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Original Article

Comparison of fully automatic analyzer and manual measurement methods in sperm analysis and clinical affect

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ABSTRACT

Aim: To investigate the clinical effect of the computer-aided sperm analyzers (CASA) by comparing the low sperm concentration semen samples evaluated by CASA with the sperm count performed on Makler Counting Chamber (MC) as a manual method.

Methods: Semen samples were taken from184 patients coming to our clinic were evaluated with CASA (SQA-V Gold sperm analyzer, MES Medical Electronic Systems Ltd. Caesarea Industrial Park, IL 3088900, UK) and MC (Makler Counting Chamber, Sefi-Medical Instruments ltd., Haifa, Israel). Samples were divided into two groups as samples containing sperms and samples without sperms, according to the CASA results.

Results: There was a very high correlation between the two measurement methods (rho = 0.982) and regression analysis formula was y=1.042x-0.104. No sperm was detected in CASA in any of the samples identified to have no sperm in MC. However, when patients who were identified with no sperm in their CASA measurements (n=51) were analyzed with MC, 29 patient samples (56.9%) had an average of 0.23±0.35 $x10^6$ /mL sperm.

Conclusion: CASA's used in routine semen analysis provide a great convenience in measuring sperm count, compared to manual methods and provide highly correlated results. Manual verification of samples can be recommended since the samples diagnosed with azoospermia provided different results with a manual method in our study.

Keywords: Infertility, azoospermia, computer-aided sperm analyzers, method comparison.

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Introduction

Infertility is a potentially life-changing diagnosis for couples who are trying to conceive. It can be defined as the condition of not being able to conceive despite regular unprotected intercourse for at least 12 consecutive months [1,2]. Male factor is suspected in approximately half of the cases [3]. The most common and precise diagnostic step in male infertility is semen analysis. Semen analysis helps to investigate male infertility and provides basic data on the contribution of the male factor for an infertile couple [4]. It also

helps to identify reversible medical conditions that can affect fertility [5]. The subjectivity of the evaluation and interpersonal variation of sperm concentration and motility are the most significant limitations of this technique [6]. The diagnostic and prognostic effectiveness of semen analysis is correlated with strict compliance to the guidelines recommended by the World Health Organization. Neubauer slide (NS), Makler counting chamber (MC), spectrophotometric methods and fully automated (or computer-aided) sperm analyzers (CASA) can be used for sperm counting [3,6,7]. Spermatozoa motility, morphology and concentration can be analyzed simultaneously on modern CASA systems but such assessments are not as reliable as traditional methods (such as NS or MC) [8,9]. Computerized systems such as CASA, are more convenient for analyzing complex parameters such as sperm motility and offer an objective and fast method for semen analysis. CASA uses a microscope, camera and computer software for sperm motility analysis [7,8]. In traditional semen analysis methods, sperm cells in the semen placed on a slide such as NS or MC are counted on the microscope [10]. The complete absence of sperm in semen analysis is defined as azoospermia [6,11]. Azoospermia is the definition of the semen rather than the basis of diagnosis and treatment or the cause of sperm absence [10]. However, azoospermia is not the case even if there is a single sperm in the semen. Even the case of a single sperm can affect the treatment and this becomes a condition of subfertility rather than infertility [12]. Although seemingly simple, the diagnosis of azoospermia is complicated by many factors, such as significant errors associated with counting a small number of spermatozoa, a large number of microscopic fields to be examined, and the difficulty of examining debris-loaded sperm pellets. It is recommended to examine fixed but non-centrifuged samples to overcome these situations [10].

In the widely used CASA, patients with a very low amount of sperm in their semen who are diagnosed with azoospermia is a frequent situation. This study investigates the clinical effect of CASA by comparing the low sperm concentration semen samples evaluated by CASA with the sperm count performed on MC.

Materials and Methods

The study was approved by Bolu Abant Izzet Baysal University, Clinical Research Ethics Committee, decision number 2020/60, dated 07/04/2020. Semen samples were taken from184 patients, who applied and gave written consent to the male infertility laboratory. The samples were macroscopically confirmed to be semen samples. Semen samples were obtained through masturbation by dry method in sterile containers. Samples were analyzed after liquefaction in the incubator (Heraeus, Thermo Electron Corporation, Langenselbold, Germany) for about 30 minutes at 37°C and thorough mixing. Samples which were less than 1 ml and more than 4 ml, samples that were not treated with liquefaction within 30 minutes and samples showing hyperviscosity were excluded from the study [10,13-15]. Fresh semen samples were evaluated without dilution and processing on the samples included in the study. All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later ammendense or comparable ethical standards.

