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Decline in semen parameters from 2000 to 2016 among Bangladeshi men attending a tertiary care hospital

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Abstract

Introduction: The objective of this study was to analyze longitudinal changes in sperm parameters of Bangladeshi men. We hypothesized that semen parameters declined for this population.

Methods: We retrospectively analyzed semen data from men aged 18-44 years who sought care for general sperm quality or updates on fertility status at an infertility clinic in Dhaka, Bangladesh, from January 2000 to June 2016 (n = 13,853). Samples with incomplete data were excluded (n = 143). The WHO normal criteria and semen analysis procedures were used to evaluate parameters of the remaining 13,810 specimens. Samples with missing values on sperm concentration (n = 6187) were excluded from concentration analyses. Age and duration of abstinence at testing were recorded and adjusted for. Data were imported into SAS® 9.4 statistical software. Temporal significance was investigated using one-way ANOVA for mobility parameters and Chi-square test for raw concentration. Logistic regression analysis investigated the effects of confounders on azoospermia and raw concentration, while median regression modeling adjusted confounders for concentration, total motility, and rapid linear (RL) motility. Results: Age distribution was significantly correlated with annual parameter changes (concentration, total motility, and RL motility; P = 0.0001). Adjusted total motility and RL motility declined by 20% from their maximum values to end of the study (P < 0.0001). Raw concentration lacked clear trends and was unaffected by adjustment. Azoospermia increased by 18% between the 2000-2010 and 2011-2016 participants (odds ratio = 0.16 [0.14–0.16]).

Conclusion: In agreement with the hypothesis, Bangladesh males attending this clinic have experienced decline in semen parameters (total motility and RL motility) and increased frequency of azoospermia.

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Introduction

Changes in sperm quality indicators – sperm count, percentage of sperm motility, sperm density, and normal sperm morphology – have been explored globally over the last two to three decades.[1] Longitudinal and cross-sectional studies in Israel showed that the average sperm parameters in the nation have dropped over the last 25 years, with significant decrease of total motile sperm counts per ejaculate and percentage motility.[2] A retrospective analysis of semen in healthy Belgian men identified a significant decrease in sperm motility and increased in immotile sperm from 1997 to 1995.[4] Another study highlighted Japan and Denmark as having the lowest semen indicators in the world. This study, as well as a review on all sperm-density studies done from 1934 to 1996, concluded that while geographical location of nations may result in regional dispersity for semen quality, parameters have declined overall in many parts and in both regions.[5][6]

While semen data are available for most of the global communities, South-Asian countries lack research studies. A 2007 study conducted by the infertility unit at the Bangabandhu Sheikh Mujib Medical University found that almost 62% of couples attending the infertility unit faced primary infertility, while 39% experienced secondary infertility. Semen analysis results from this study indicated that among the male partner, oligospermia, or sperm concentration of < 20 × 106 sperm/mL, asthenospermia, or sperm concentration of < 20 × 106 sperm/mL, and morphological defects in the semen were increased.[7][8][9] In 2010, an estimated 3 million Bangladesh couples were infertile, and for 60% of those couples, the male partner was responsible.[9]

The objective of this study was to analyze changes in semen quality of a subset of the Bangladesh male population attending an infertility clinic between 2000 and 2016. Through this study, we hope to evaluate whether there is an observable decline of semen parameters in Bangladesh males, as determined by trends recorded for motility, morphology, and concentration. Based on trends observed in the global community, we hypothesized that there is a temporal decline in semen parameters for the study population.

Materials and Methods

The Ethical Review Committee of the Diabetes Association of Bangladesh approved the protocol of this study (Memo no: BADAS-ER<small>REC</small>16/0091). Participants were required to provide signed consent for their analysis results to be included in the study database and received signed analysis reports for their personal records.

Study population and participants

Data collection for this study was conducted in the Centre for Assisted Reproduction (CARE) at the Bangladesh Institute of Research and Rehabilitation for Diabetes, Endocrine and Metabolic Disorders (BIRDEM) from January 2000 to June 2016. CARE is one of the largest infertility clinics in Bangladesh and a major center for infertility referrals. A majority of patients at CARE reside in Dhaka, Bangladesh, but services are also provided to patients from other regions in the country and those visiting from overseas.

The overall study population consisted of 13,853 participants. A total of 143 participants were excluded from analysis due to incomplete data; 6187 datasets did not have quantitative sperm concentration values and were excluded from raw concentration analyses. Data is not available for 2006 and thus data from 2000 to 2005 and 2007 to 2016 is included.

