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Original Article

First-trimester Combined Screening for Trisomies 21, 18, and 13 by Three Closed Chemiluminescence Immunoassay Analyzers (an Experiment on Iranian Pregnant Women)

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ABSTRACT

Background: Pregnancy-associated plasma protein-A (PAPP-A) and free β-human chorionic gonadotropin (free β-hCG) as valuable biochemical biomarkers are used to screen down syndrome, Edwards syndrome, and Patau syndrome in the first trimester of pregnancy. Closed immunoassay analyzers are regarded as sophisticated platforms to measure biochemical biomarkers. This study compared the performance of three chemiluminescence analyzers when used for combined screening.

Methods: The present cross-sectional study was conducted on 371 pregnant women within the age range of 20-47 years during 11^{+0} to 13^{+6} weeks of pregnancy referring to Dena laboratory in Tehran, Iran, during July 2018 and August 2018 using random selection. The biochemical biomarkers of PAPP-A and free β-hCG were assayed on Cobas, Immulite, and Maglumi analyzers. Benetech software as a commercial screening software was used to calculate the risks of trisomy 21 (T21), trisomy 18 (T18), and trisomy 13 (T13). Deming regression, nonparametric spearman analysis, analysis of variance, and Chi-square test were performed to analyze the data.

Results: For the screening population, although the three systems well correlated to PAPP-A and free β-hCG, the values of Maglumi were slightly higher than those reported for Cobas and Immulite. The multiples of the median (MoM) of PAPP-A and free β-hCG had a significant correlation on three platforms. There were no significant differences between the calculated risks of T21, T18, and T13 on the three systems. The sensitivity for all systems was reported as 50%. In addition, specificity and negative predictive value (NPV) were higher than 99% and 95%, respectively. Positive predictive value (PPV) was reported as less than 50%.

Conclusion: The obtained results of the present study demonstrated that there were significant correlations between three different systems in terms of PAPP-A and free β-hCG values and MoMs. The sensitivity of all systems for all trisomies was 50%; however, the specificity of all systems was almost the same. The best PPV and NPV for T21 were on Cobas, Immulite, and Maglumi, respectively. The PPV and NPV of all systems for T18/13 were almost the same.

Keywords: Chorionic gonadotropin, First pregnancy trimester, Pregnancy-associated plasma protein-A, Risk assessment

Introduction

Pregnancy-associated plasma protein-A (PAPP-A), free β-human chorionic gonadotropin (free β-hCG), nuchal translucency (NT) thickness, and maternal age are important markers used for the most common chromosomal aneuploidies, including down syndrome (trisomy 21 [T21]), Edwards syndrome (trisomy 18 [T18]), and Patau syndrome (trisomy 13 [T13]). In addition, regarding the complexity of risk calculation, the use of software is necessary for screening programs (1). Furthermore, the first trimester of screening (FTS) offers better detection rates (DRs)

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than the second trimester of screening and let the clinicians have adequate time to take further action (2). Considering these important points and accessibility importance of diagnostic tests, the FTS has been known as the first line of trisomy screening for all Iranian pregnant women.

Human chorionic gonadotropin (hCG) has two kinds of subunits, namely α- and β-subunits, which non-covalently join to each other to form intact hCG. Actually, β-subunit spreads throughout the bloodstream as a non-biologically active form. In combined testing, free β-hCG is measured using capture and detection antibodies. Those binding with the α-subunit cannot be detectable by these antibodies. In fact, the levels of free β-hCG increase in T21 cases when the total hCG levels are still normal (3). In addition, free β-hCG levels in T18 and T13 reduce (4). Since intact hCG is unstable, it can be dissociated to α- and β-subunits.

The PAPP-A secreted from trophoblasts is a large glycoprotein belonging to the metzincin superfamily of zinc metalloproteinase (5). The PAPP-A bound to the major basic protein is detectable in the maternal bloodstream in a heterotetrameric complex of two PAPP-A to two proBMP molecules which have been connected by a disulfide bond (6). In T21, T18, and T13 pregnancies, PAPP-A levels decrease in the first trimester of pregnancies (4).

In the FTS, the risks at or above 1:250 for all three trisomies considering high-risk and invasive tests, such as amniocentesis or chorionic villus sampling, are most frequently offered. The risk of fetal loss for both invasive procedures is within the range of 0.5-1% (7). It has been understood that the average DR and false positive rate (FPR) of the first trimester combined screening for T21 were 89.5% and 3.6%, respectively (8). The DR for T18 and T13 was observed to be 78.8% when the cut-off was 1:150 (9).

