SHBG [19,25] with increased BMI. Similarly, another study found that increased BMI decreased the level of testosterone, SHBG, FSH, and inhibin B, but increased the level of free androgen [10].

The current study did not evaluate the outcomes of azoospermic couples who underwent TESE–ICSI. BMI and baseline hormonal levels were the only parameters that were evaluated for the influence on male sperm retrieval. Evidence varies regarding male obesity and subfertility. According to the literature, obesity increases DNA damage of sperm, decreases mitochondrial activity, increases oxidative stress in testis, and shows poorer TESE outcomes. Conflicting evidence has been presented in studies discussing the influence of BMI on sperm quality, but was related to decreased ejaculate volume, lower sperm concentration, and increased DNA damage [4,18,26]. Other studies reported no correlations between BMI and sperm parameters [22]. A meta-analysis [27] demonstrated that obesity in males had no negative effect on clinical pregnancy and live births. As aforementioned, the current study did not investigate the effect of overweight status on sperm parameters after TESE or ICSI outcomes. However, hormonal levels, namely FSH, LH, testosterone, SHBG, and prolactin, did not correlate and could not significantly predict TESE outcomes. However, Cissen et al. [28] developed a prediction for positive results obtaining sperm with TESE. A retrospective, multicentre study,

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including data from 1371 TESE procedures, found that older males, higher testosterone levels, and lower FSH and LH serum levels were predictive for successful retrieval of sperm [28].

A main limitation of our current study was its retrospective analysis. It included a relatively low sample size of azoospermic patients and was designed as a single-center study. Moreover, we did not divide our patients into patient groups with or without KS, and only included azoospermic patients who underwent TESE without a reference to the etiology of azoospermia. With the retrospective design, the study also lacked an evaluation of E2 levels in obese and infertile men. High levels of E2 inhibit testosterone biosynthesis, in addition to amplitude reduction of cycle secretion of LH and FSH by hypophysis. The benefits of inhibitor aromatase usage when a patient shows a relation between testosterone and E2 lower than 10 are already known. The normalization of this relation can improve significantly, and it has been extensively proven that E2 levels are significantly increased in overweight and obese patients. Lastly, we did not investigate how increased BMI could have altered semen quality as this has been observed in other studies.

Conclusions

In conclusion, the association between higher BMI and positive sperm retrieval in infertile males were evaluated, but we found that obesity with high BMI had no effect. This study also demonstrated that hormonal levels could not predict positive TESE outcomes in male patients with azoospermia. However, a negative association between BMI and serum testosterone levels was observed, which could potentially implicate the influence of obesity on fertility of men.

Conflicts of interest

None.

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