



Original Article

Comparison of semen analysis by manual and automated method

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ABSTRACT

Background: Semen analysis is used to evaluate male fertility. The aim of this study was to compare the results of semen analysis using manual method and automated sperm analyzer.

Materials and Methods: This was a comparative study of 50 cases of semen analysis done in the Department of Pathology at the B.P. Koirala Institute of Health Sciences from March 2009 to March 2010. The automated sperm analyzer did not show the WHO parameters of patients who had functional sperm count (FSC) less than five hundred thousand (500,000). Semen analysis of each of the case included in the study was done by manual and automated method (using SQAI-P analyzer).

Results: Out of 31 patients, the mean age of the patients was 28.56 years with youngest patient of 20 years and eldest of 45 year. Sensitivity and specificity was 100% in analysis of sperm concentration by both the methods. Sperm motility analysis showed 100% sensitivity and 81.81% specificity. Sperm morphology analysis showed 100% sensitivity and 34.48% specificity.

Conclusion: It was observed that the automated method is much quicker and precise than the conventional, manual method for semen analysis.

INTRODUCTION

Semen analysis is the first diagnostic tool to evaluate the male factor in an infertile couple. Conventional manual semen analysis is the routine method in most laboratories, but this method suffers from subjectivity and lack of standardization. Despite widespread use, the test is not perfect in universally predicting the exact fertility status. Manual method is widely used in most laboratories to evaluate semen volume, sperm count, motility and morphology. Automated method

of semen analysis when compared to manual method is not only quicker and precise, parameters such as sperm concentration, motility and normal morphology correlates well.¹

Introduction of automated sperm analyzer demonstrated that they could be alternative to manual method of semen analysis and that can promote laboratory standardization. Modern automated techniques are capable of analyzing sperm motility and kinetics with greater accuracy even in presence of round cells and debris.²

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MATERIALS AND METHODS

All the semen samples of the male partners attending outpatient department of Obstetrics and gynaecology of BPKIHS during one year period (March 2009 to February 2010) were included in the study. Permission was obtained from ethical review committee. Brief history was taken from the patients regarding personal habits (smoking and eating habits). It was found that 15 people were smokers and rest were non-smokers. Eating habits of all the patients were almost the same. Samples were collected in the vicinity of the laboratory in specified containers except for 10 samples which the patients collected at home and brought for analysis. The following physical parameters were recorded: appearance, liquefaction, volume, viscosity and pH. The samples were then separated in two containers. One sample was processed manually (conventional method) and the other in the automated sperm analyzer.

Manual method: In this method microscopic examination was done and the following parameters were recorded in each sample: sperm count was done in RBC square of the Naeubauers counting chamber (million/ml). Sperm motility was assessed as actively motile, sluggishly motile and non-motile. A sample was regarded to be having sperms with normal motility if the value was more than 50%. Sperm morphology was assessed as normal or abnormal after staining the slides by May Grunwald's Giemsa and Papanicolau stain. Sperms having more than 30% normal morphology were regarded as normal.

Automated Method: The following WHO parameters were analyzed using SQAI-P analyzer: sperm concentration, motility, and morphology. The above mentioned three parameters were compared with manual methods. Following additional parameters recorded by automated analyzer which were not assessed by manual method: motile sperm concentration, functional sperm concentration and sperm motility index. The automated sperm analyzer did not give the WHO parameters of the patients whose functional sperm count were less than 500000/ml.

Statistical analysis was performed using EXCEL wherever applicable. Sensitivity and specificity was calculated and compared.

RESULTS

A total of 50 patients were analyzed during the study period. Age of the individual ranged from 22 to 45 years with the mean age of 28.56 years. Seventy eight percent (n=39) were between 20-30 years. Nineteen patients had functional sperm count of less than 500,000/ml and WHO parameters of samples from these patients were not recordable by automated analyzer. Therefore the parameters of these samples could not be compared with manual method. Thus statistical analysis of only 31 samples was possible.