Commercial prenatal screening software uses the measurements of PAPP-A and free β-hCG to convert concentrations to multiple of the median (MoM) using a previous database. Maternal age and NT thickness are combined with MoM values by the program in order to calculate the final risks of trisomies. When the calculated risks are lower than the cut-off, pregnancies are considered high-risk. In addition, when the risks are higher than the threshold, pregnancies are considered low-risk. Therefore, setting an impeccable median is a significant factor to achieve a more reliable risk of trisomy (10).

The National Institute of Health and Care Excellence has a specific guideline for antenatal care and antenatal screening working standards of the national Down's syndrome screening program. The aforementioned guideline suggests all women undergo screening during pregnancy by a combined test for a DR of 75% at an FPR of 3% (11, 12). Furthermore, the Reference Laboratory of Iran's Ministry of Health has laid down a rule for those laboratories to perform the FTS using closed dedicated automated platforms in order to improve precision and accuracy and decrease risks variation between laboratories. As a result, screening can be carried out solely by Cobas e411, Immulite 2000, and Maglumi 4000 which have a significant market share in Iran.

Until recently, manual enzyme-linked immunosorbent assay has been routinely used for measuring PAPP-A and free β-hCG, and only big laboratories can afford high-throughput immunoassay analyzers. Currently, in spite of using these kinds of platforms, there have been still discrepancies between measurements. With this background in mind, this study aimed to evaluate the correlations between measurements, MoM values, and final risks of three different common closed chemiluminescence immunoassay (CLIA) analyzers in the FTS.

Methods

The present cross-sectional study was performed on 371 singleton pregnant women referring to Dena laboratory in Tehran, Iran, for the combined testing during July 2018 and August 2018 using convenience sampling. According to the Clinical and Laboratory Standard Institute I/LA25-A2, at least 100 pregnant women should be randomly selected for each analyte. In this regard, a larger number is recommended whenever it is practical; therefore, 371 pregnant women were selected for 3 weeks of pregnancy. The median of maternal age was 34 years (range: 20-47 years) at the time of sampling.

Morning blood sampling was conducted owing to fasting requirements. About 10 ml of peripheral blood was drawn in a clot activator blood collection tube (Improve Medical Corp., Guangzhou, Guangdong China) and centrifuged at 1850 g for 10 min within a few hours of collection. The PAPP-A and free β-hCG were routinely analyzed within 4 h of separation through three various closed fully automated analyzers, including Maglumi 4000 (Snibe Company, Shenzhen, Guangdong, China), Cobas e411 (Roche Diagnostics, Penzberg, Germany), and Immulite 2000 (Siemens healthcare diagnostics,

Deerfield, IL, USA). Both Maglumi 4000 and Immulite 2000 analyzers are CLIA systems, while the Cobas e411 is an electrochemiluminescence immunoassay analyzer.

Initially, the mothers with a history of smoking, insulin-dependent diabetes mellitus, pre-existing illness, history of blood transfusion or bone marrow transplantation, and pregnancy with donated ovum were excluded from the study. Then, the median for each instrument was calculated using the recorded data. The measurements of PAPP-A and free β-hCG from different platforms were converted to their MoM equivalence by commercial prenatal risk assessment software (Benetech Inc., Toronto, ON, Canada). Informed consent was obtained from all the participants, and the subjects were assured that all the information would not be revealed unless for research purposes in an anonymous form. Furthermore, this study was approved by the Ethics Committee of Islamic Azad University, Science and Research Branch, Tehran, Iran (IR.IAU.SRB.REC.1397.163).

Statistical analysis

The data were analyzed using Microsoft Excel (version 2016; Microsoft Corp., Redmond, WA, USA), GraphPad Prism software (version 7; GraphPad Software, San Diego, CA, USA), and SPSS software (version 22; IBM Corp., Armonk, NY, USA). The Shapiro-Wilk test was performed to verify the normalization of data distribution. Since all the variables were not normal (P<0.05), Spearman's correlation was utilized to assess the bivariate correlation. The biochemical biomarkers' values and MoMs from Maglumi 4000, Cobas e411, and Immulite 2000 were compared by plotting the data into a scatter plot. In addition, the slopes were assessed using the Deming regression analysis. Analysis of variance (ANOVA) and Chi-square test were used to compare MoM values and final risk of trisomies, respectively. A p-value of < 0.05 was assumed statistically significant.