Semen analysis procedures and calculations

All semen analyses were conducted by a single laboratory technician who used the same type of laboratory materials for the entire duration of the study. The methods used for semen analysis are outlined in the WHO’s Laboratory Manual for Examination and Processing of Human Semen (4th and 5th ed.)([10]) Participants provided semen samples through masturbation or intercourse at the on-site masturbation. 3-5 days of abstinence before sampling was advised, and duration was recorded. Samples were liquefied for 30 min and then gently vortexed before 2-3 drops were extracted. Drops were loaded into a Makler counting chamber (0.01 mm × 0.01 mm grid) and observed under a phase-contrast microscope at ×10 magnification. The laboratory technician determined morphology by adjusting the microscopic view to a higher magnification so that physical characteristics of the spermatozoa were visible. The count of sperm that were not in the normal tadpole shape or swim abnormally were considered to have abnormal morphology. The percentage of sperm with abnormal morphology was estimated based on the magnification of the grid proportional to the overall 10 × Makler chamber grid.

Statistical analysis

The dataset (n = 13,810) was imported from an electronic database into SAS® 9.4 statistical software (Cary, North Carolina) for analysis. Because normally tested show the random parameters to be severely skewed, median (interquartile range) was reported for parameter baseline. Age, duration of abstinence, and liquefaction were reported as mean ± standard deviation. Significance between annual means and medians was found through parametric one-way ANOVA and non-parametric Chi-square distribution analysis. P < 0.05 was considered statistically significant. Parameters with missing quantitative concentration data or incomplete records (n = 6187) were excluded from the logistic regression analyses of this variable. To adjust for confounding, concentration, motility, and RL motility were adjusted by median age of patients at the time of testing and median duration of abstinence before testing through median regression modeling.

Due to the incompleteness of the quantitative concentration dataset, qualitative azoospermia concentration diagnosis was evaluated in logistic regression analysis since it is definitive. Other qualitative diagnoses such as normospermia and oligoasthenoteratozoospermia were not used in analysis because the WHO criteria changed between 1989 and 2010 affected diagnosis frequencies. [11] To account for the criteria change, concentration diagnosis frequencies were adjusted separately based on the WHO 2010 criteria. Morphology reporting in the dataset was inconsistent with the WHO grading criteria and therefore omitted from analysis beyond baseline.

Results

Baseline semen characteristics, age, and duration of abstinence of the study population (n = 13,810) are shown in Table 1. The average age of participants was 35 ± 6.6 years, and age distribution was significantly correlated with annual parameter changes (P < 0.0001) [Table 1]. Duration of abstinence (P = 0.05) and liquefaction (P = 0.07) remained unchanged in annual comparisons, both averaging around 5.2 ± 3.8 days and 1.0 ± 0.8, respectively. All semen parameters (concentration, total motility, RL motility, and normal morphology) appeared to vary...
drastically (P < 0.001) over time. Total motility was severely reduced from the beginning of the study to the end, where the peak median of 50% decreases to consistent 30% median motility in the study population. RL motility was similar, where the median became a consistently low value of 10% from 2011 to 2016 (Table 1).

To control for potential confounding from age and duration of abstinence, adjusted medians for total motility and RL motility were estimated using median regression models. Unadjusted versus adjusted p-values for quantitative concentration and both motility variables are shown in (Figure 1). The unadjusted median graphs simply indicate annual median, while adjusted median graphs control for age and abstinence time. Crude total motility medians decreased temporarily from 2000 to 2016, whereas the adjusted medians stayed relatively flat over the same period. There was a significant trend for decreasing total motility, RL motility, and both clinical diagnoses (Table 1).

Concentration diagnosis frequencies for normozoospermia, azoospermia, and oligozoospermia (severe, moderate, and mild) are impacted by the change in WHO parameters (Table 2). The WHO 1999 parameters apply to data from 2000 to 2010, while 2010 parameters were standardized for the duration of 2011-2016. With this change, normozoospermia reflected as 220 × 10^6/mL to >15 × 10^6/mL. Oligozoospermia parameters decreased to values outlined in (Table 2). Because frequencies were adjusted to the WHO 2010 parameters, normozoospermia frequency increased from 69.5% to 68.1% of the study population, while both oligozoospermia diagnosis decreased from 4.4% to 3.1%. Azoospermia and moderate and severe oligozoospermia remained constant between crude and adjusted calculations for median years of age (25 years) and median duration of abstinence (5 days). The odds of an individual developing azoospermia with increasing age was 0.16 times greater than the risk for younger individuals while adjusting for age and duration of abstinence (odds ratio, OR = 0.16 [0.14-0.18]). P < 0.001 (Table 2).

