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Short Communication

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Comparison of Total Bilirubin Values Measured with ABL 735 Blood Gas Analyzer and Roche Cobas C8000 Chemistry Analyzer in Age-Segregated Pediatric Patients

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Abstract

***Aim**: Measurement of blood bilirubin levels is a crucial analysis because of the toxic effects of bilirubin on brain tissue, particularly in preterm neonates. The aim of this study was to investigate the consistency of the total bilirubin values obtained by the blood gas analyzer and the autoanalyzer.*

***Material and Methods**: In this study, we used total bilirubin data of 407 pediatric patients from Kocaeli University Medical Faculty Education and Research Hospital Central Laboratory System. Total bilirubin data, provided that it was measured simultaneously, was obtained from ABL 735 blood gas analyzer and Roche Cobas C8000 chemistry analyzer. Pediatric patients (neonates, infant and children under 17 years old) were selected retrospectively by year between 2015-2017.*

***Results**: Under a cut-off value (14.6 mg/dL) ABL 735 blood gas analyzer and Roche COBAS C8000 chemistry analyzer had strong correlation (r = 0.939) for total bilirubin measurements. It was found that 2-15 days old neonates give more scattered total bilirubin data by Bland Altman analysis in two measurements. Statistical analysis performed to compare whole total bilirubin data identity between two measurements: correlation coefficient was found r = 0.949 a statistically significant positive correlation (p < 0.001).*

***Conclusion**: According to our analysis which was supported by previous studies in the literature, we can say that the compatibility between the blood gas analyzer (multi-wave-length spectrophotometric technique) and the chemistry analyzer becomes weaker when the total bilirubin levels exceed 14.6 mg/dL.*

***Keywords**: bilirubin, blood gas analyzer, chemistry analizer, pediatric patients*

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Introduction

Jaundice is a common clinical entity in most neonates. Predominantly, jaundice is benign, whereas due to the potential toxicity of bilirubin, almost whole neonates are rigorously evaluated in terms of bilirubin levels to determine occurrence of a severe or pathological hyperbilirubinemia (1). If blood bilirubin levels reach excessive amounts, that will cause bilirubin-induced neurologic dysfunction (such as kernicterus, acute bilirubin encephalopathy) with destructive outcomes in newborns (2).

Laboratory results are of great importance in the diagnosis and management of patients admitted to the emergency department (ED). Emergency physicians need rapid and reliable laboratory outcomes to give fast decision management of treatment of critical patients (3, 4).

Point-of-care testing (POCT) means of delivering laboratory testing is an increasingly popular issue in clinical laboratory medicine. Due to the clinical importance of quick and accurate measurement of the parameters, moreover rising pressure on physicians to see more patients and short examination time for each patient, POCT stands for the progressively trendy way of laboratory test (5, 6). A broad list of POCT device analytes are available, including blood gas, electrolytes, pregnancy, cardiac, and infectious disease testing. As a substantial POCT device, the blood gas analyzer has a wide range of measurements with such parameters as bilirubin, hemoglobin, hematocrit, glucose, sodium, potassium, chloride, and calcium in addition to pH and blood gases (7, 8). Blood gas analysis is an essential test which is applied frequently for nearly all clinics, especially emergency departments.

Bilirubin is an end product of heme metabolism. High bilirubin levels in human serum can lead to bilirubin accumulation in the brain (9, 10). The timely detection of bilirubin level has great importance in especially vulnerable age and patient groups for instance in premature and term neonates, intrauterine growth retardation (IUGR), small for gestation age (SGA), infant of the diabetic mother. Besides that, in most cases, newborn jaundice is a benign condition (11). However, newborn jaundice is considered pathological, on condition it presents within the first 24 hours after birth. If serum total bilirubin (TB) level rises by more than 5 mg/dL per day or it is higher than 17 mg/dL and it lasts longer than 2 weeks, it requires an urgent management due to the risk for bilirubin encephalopathy and persistent neurological sequelae (12).