Results

In the present cross-sectional study, the serum samples of 371 singleton pregnant women within the age range of 20-47 years during 11^{+0} to 13^{+6} weeks of pregnancy referring to Dena Laboratory within July 2018 and August 2018 were randomly selected. The results of the Shapiro-Wilk test showed that all the variables did not follow a normal distribution (P<0.05). Spearman's correlation also showed associations between different systems for both values and MoM (P<0.001 for each of two-to-two relationships) as presented in Table 1.

Table 1. Spearman's correlation between biochemical biomarkers' values and calculated multiples of the median

				DPC	Cobas	M4000
PAPP-A[a]	DPC[c]	Concentration	Correlation coefficient	1.000	0.938**	0.925**
			P-value	-	0.000***	0.000***
		MoM[f]	Correlation coefficient	1.000	0.946**	0.913**
			P-value	-	0.000***	0.000***
	Cobas[d]	Concentration	Correlation coefficient	0.938**	1.000	0.978**
			P-value	0.000***	-	0.000***
		MoM	Correlation coefficient	0.946**	1.000	0.951**
			P-value	0.000***	-	0.000***
	M4000[e]	Concentration	Correlation coefficient	0.925**	0.978**	1.000
			P-value	0.000***	0.000***	-
		MoM	Correlation coefficient	0.913**	0.951**	1.000
			P-value	0.000***	0.000***	-
Free β-hCG[b]	DPC	Concentration	Correlation coefficient	1.000	0.977**	0.968**
			P-value	-	0.000***	0.000***
		MoM	Correlation coefficient	1.000	0.978**	0.969**
			P-value	-	0.000***	0.000***
	Cobas	Concentration	Correlation coefficient	0.977**	1.000	0.963**
			P-value	0.000***	-	0.000***
		MoM	Correlation coefficient	0.978**	1.000	0.967**
			P-value	0.000***	-	0.000***
	M4000	Concentration	Correlation coefficient	0.968**	0.963**	1.000
			P-value	0.000***	0.000***	-
		MoM	Correlation coefficient	0.969**	0.967**	1.000
			P-value	0.000***	0.000***	-

a: Pregnancy-associated Plasma Protein-A; b: Free β-human chorionic gonadotropin; c: Immulite 2000; d: Cobas e411; e: Maglumi 4000; f: Multiple of the median; ** P<0.01; *** P<0.001

The association of PAPP-A between Maglumi 4000 and Cobas e411 was better than that reported for Immulite 2000 with Maglumi 4000 and Cobas e411. Moreover, the slope for the Deming regression line was 0.9 (95% CI: 0.804-1.008); however, the slopes for the Deming regression lines for Cobas e411-Immulite 2000 and Immulite 2000-Maglumi 4000 were 1.23 (95% CI: 1.175-1.285) and 1.25 (95% CI: 1.103-1.415), respectively (Figure 1).

The association of free β-hCG between Cobas e411 and Immulite 2000 was better than that reported for Maglumi 4000 with Cobas e411 and Immulite 2000; however, the slope for the Deming regression line was 0.954 (95% CI: 0.920-0.988). Nevertheless, the slopes for the Deming regression lines for Immulite 2000-Maglumi 4000 and Cobas e411-Maglumi 4000 were 1.02 (95% CI: 0.959-1.089) and 1.05 (95% CI: 0.958-1.145), respectively (Figure 2).

The association of PAPP-A MoM between Cobas e411 and Maglumi 4000 was better than that reported for Immulite 2000 with Maglumi 4000 and Cobas e411. However, the slopes for the Deming regression lines obtained from PAPP-A MoM on three different platforms of Cobas e411-Maglumi 4000, Cobas e411-Immulite 2000, and Immulite 2000-Maglumi 4000 were 0.975 (95% CI: 0.926-1.022), 1.02 (95% CI: 0.883-1.151), and 0.86 (95% CI: 0.717-1.003), respectively (Figure 3).

Pearson's correlation did show a significant relationship between free β-hCG MoM on Maglumi 4000 with those on Cobas e411 and Immulite 2000. Furthermore, the slopes for the Deming regression lines obtained from free β-hCG MoM on the three different platforms of Cobas e411-Maglumi 4000, Cobas e411-Immulite 2000, and Immulite 2000-Maglumi 4000 were 0.958 (95% CI: 0.853-1.063), 0.933 (95% CI: 0.897-0.968), and 1.02 (95% CI: 0.936-1.111), respectively (Figure 4).