Table 1: Distribution of patients according to sperm motility

Percentage (%)	Manual Method	Automated Method
1-10	00	00
11-20	05	06
21-30	14	07
31-40	03	11
41-50	07	02
51-60	00	03
61-70	02	01
71-80	00	01

Among these patients 16 patients had oligospermia. Semen analysis by the manual method showed that the sperm concentration was in the range of 11-20 million/ml in majority of patients (n=10), followed by 6 patients having sperm concentration in the range of 1-10 million/ml. None of the patients were detected with sperms concentration in the range of 71 - 80 million/ml by manual method in comparison to 3 patients by automated method. In automated sperm analyzer 10 patients had oligospermia which showed sperm concentration in the range of 21-30 million/ml and 31-40 million/ml in 5 patients each. Fifteen patients had normal sperm concentration (> 20million/ml) by the manual method and twenty-one patients by the automated method respectively.

Among 31 patients 29 had less than 50% motile spermatozoa according to manual method in comparison to 22 in automated method. Percentage of motility among study group is listed in Table 1.

Semen analysis by the manual method showed that majority of patients (n=15) had normal sperm morphology which ranged from 21-30%. Eight patients had sperms with normal morphology (>30%) by the manual method and 8 patients had 11-20% spermatozoa with normal morphology.

Semen analysis by the automated method showed that 11-20% normal sperm morphology was observed in 13 cases. Nine patients had sperms with normal morphology (> 30%) by the automated method and 9 patients had normal sperm morphology ranging between 21-30%. The personal habits of the patients did not seem to have significant effects as smokers and non-smokers did not have wide range of differences in the semen parameters.

In analysis of sperm concentration by both the methods, the sensitivity and specificity was 100%. Sperm motility analysis showed 100% sensitivity and 81.81% specificity. Sperm morphology analysis showed 100% sensitivity & 34.48% specificity. Thus, in analysis of sperm concentration by both the methods, the accuracy was 100 % Sperm motility analysis showed 87.09 % accuracy. Sperm morphology analysis showed 38.70 % accuracy. The

mean value by manual method was $28.80 \pm 18.87\%$. The coefficient of variation was 65.55% by the manual method. The mean value by automated method was $33.54 \pm 20.20\%$. The coefficient of variation was 60.23% by the automated method.

DISCUSSION

In this study it was observed that the maximum number of patients were in the age group of 20-30 years with a mean age of 28.56 years. The youngest patient was 20 years old and the eldest was 45 years old. This correlates with the findings of Jensen TK et al who also observed that the patients were in the age group of 20-45 years.³

In our study it was seen that sperm concentration, motility and morphology was directly proportional to the age of the patients. These parameters were decreased in patients who were comparatively elder. One patient 20 years of age had sperm concentration of 70 million/ml (manually) and 79 million/ml (by automated analyzer) compared to a patient aged 32 years whose sperm concentration was 7 million/ml (manually) and 9 million/ml (by automated analyzer). Similarly, sperm motility of the youngest patient (20 years) was 50% (manually) and 52% (automated method) respectively compared to the elder patient (32 years) whose sperm motility was 15% and 14%. Sperm morphology also showed a decrease with increasing age. The younger patient had 30% (manually) and 36% (automated method) normal sperm morphology compared to the elder patient who had 20% and 16% normal sperm morphology. Eskanzi et al. and Centola et al. also observed that sperm concentration, motility and morphology decreased with the advancing age of the patients in their study.^{4,5}

In our study lengthy sexual abstinence was found to affect all semen characteristics. In a patient with a sexual abstinence of 5 days semen concentration and total sperm count showed mild increases (20 million/ml and 24 million/ml) whereas motility (35% and 28%) normal morphology (20% and 19%) decreased significantly. Pellestor et al also concluded that length of sexual abstinence affect semen parameters.⁶

In our study we observed that sperm motility results of automated analyzer and manual method correlated with each other but not as strong as sperm concentration results. Correlation was seen between the results of automated analyzer and manual method in case of sperm motility in patients who had sperm motility ranging from 11-20%. The automated analyzer showed 6 patients and manual method showed 5 patients who were having sperm motility in this range. The highest percentage of progressive motile sperm was observed in one patient (75% motility) by the automated analyzer. Two patients had progressive motile sperm in the range of 61-70% by manual method and 1 by automated analyzer. In analysis of sperm motility by manual

method the sensitivity was 100% and 81.81% specificity. Thus, the specificity of both the methods was less compared to sensitivity in case of sperm motility analysis. Both the methods showed 87.09% accuracy in analysis of sperm motility. Similar correlation was seen in a study done by Komori et al, in their study "Comparative study of Sperm Motility Analysis System (SMAS) and conventional microscopic semen analysis" in which sperm motility percentage obtained by Sperm Motility Analysis System and manual analysis on WHO guidelines were strongly correlated.⁷