As the other parameters for point of care testing in clinical chemistry, bilirubin measurement methods are expected to provide quick, valid, accurate and reliable results with small sample volumes (5, 13, 14).

In clinical laboratory medicine, the reference method for the measurement of TB is applied to the automated chemistry analyzer. Therefore, in this study, our aim was to evaluate whether or not the TB values measured by the ABL 735 blood gas analyzer and the Roche Cobas C8000 chemistry analyzer are compatible with each other, and if so determining in which condition they take place.

Materials and Methods

This retrospective study was approved by the Ethics Committee of Kocaeli University (Project number: KÜ GOKAEK 2017/272).

TB outcomes of samples of 407 patients - analyzed simultaneously on both devices - were selected from the laboratory information system. The data were selected from patients - 0 to 17 years old - who were admitted to our pediatric hospital and newborn clinics between January 2015 and May 2017. The agreement of TB levels was evaluated between Radiometer ABL 735 blood gas analyzer and Roche Cobas C8000 chemistry analyzer. We described 3 main groups

as neonate, infant, and child group. Neonates were also divided into 3 subgroups as 0-2 days old, 2-15 days old and 15-28 days old. All main age groups and subgroups were also divided into two groups according to the accepted upper and lower TB limits (For neonates: 0-2 days-5 mg/dL, 2-15 days - 12.9 mg/dL, 15-28 days - 10 mg/dL. For infants and children - 0.99 mg/dL) (15).
In our laboratory, the blood samples for the chemistry analizer were centrifuged (NUVE NF1200) immediately for 10 minutes at 3000 x g and the obtained serum samples were examined on the Roche Cobas C8000 chemistry analizer by the Diazo enzymatic method. In this method, the presence of a suitable solubilizing agent, TB is coupled with a diazonium ion (3,5- Dichlorophenyl diazonium) in a strongly acidic medium. The color intensity of the red azo dye formed is directly proportional to the TB and can be determined photometrically.
Undiluted whole blood samples which were collected with standard heparinized blood gas injectors in accordance with the standards recommended by IFCC were handled in the blood gas analyzer (16). ABL 700 series blood gas analyzer provides optimal optical performance on condition that the same device and aspiration mode measures TB in whole blood. The optical system is based on visible absorption spectroscopy measuring at 128-wavelength spectrophotometer with a measuring range of 478 - 672 nm. It was shown in the literature that both heparinized plasma and serum samples gave the statistically same results for bilirubin (17). Moreover, Lano et al. indicate that measurement of TB in whole blood, plasma and serum samples are given accurate and reliable results that are compatible with each other (13).

Statistical analysis

The statistical analysis was performed with IBM SPSS 20.0 Software. The normal distribution of the data for the parametric-nonparametric test selection was investigated by statistical evaluation. Wilcoxon signed rank test and Spearman correlation analysis was used for nonparametric tests. The data were evaluated by the Bland-Altman method with MedCalc program to compare blood gas and chemistry analizer results (18). The p-value of 0.05 ($p < 0.05$) was considered significant in the analysis.

Results

We conducted the TB concentration data, a total number of 407 newborns and pediatric patients who underwent simultaneous whole blood and serum analysis with ABL 735 blood gas analyzer and Roche COBAS 6000 chemistry analyzer. The mean TB levels which were measured by the chemical analyzer were 2.3 (0.52-6.79) and minimum; 0.09, maximum; 19.12 mg/dL. At the same time, the mean TB levels which were studied by the blood gas analyzer were 2.1 (0.4-7) and min; 0, max; 18.7 mg/dL. The mean age was 28 days old (3 days – 5.16 years) and min; 0 days, max; 17.0 years .
The Spearman correlation coefficient was $r = 0.949$ between the two measurements indicating a statistically significant positive correlation ($p < 0.001$). We also found the statistical similarity between the bilirubin values of the analyzers. ($p > 0.05$). Furthermore, the regression equation was found as $y=0.977x+0.006$, according to Deming (19).
All data were also analyzed with Bland-Altman graph. In the Bland-Altman analysis, the mean bias was -0.0031 ±2.29 (-2.32 to 2.28 95% Cl; Confidence Interval). These results show a significant agreement between the results of the two measurement methods.
Descriptive statistics of three main groups and subgroups with normal TB values are shown in Table 1.
Descriptive statistics of three main groups and subgroups with the upper limit of TB level for

each age groups are also shown in Table 2.