Semen samples were analyzed with SQA-V Gold sperm analyzer (SQA-V Gold sperm analyzer, MES Medical Electronic Systems Ltd. Caesarea Industrial Park, IL 3088900, UK)

for CASA method, and MC (Makler Counting Chamber, Sefi-Medical Instruments ltd., Haifa, Israel) for manual method. Samples were taken blindly from the same pool to be evaluated blindly at the same time and transferred to the device for CASA and to the microscope with MC for manual method. In sperm analysis, the sperm concentration was determined as 10^6/mL. Motility in sperm analysis was evaluated as; progressively motile, non-progressively motile and immotile. Motility rates were given as a percentage of total sperm count. Samples were divided into two groups as samples containing sperms and samples without sperms, according to the CASA results. The manual microscopic method was performed in compliance with the standard protocol in the WHO 2010 guideline [6,10].

Automated analysis was performed by using the laboratory-based CASA system, SQA-V Gold sperm analyzer. Automatic semen analysis was performed in accordance with the protocol of the manufacturing company. In summary, samples were mixed thoroughly and inserted in the device's electro-optic chamber with a capillary for CASA counting. Sperm counts and movements are reported automatically after the data are analyzed through special algorithms in the computer system by translating the light-beams into electrical signals. The measurement range for the sperm concentration of the SQA-V Gold sperm analyzer was specified as 0-700 10^6/mL by the manufacturer.

Statistical analysis

Statistical analysis of the data was performed through the SPSS program (version 17.0, SPSS Inc., Chicago, IL, USA). The conformity of the numerical values to normal distribution was evaluated through the Kolmogorov-Smirnov test. Descriptive data were presented as mean ± standard deviation and median (1^{st} - 3^{rd} quarter). Wilcoxon-rank test was used in the comparison of dependent variables after it was determined that the data did not conform to normal distribution. McNemar test was used in the comparison of paired nominal data. Passing-Bablok regression analysis and Spearman correlation analysis were used to evaluate the compatibility between the two methods. $p<0.05$ was considered to be statistically significant.

Results

CASA and MC semen analysis results are shown in Table 1. The median sperm count values according to CASA and MC were 16.4

Table 1. Comparison of CASA and MC semen analysis results.

Parameters	CASA		MC		*p value**
	x̄±SD	Md (Q1-Q3)	x̄±SD	Md (Q1-Q3)	
Sperm number (x10^6/mL)	29±33.9	16.4 (0-46.8)	28±31.8	16 (0.2-40)	*0.066*
Immotile (%)	37.5±33.3	35 (0-68.8)	51.3±31.7	50 (25.3-79)	*<0.001*
Non-progressive motile (%)	10.7±10.9	10 (0-17)	19.2±17.1	20 (0.8-30)	*<0.001*
Progressively motile (%)	24.4±26.6	15 (0-44.8)	16.1±20.2	10 (0-30)	*<0.001*

x̄±SD: mean ± Standart deviation, Md (Q1-Q3): Median (1^{st}-3^{rd} quartile),: Wilcoxon signed-rank test. CASA: computer-aided sperm analyzers. MC: Makler Counting Chamber.*

(0.0 - 46.8) and 16.0 (0.2 - 40.0), respectively, and there was no statistical difference between the two values *(p = 0.066)*. There was a very high correlation between the two measurement methods *(rho = 0.982)* and the Passing-Bablok regression analysis formula was y = 1.042x-0.104 (Figure 1). Comparison of groups with and without sperm according to CASA and MC is shown in Table 2. No sperm was detected in CASA in any of the samples identified to have no sperm in MC. However, when patients who were identified with no sperm in their CASA measurements (n = 51) were analyzed with MC, 29 patient samples (56.9%) had an average (min-max) of 0.23 ± 0.35 (0.1-2.0)x10^6 /mL sperm.

Discussion

In our study where sperm concentrations in semen analysis were evaluated, the semen samples that arrived at our laboratory were examined with CASA and MC and being the diagnostic criteria in the diagnosis of azoospermia, only sperm concentrations were compared. In the measurements performed by CASA and MC, there was a high correlation with regards to sperm concentration. In 57% of the samples that would be diagnosed as azoospermia through CASA, the presence of sperm was detected through MC.

In studies have shown high correlations between CASA and manual methods with regards to sperm parameters [16,17]. Kose et al.