The medians for PAPP-A MoMs were 1, 0.98, and 0.97 for Immulite 2000, Cobas e411, and Maglumi 4000, respectively. In addition, the free β-hCG MoMs were 1.01, 1.02, and 1.03 for Immulite 2000, Cobas e411, and Maglumi 4000, respectively. In terms of PAPP-A and free β-hCG

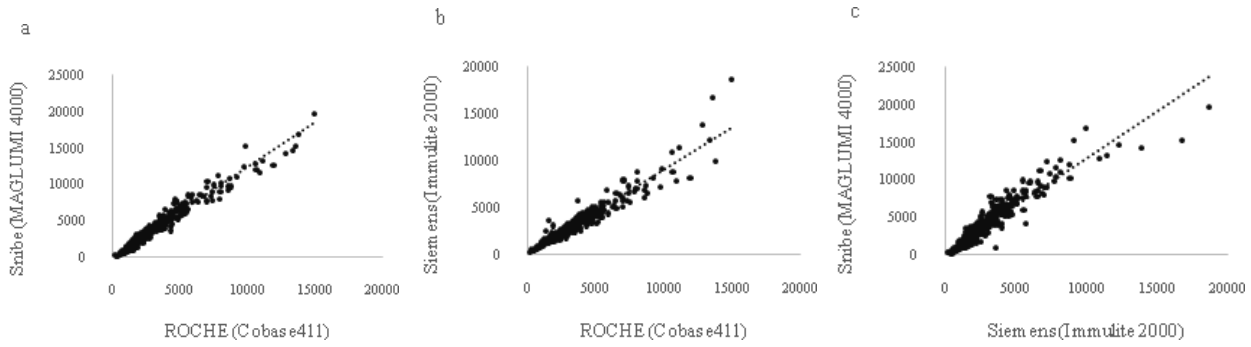

Figure 1. Illustration of different correlations in screening population (n=371) through comparison of plots of pregnancy-associated plasma protein-A values (mIU/L) analyzed on Cobas e411 and Maglumi 4000 (a), Cobas e411 and Immulite 2000 (b), and Immulite 2000 and Maglumi 4000 (c)

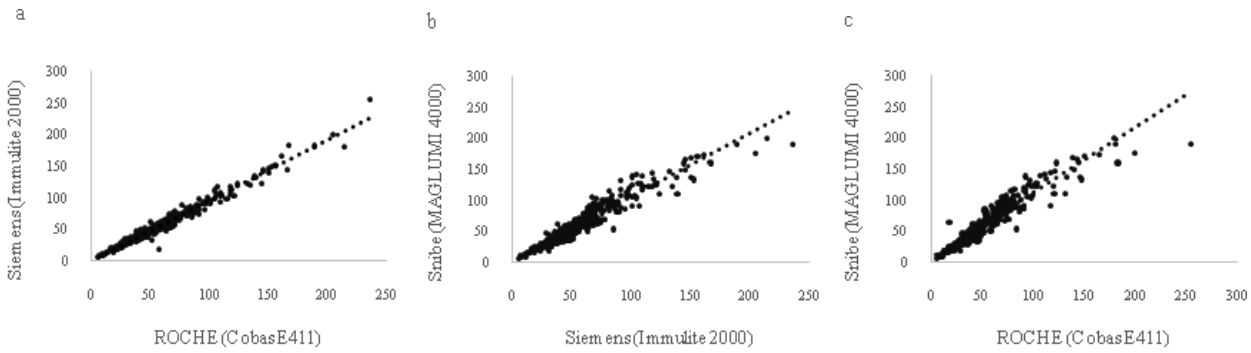

Figure 2. Illustration of different correlations in screening population (n=371) through comparison of plots of free beta-human chorionic gonadotropin values (ng/mL) analyzed on Cobas e411 and Immulite 2000 (a), Immulite 2000 and Maglumi 4000 (b), and Cobas e411 and Maglumi 4000 (c)