The values measured by both analyzers are shown for each age groups in the Bland-Altman graphs (Figure 1).

Regression analysis of the TB was performed according to each group. The equation of the TB values up to 5mg/dL for 0-2 days old neonates was y= 1.065x-0.453, r = 0.832. The equation of the TB values higher than 5mg/dL for 0-2 days old neonates was y=0.715x+1.907, r=0.832. The equation of the TB values up to 12.9mg/dL for 2-15 days old neonates was y=0.976x+0.183, r=0.815 and higher than 12.9mg/dL for 2-15 days old neonates was y= 0.956x+0.173, r=0.580. The equation of the TB values up to 10 mg/dL for 15-28 days old neonates was y=1.024x+0.085, r=0.929 and higher than 10 mg/dL for 15-28 days old neonates was y=0.763x+2.826, r=0.986. The equation of the TB values up to 0.99 mg/dL for infants was y=0.967x-0.116, r=0.602 and higher than 0.99 mg/dL for infants was y=1.114x-0.441, r=0.990. The equation of the values up to 0.99 mg/dL for children was y=0.820x-0.012, r=0.660 and higher than 0.99 mg/dL for children was y=1.050x-0.108, r=0.977.

In addition, we statistically analyzed our data based on the cut-off value given in the literature (20). The median (25-75 percentile) of the 14.6 mg/dl below bilirubin levels which were studied by the chemical analyzer was 1.92 (0.5-6.3) and min; 0.09, max; 14.54 mg/dL (n = 394). The median of the 14.6 mg/dL below bilirubin levels

Table 1. Descriptive statistics of the groups with TB level for each age groups

	Blood Gas Analyzer			Chemistry Analyzer		
	min	max	median (25-75 percentile)	min	max	median (25-75 percentile)
Neonates (n=148)						
0-2 days old (n=22)	0.00	5.80	2.80 (0.65 - 5.09)	0.29	4.99	3.76 (1.7 - 4.54)
2-15 days old (n=89)	0.00	16.10	6.60 (4.15 - 9.35)	1.20	12.82	7.01 (4.86 - 9.35)
15-28 days old (n=37)	0.50	10.00	4.60 (2.35 - 7.15)	0.60	9.72	4.99 (2.26 - 6.54)
Infants (n=21)	0.00	1.10	0.23 (0.10 - 0.60)	0.10	0.85	0.50 (0.34 - 0.60)
Children (n=128)	0.00	1.29	0.29 (0.12 - 0.47)	0.09	0.90	0.40 (0.29 – 0.6)

* The TB reference interval for the newborn group is 0-2 days <5 mg / dL, 2-15 days <12.9 mg / dL, 15-28 days <10 mg / dL. The TB reference interval for infants and children was accepted as <0.99mg / dL.

Table 2.Descriptive statistics of the upper limit of TB level for each age groups

	Blood Gas Analyzer			Chemistry Analyzer		
	min	max	median (25-75 percentile)	min	max	median (25-75 percentile)
Neonates (n=57)						
0-2 days old(n=32)	0.00	16.50	7.45 (5.73 - 10.15)	5.00	15.38	7.88 (5.44 - 11.00)
2-15 days old(n=22)	8.20	18.70	14.10 (12.38 - 16.78)	10.70	19.12	14.63 (13.84 - 15.30)
15-28 days old (n=3)	11.00	14.70	13.40 (n/a – n/a)	10.95	15.80	13.38 (n/a – n/a)
Infants (n=23)	0.80	14.38	3.91 (1.70 - 7.40)	1.03	13.10	4.50 (1.60 - 6.30)
Children(n=30)	0.20	8.60	1.40 (0.95 - 1.78)	0.99	8.50	1.25 (1.10 - 1.64)