The OR for males at median age to have normal sperm is 1.02 times more likely (P < 0.001) as opposed to males of other age groups. Holding age constant, OR for males who have abstinence for 5 days and present normal sperm concentration in semen analysis is 1.02 times higher (P < 0.005) than men who have abstained <5 days. Coupled with abstinence, males of median age are 2.2 times more likely to have normozoospermia compared to males of other ages (P < 0.001).

Discussion

Our study shows that for Bangladesh men, there has been a decline in total motility and RL motility on semen analysis from 2000 to 2016, and the trends and magnitude of decline are more evident upon adjusting for age and duration of abstinence. The incidence of azoospermia also increased when adjusted for age and duration of abstinence.

Interestingly, the overall frequency of normozoospermia increased upon adjusting for the WHO 2010 concentration diagnosis. Though this finding does not indicate that the actual frequency of fertile males increased. The criteria changed from 220 × 10^6/mL to >15 × 10^6/mL, and may be solely responsible for the shift in normozoospermia because many participants qualified for the new criteria. The median trend between the WHO 1999 and 2010 parameters, frequencies remained consistent with the expectation of a more reliable comparison. The increase in frequency of azoospermic participants from 10.9% of the 2000–2010 subset to 14.9% of the 2011-2016 indicates a slight decrease in concentration across the population. This finding is consistent with past meta-analyses from international literature that describe semen concentration trends over the last few decades.[1] More recent studies on concentration trends also show decreased parameters in countries not previously studied, such as Israel, India, and New Zealand.[12][13] However, adjusted and unadjusted new concentration analyses do not indicate a clear trend of decline in concentration for our study population.

The effect of risk factors on semen quality in our study participants may not be conclusive because there was only a clear trend of decline in motility. However, it is important to acknowledge risk factors as potentially associated with semen quality. Literature extrapolates that there is a potential for decline in semen quality due to endocrine disruptor exposure, which is associated with increased industrialization, especially in developing countries.[1] Regions of widespread industrialization generally experience higher rates of oligozoospermia than other areas.[14] Occupational and environmental exposure to toxicants also alters sperm counts industrially and may have degenerative effects directly on reproductive organs or hormonal balance that is crucial for growth, sexual development, and physiological functions.[15][16][17]

The longevity of data collection provide strength to this study and show that studies of this nature are feasible despite Bangladesh being a low-resource setting. Moreover, semen analysis readings and methodology should be consistent because the analyses were conducted by a single observer who used the same type of laboratory materials for the entire duration of the study.

Conversely, several improvements were needed in the study design and dataset. Although it was previously described that normozoospermia and acrospermia increased with time, characterization of the diagnoses was limited by the absence of raw concentration readings from these data. If these data were available, the quantification for oligozoospermia stratification could be described more accurately. It is also important to note that sample size did not remain consistent throughout the study. There was a fluctuation of participants during the second half of the study. Therefore, the increase in frequency of acrospermia may have offset the expected decrease in normozoospermia. Moreover, there appeared to be a decrease in median parameters between 2000 and 2004, which may or may not be associated with the reduced sample size compared to post-2008 data. Association could be explained for this observation because the parameters would not have been affected by the WHO criteria changes until 2010, but sample size is not a conclusive factor because none of the factors other than mobility showed association.

Among the correlated dataset, outliers who do not reside in Dhaka or have drastically different life course exposures are not eliminated. Therefore, the effect of confounding due to influential risk factors (i.e. exposure to toxins, preceding health conditions, environmental factors, and drug use) is not clear. All the study participants were from a single clinic, thus limiting generalizability of our results. Moreover, we were unable to follow single participants over time and determine whether multiple datasets represented a single participant due to a lack of patient identifiers. Significant measurement bias exists where the WHO 2010 semen parameters have been deemed as unreliable from emerging studies because they are determined by the world population at large, thus potentially not providing a true measure for the burden of infertility as differentially regional.[10] An absence of raw concentration counts for 618 participants makes it difficult to assess whether the laboratory technician’s classification of oligozoospermia versus normozoospermia is consistent over time. Although consistently reported by a single technician, laboratory error could result in the misclassification of semen parameters over time.

This study provides a rationale for conducting observational studies on male infertility in the context of Bangladesh and neighboring South Asian countries. As we established the trend of decline in semen quality, there may be a need for improvement of the WHO parameters to provide a clearer definition for semen quality. Literature extrapolates that there is a potential for decline in semen quality due to endocrine disruptor exposure, which is associated with increased industrialization, especially in developing countries.[1] Regions of widespread industrialization generally experience higher rates of oligozoospermia than other areas.[14] Occupational and environmental exposure to toxicants also alters sperm counts industrially and may have degenerative effects directly on reproductive organs or hormonal balance that is crucial for growth, sexual development, and physiological functions.[15][16][17]

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