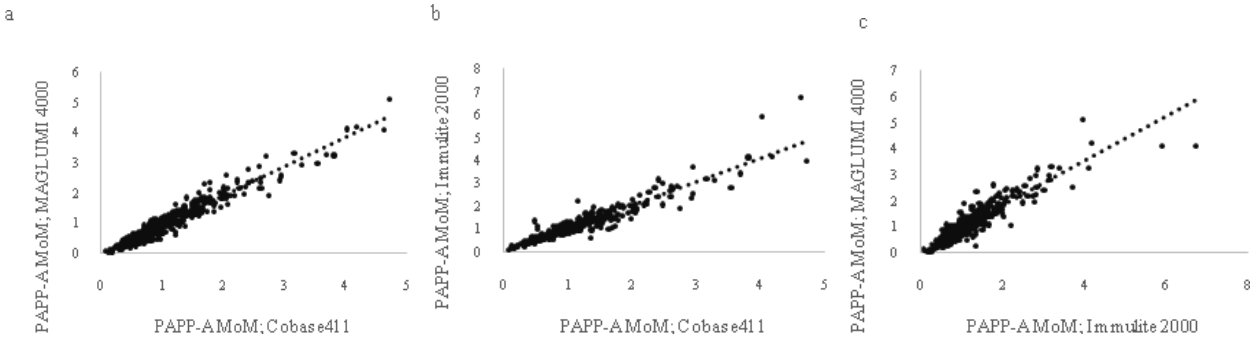

Figure 3. Illustration of different correlations on different platforms while using software in screening population (n=371) through comparison of plots of pregnancy-associated plasma protein-A multiple of median values analyzed on Cobas e411 and Maglumi 4000 (a), Cobas e411 and Immulite 2000 (b), and Immulite 2000 and Maglumi 4000 (c). PAPP-A: Pregnancy-associated Plasma Protein-A, MoM: Multiple of Median

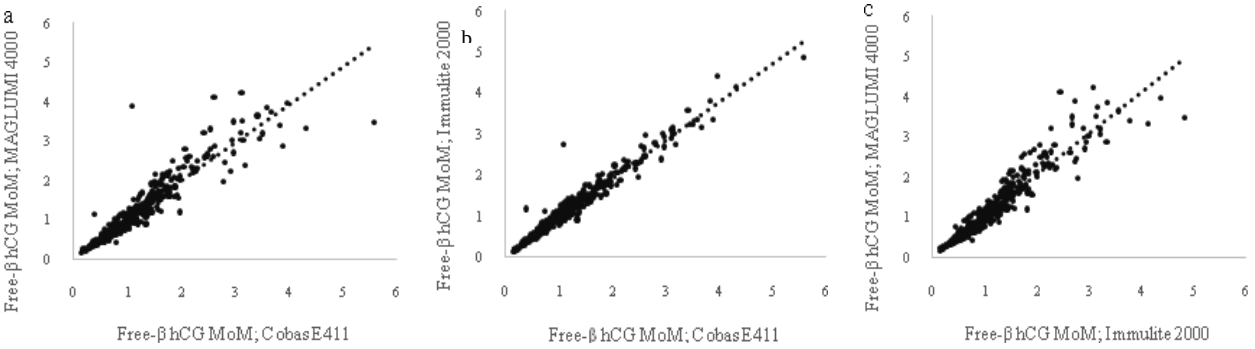

Figure 4. Illustration of different correlations in screening population (n=371) through comparison of plots of free beta-human chorionic gonadotropin multiple of median values analyzed on Cobas e411 and Maglumi 4000 (a), Cobas e411 and Immulite 2000 (b), and Immulite 2000 and Maglumi 4000 (c). Free β-hCG: free beta-human chorionic gonadotropin, MoM: Multiple of Median

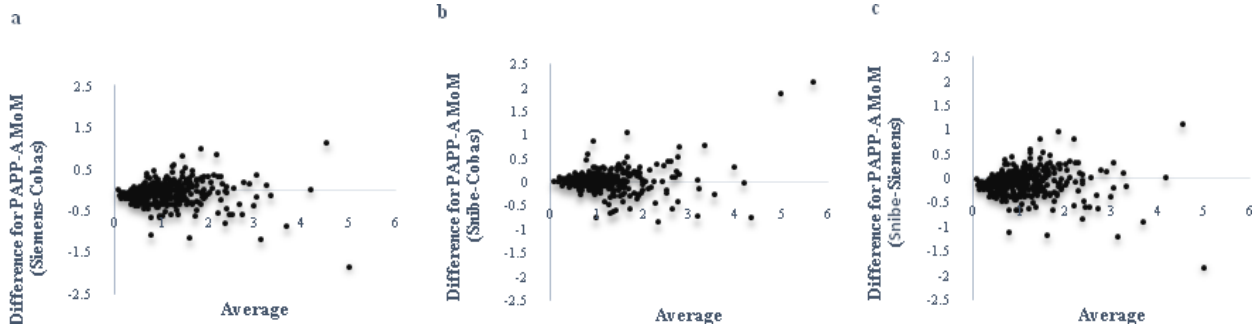

Figure 5. Illustration of differences in different averages of multiple of the median through different plots for pregnancy-associated plasma protein-A multiple of median values for Cobas e411, Immulite 2000, and Maglumi 4000

MoMs, no significant differences were observed between all the platforms as it can be observed in the different plots (Figure 5 for PAPP-A and Figure 6 for free β-hCG; P=0.18 and P=0.7, respectively).