* Upper limit of blood bilirubin level for each age group for neonates group are 0-2 days old > 5mg/dL, 2-15 days old >12.9mg/dL, 15-28 days old > 10mg/dL. For infants and children groups TB reference interval > 0.99mg/dL.

which were studied by the blood gas analyzer was 1.7 (0.35-6.43) and min; 0.0, max; 16.5 mg/dL (n = 394). The median of the age was 34.4 days old (3.0 days- 5.46 years) and min; 0.0, max; 17 years old. (n = 394). The regression equation of the TB values below the 14.6mg/dL was y = 0.968x + 0.025, r = 0.939. When we looked at the median of the 14.6mg/dL above bilirubin levels which were studied by the chemical analyzer was 15.38 (15.04-16.27) and min; 14.71, max; 19.12 mg/dL (n = 13). The median of the 14.6mg/dl above bilirubin levels which were studied by the blood gas analyzer was 15.8 (14.5-17.7) and min; 14.0, max; 18.7 mg/dL (n = 13). The median of the age was also 6 days old (3-8 days) and min;1, max; 20 days old. (n = 13). The regression equation of the TB values above the 14.6 mg/dL was y = 0.26x + 15.657, r = 0.020. This cut-off value (14.6mg/dL) is shown in the Bland-Altman graphs (Figure 2).

Discussion

One of the biggest problems that must be overcome for newborn blood tests is to reduce the pain and discomfort during blood collection (21, 22). It is also necessary to obtain fast results from the analyzers used in emergency laboratories. In our study, the turnaround time of the ABL 735