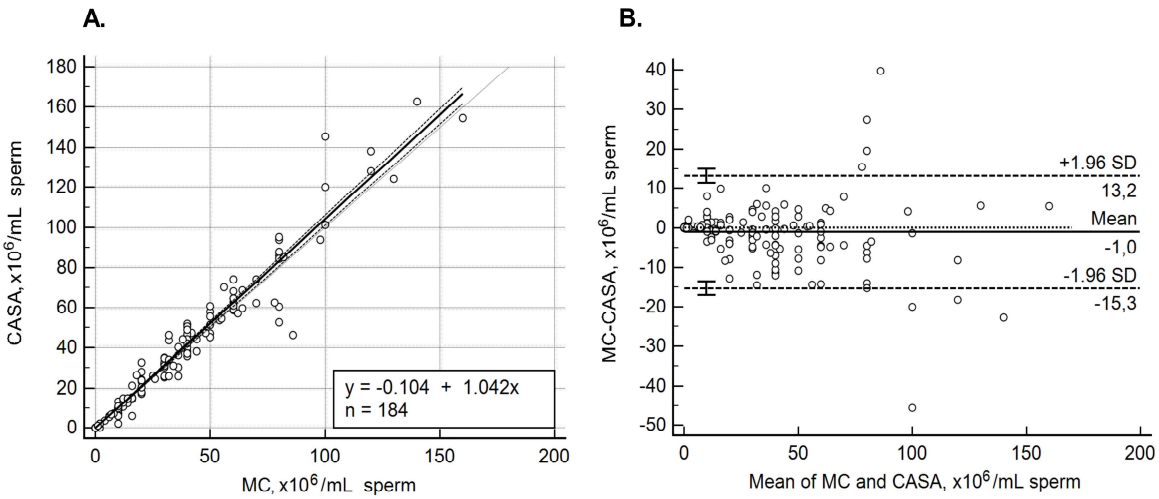

Figure 1. Passing-Bablok regression (A) and Blant-Altman (B) plots of CASA and MC sperm numbers.

Table 2. Comparison of the groups with and without sperm according to CASA and MC.

Parameters	No-sperm in MC	Sperm in MC	*P value**
No-sperm in CASA	22 (%43.1)	29 (%56.9)	*<0,001*
Sperm in CASA	0 (%0)	133 (%100)	

**McNemar's test. CASA: computer-aided sperm analyzers. MC: Makler Counting Chamber.*

found a correlation of 0.84 between methods in terms of sperm concentration [16]. Similarly, Lammers et al. showed that there was a 0.95 correlation between various CASA methods and manual method in terms of sperm count [17]. In parallel to the literature, a correlation of 0.98 was found between CASA and the manual method in our study.

Wang et al. [7] stated that sperm motility and morphology were associated with the time until

natural pregnancy, while sperm motility might be less predictive. Gnoth et al. [12] named the absence of sperm as azoospermia and classified the prolonged time to conceive as subfertility. In our study, sperm count was evaluated and it was observed that when the same samples were examined with two different methods with regards to azoospermia, sperms could be found in the samples that were reported as azoospermia with CASA when analyzed in detail with the manual method.

Bjorndahl et al. [18] prepared a guideline to journals for better sperm analysis evaluations. They developed criteria for evaluation of the general analysis, concentration, motility, morphology, sperm viability, other findings and analysis data in the evaluation of semen analysis. Our study fulfilled all seven criteria of sperm concentration evaluation in this guide.

As a result of the improvements in CASA systems in parallel with the development of hardware, the capability to gradually analyze the concentration of moving spermatozoa by using fluorescent DNA stain and a tail detection algorithm in addition to sperm concentration provided a superiority over manual methods in motility measurement [9,19]. However, in our study, different clinical findings were shown in semen analysis in very low concentrations which could not be measured by CASA. Detecting sperms in 57% of the samples that cannot be measured by CASA through manual evaluation demonstrates the importance of verification of sperm analysis in very low concentrations with a manual method, despite the current improvements and superiority of CASA over manual methods in certain parameters.

Although it is known that sperm parameters can be extremely variable even if the sperm analysis results of the same individuals do not differ significantly at various times, it is stated that sperm concentration analysis is one of the most reliable methods [20]. Variability is even more important at low sperm concentrations, and our study recommends the analysis of these low-concentration samples with more than one method.

Currently, in clinical laboratories worldwide, a semen analysis is still based on a manual microscopy method. However, some of the major disadvantages of this technique are that it is labour-intensive, subjective, laboratory-based, and time-consuming. Although partial automation of routine semen analysis with CASA is adopted in clinical use, it is reported in studies that it is still in the development phase to receive wider acceptance [15,19,21,22]. Our study on the other hand, emphasizes that a manual microscopy method is required clinically, specifically in approaching the azoospermia cases.

Conclusions

CASA's used in routine semen analysis provide a great convenience in measuring sperm count, compared to manual methods and provide highly correlated results. However, in the evaluation of azoospermia, it is known that the presence of even a single sperm in the sample may change the clinic and treatment. Manual verification of samples can be recommended since the samples diagnosed with azoospermia provided different results with a manual method in our study.

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