The ANOVA was performed for the comparisons between PAPP-A and free β-hCG MoMs on three different instruments. In this regard, there were no significant differences between PAPP-A and free β-hCG MoM (0.2 and 0.26, respectively).

The risks of chromosomal aneuploidies (i.e., T21, T18, and T13) were calculated by antenatal risk assessment Benetech software (version 3.3.0.0) in the screening population using measurements from Cobas e411, Immulite 2000, and Maglumi 4000 (Figure 7). The Chi-squared test was applied to compare the final risk of trisomies, and there were no significant differences between final risks derived from the three different instruments (P-values for T21,

T18, and T13 as 0.62, 0.41, and 0.41, respectively). Out of 10 pregnancies with a high risk of T21, regardless of using which platform, 10 pregnancies had an increased risk of T21. Out of that 10 high-risk pregnancies, 2 cases carried fetuses affected by T21.

When the measurements of Immulite 2000 and Maglumi 4000 were used, two and five more pregnancies were high-risk, respectively, compared to those reported for Cobas e411. All low-risk pregnancies calculated by the software, regardless of using which platforms, had no sign of T21 after birth, except for two cases detected through non-invasive prenatal testing (NIPT), verified by amniocentesis, and were consequently aborted. For T18 and T13, five, four, and nine pregnancies were high-risk; however, two of them were approved by NIPT and amniocentesis. The descriptions of all platforms are shown in Table 2.

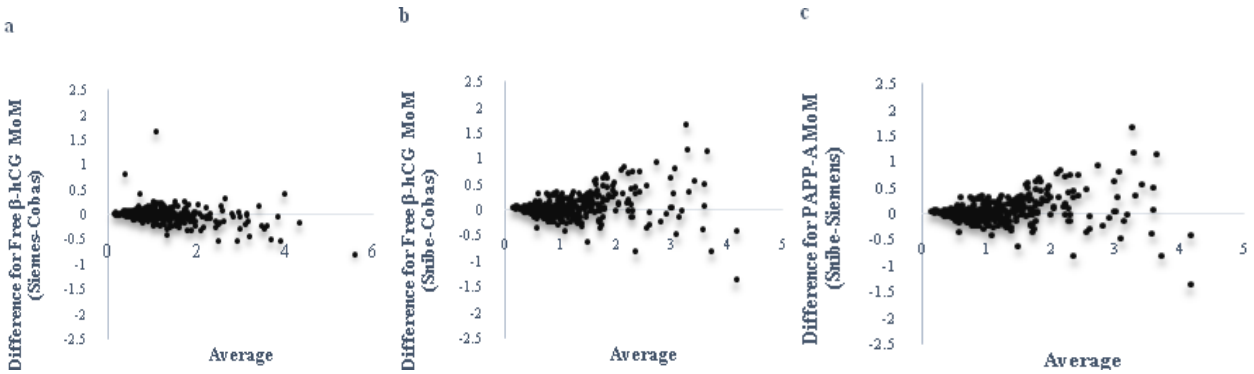

Figure 6. Illustration of differences in different averages of multiple of the median through different plots for free beta-human chorionic gonadotropin multiple of median values for Cobas e411, Immulite 2000, and Maglumi 4000