A strong correlation was seen between the results of automated analyzer and manual method in case of sperm count in patients who had sperm concentration ranging from 1-10 million/ml. The automated analyzer showed 5 patients and manual method showed 6 patients who were having sperm concentration in this range. The patients having sperm concentration ranging from 1-10 million/ml were in the age group of 21-37 years. In analysis of sperm concentration by both the methods (automated analyzer and manual method) the sensitivity and specificity was 100%. Both the methods were very accurate in analysis of sperm concentration (100%). A similar agreement was seen in the study by Komori et al, in year of 2006, in which a new system for sperm analysis, SMAS, was compared with manual semen analysis based on WHO guidelines.⁷

A good agreement was seen between the results of sperm concentration reported by the SQA-V automated analyzer and those obtained manually in a double-blind prospective study "Automation is the key to standardized semen analysis using the automated SQA-V sperm quality analyzer" done by Agarwal et al, in year of 2007, of semen samples donated by fifty healthy men.⁸

Our study shows that sperm concentration results provided by the SQA IIC- P are in correlation with manual results, and our findings are similar to those reported earlier by Agarwal et al, who also observed that the sperm concentration results obtained by the SQA - V are in agreement with manual results.⁸

In the present study it was seen that the automated method of semen analysis is more reliable and fast compared to manual method. A study conducted by Goulart AR concluded the same.⁹

Samples were collected in the laboratory in containers except for 10 samples (which were collected at home). A study by Lichet et al. stated that the semen sample can be collected in office and at home. There was no statistically significant difference in sperm parameters according to the site of collection.¹⁰

According to Vogt et al. there was no significant effect of smoking on sperm quality. They reached this conclusion

after analyzing the sperms of 150 smokers, 37 ex-smokers and 52 never-smokers. In the present study the similar things were noted.¹¹

We can therefore say that for analyzing sperm concentration, the SQA II C-P can be used in place of manual analysis and vice versa. Study "Automation is the key to standardized semen analysis using the automated SQA-V sperm quality analyzer" was done by Agarwal et al, in year of 2007. The automated motility readings when compared by those obtained manually showed good agreement and only marginal differences were found. Manual analysis of motility may be overestimated. Agarwal et al also stated that scoring of motility manually is prone to overestimation. This may be attributed to the fact that manual assessment of motility is subjective and generally overestimated because of the attraction of the eye to movement.⁷

The assessment of morphology showed high sensitivity (100%) for identifying normal morphology compared to specificity which was only (34.48%). The accuracy in sperm morphology analysis was 38.70%. Thus, semen analysis by both the methods has low accuracy in sperm morphology analysis. In a study done by Agarwal et al, percent normal morphology by automated method showed a sensitivity of 89.9% and a specificity of 50% when compared with the average manual results.

The SQA IIC- P only provides percent normal morphology results without quantifying specific abnormalities. As such, it is limited when compared with manual methodology where morphological defects need to be identified and quantified. Statistically, the agreement between the percent normal morphology readings of the SQA IIC- P versus manual data is moderate. The SQA IIC- P shows high sensitivity to accurately detect abnormal morphology and greater precision and speed compared with the manual method for determining percent normal morphology. Therefore, although limited, the SQA IIC- P is useful as a screening tool for distinguishing between samples with normal versus abnormal morphology.

CONCLUSION

Automated method of semen analysis is a quicker method for the assessment of male infertility. Sperm concentration analysis by both the methods has 100% sensitivity and specificity. Motility is often over estimated by manual method. Automated sperm analyzer only provides percent normal morphology results without quantifying specific abnormalities. As such, it is limited when compared with manual methodology where morphological defects need to be identified and quantified. Thus, automated sperm analyzer can be used interchangeably with manual semen analysis for examining sperm concentration, motility and morphology.

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