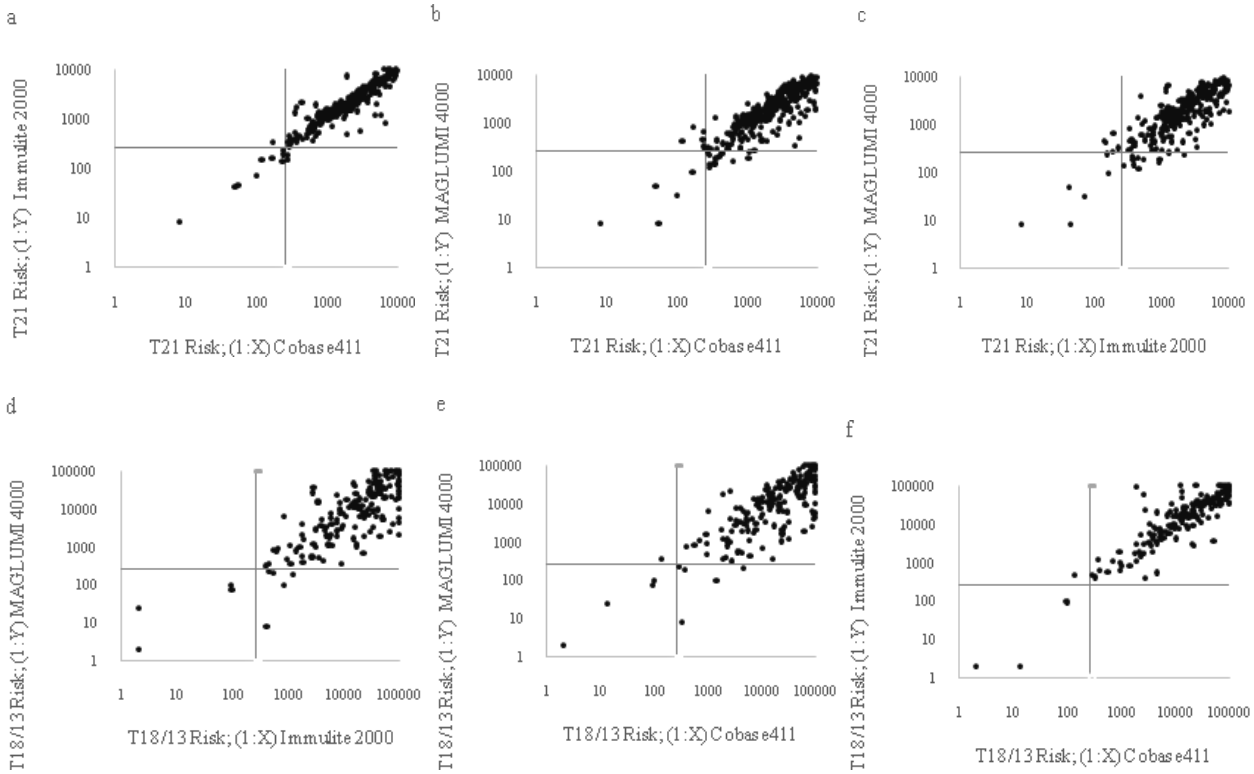

Figure 7. Comparison of calculated risk of trisomy 21 between Cobas e411 and Immulite 2000 (a), Cobas e411 and Maglumi 4000 (b), as well as Immulite 2000 and Maglumi 4000 (c); comparison of calculated risk of trisomy 18/13 between Immulite 2000 and Maglumi 4000 (d), Cobas e411 and Maglumi 4000 (e), as well as Cobas e411 and Immulite 2000 (f) used by cut-off of 1:250 for trisomies 21, 18, and 13

Table 2. Clinical performance in detecting trisomies 21, 18, and 13 in first trimester of pregnancy using different platforms and prenatal risk assessment Benetech software (n=371)

	Benetech software					
	Cobas e411		Immulite 2000		Maglumi 4000	
	Trisomy 21	Trisomy 18/13	Trisomy 21	Trisomy 18/13	Trisomy 21	Trisomy 18/13
Sensitivity	50	50	50	50	50	50
Specificity	99.4	99.5	99.4	99.5	99.4	99.4
PPV	20	40	16.7	50	13.3	22.2
NPV	97.8	99.2	97.3	99.5	96.5	98.1

PPV: Positive predictive value; NPV: Negative predictive value

Discussion

The present study compared the measurements of PAPP-A and free β-hCG derived from the three different analyzers, including Cobas e411, Immulite 2000, and Maglumi 4000, in Iranian singleton pregnant women participating in the first trimester of pregnancy screening for three most common chromosomal aneuploidies (i.e., T21, T18, and T13). In the screening population, although the PAPP-A values on Maglumi 4000 were higher than those reported for the other two systems, there was a significant correlation between all the platforms, especially Maglumi 4000 and Cobas e411 as a valid system (correlation coefficient=0.978) (13, 14). Furthermore, the measurements of free β-hCG from Maglumi 4000 were slightly higher than those reported for Immulite 2000 and Cobas e411. However, there was a significant correlation between all the systems, especially between Cobas e411 and Immulite 2000.

Previous studies also observed a significant correlation between Immulite 2000 and Cobas e411 (14). In the present study, analyzing the sera by all three platforms on a consecutive working day, appointing a particular oriented technician to analyze the sera, using a phlebotomist to draw the blood, a lot of reagents, and a kind of sample tube, and calculating the risks of trisomies by same prenatal screening software to minimize the bias can be the strengths of the present study. The observed differences between Cobas e411 and Immulite 2000 might be due to different operational performance, shipping conditions of reagents, instrument situations, as well as different specificity and sensitivity of the reagents. On the other hand, in contrast to those in the present study, frozen sera were used in other studies (13, 14).

Both the PAPP-A and free β-hCG MoM values derived from the three systems showed significant associations ($P<0.05$). However, the differences would be various if MoM values were compared in different weeks of pregnancy. On the other hand, the MoM values of both biochemical biomarkers differed significantly in the upward concentrations of 10000 mIU/L and 150 ng/ml, respectively. These differences varied depending on the week of gestation. Previous studies, similar to the current study, observed a significant correlation between Cobas e411 and Immulite 2000 for the MoM values of free β-hCG and PAPP-A (14, 15).

Generally, it would be presumed that no felicity's correlation between various systems would result in more pregnancies at fluctuated risks of chromosomal aneuploidies in different laboratories leading to performing more invasive diagnostic tests. However, the results of the current study demonstrated that if laboratories use impeccable medians to change biochemical biomarkers into MoM values, these discrepancies between different platforms would dramatically decrease. On the other hand, there are some other variables, such as software proficiency, NT thickness, crown-rump length, nasal bone, and maternal age, which have considerable effects on the calculated risks of trisomies (16, 17).

Regarding the final risks of T21, there were no significant differences between the three platforms (i.e., 8, 10, and 13 false positives for Cobas e411, Immulite 2000, and Maglumi 4000, respectively, in addition to 2 false negatives for all the analyzers). It should be noticed that three of them were quite near the cut-off (1:250) indicating that reproducibility would also be effective to alter trisomy risks from high risk to low risk and vice versa. Furthermore, the final risks of T18/13 demonstrated no significant differences between the three platforms. Overall, assigning an intermediate risk for all pregnancies or further antenatal screenings, such as the second trimester of pregnancy tests, would be far preferable for those pregnant women their risks of trisomies are near to the borderline.

To the best of our knowledge, the present study was the first survey comparing PAPP-A and free β-hCG measurements on different kinds of closed CLIA analyzers, namely Cobas e411, Immulite 2000, and Maglumi 4000, in Iran.

Several studies compared two biochemical biomarkers for the first trimester of pregnancy on Cobas e411 and Immulite 2000 in their screening population. Similarly, we could find a significant association between two biochemical biomarkers in the FTS for both values and MoMs. Tørring et al. calculated the risks with different software (not Benetech) for the screening of pregnancies (14). They observed similar sensitivity and specificity to detect T21, while our results showed a fairly difference in the screening. The results of the present study showed that variability in the final risks might be due to the way the medians were set up into the software, not to the analyzers.

Brahms Kryptor (Thermo Fisher Scientific, Clinical Diagnostic, Brahms GmbH, Hennigsdorf, Germany) and AutoDELFIA (PerkinElmer, Life and Analytical Science, Wallac Oy, Turku, Finland) analyzers are other closed platforms measuring PAPP-A and free β-hCG in prenatal screening combined tests. Spencer showed that Immulite 2000 had no significant correlation with Kryptor as a well-known gold method to analyze PAPP-A and free β-hCG in the contemporary in vitro diagnostic market (18).

Engel et al. demonstrated that PAPP-A and free β-hCG MoM values on Cobas e411 were significantly lower than those reported for Kryptor. However, both platforms showed a similar positive rate (5.1%) in this regard. Therefore, even if there were differences in MoM, it would be better to evaluate the performance by the initial positive rate, DR, specificity, positive predictive value (PPV), and negative predictive value (NPV) to figure out how the system and software work (15).

Tørring et al. performed a technical validation on Cobas e411 for biochemical biomarkers in the FTS tests. In the aforementioned study, Cobas e411 and Immulite 2000 were correlated to AutoDELFIA and Kryptor, respectively (14). The results of the aforementioned study regarding the correlation of Immulite 2000 to Kryptor were in contrast to what Engel et al. observed. However, finding an association between Cobas e411 and Immulite 2000 in terms of PAPP-A and free β-hCG was similar to what was shown in the current study. The salient limitations of the present study were the expenses of NIPT tests and lack of the immunoassay reagents due to economic sanctions.

Conclusion

The obtained results of the present study demonstrated that there were significant correlations between the three different systems regarding PAPP-A and free β-hCG values and MoM. Nevertheless, in terms of PAPP-A, Maglumi 4000 correlated with Cobas e411 better than others. The sensitivity of all the systems for all trisomies was 50%; however, the specificity of all the systems was almost the same (about 99.45%). The best PPV and NPV for T21 were on Cobas e411, Immulite 2000, and Maglumi 4000, respectively. The PPV and NPV of all the systems for T18/13 were almost the same (higher than 99.1%). It is highly recommended to assign an intermediate risk to minimize the differences between the systems.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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