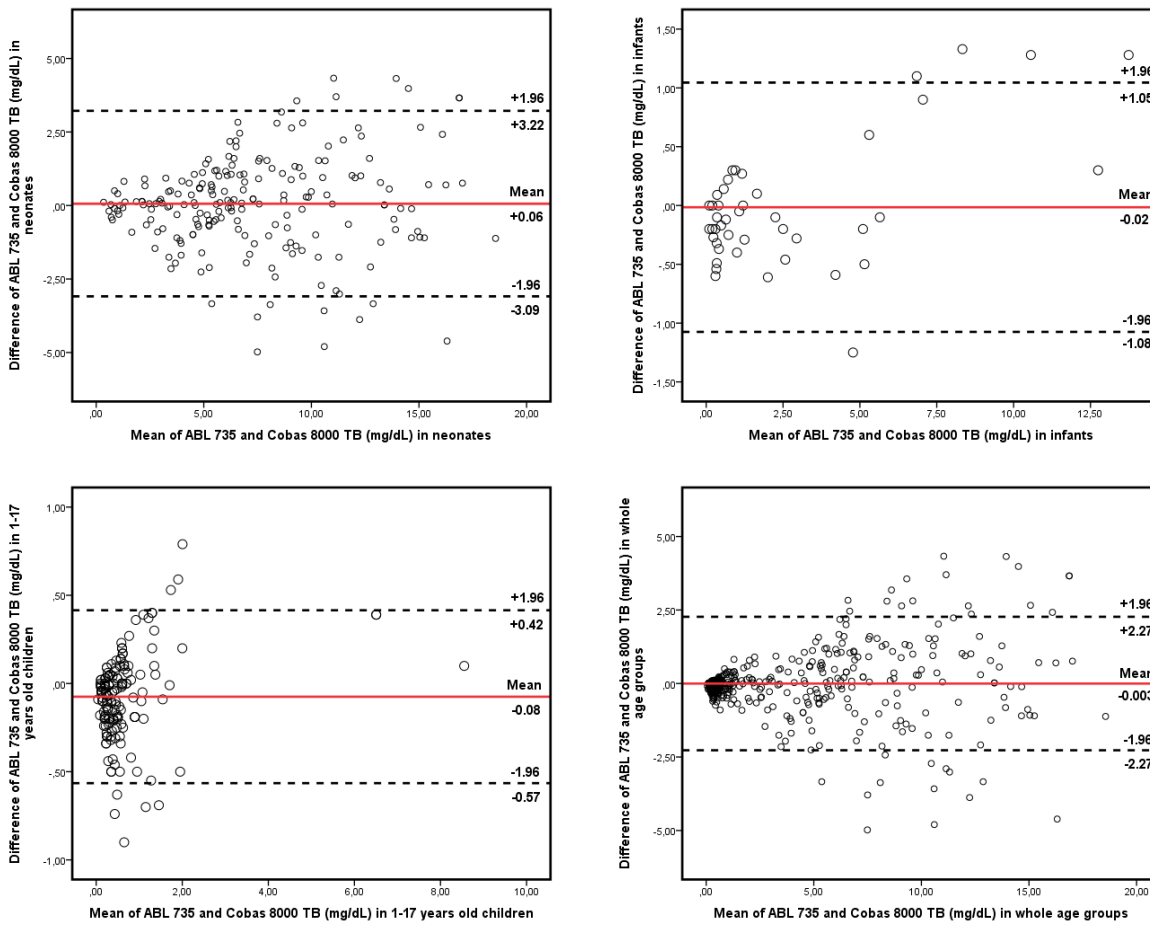

Fig. 1. Bland–Altman plots of age-segregated subgroups for total bilirubin analyzed with ABL 735 Blood Gas Analyzer and Roche Cobas C8000 Chemistry Analyzer.

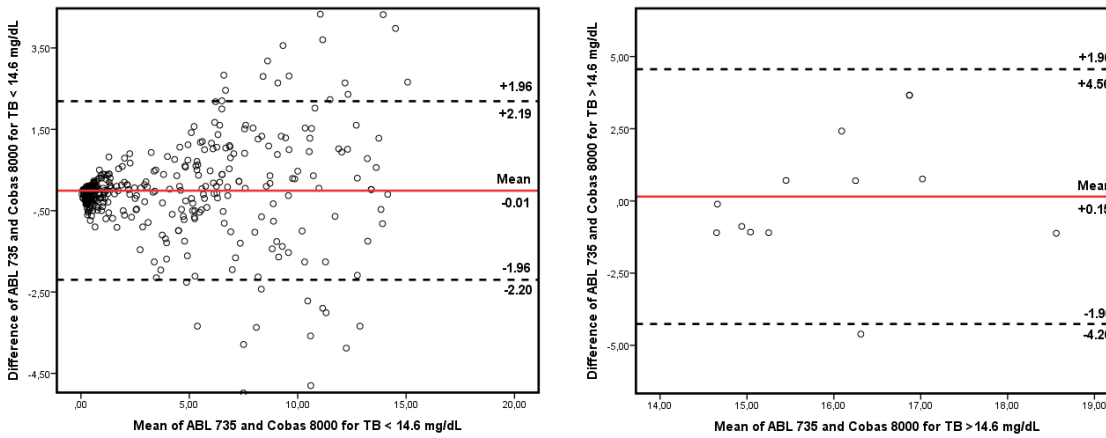

Fig. 2. Bland-Altman plots of TB concentrations (mg/dL) analyzed with ABL 735 Blood Gas Analyzer and Roche Cobas C8000 Chemistry Analyzer based on the cut-off value given in the literature (20).

blood gas analyzer is 15 minutes on average. On the other hand, the turnaround time of the Roche Cobas C8000 chemistry analyzer is 37 minutes on average. In our study, blood gas analysis turnaround time of bilirubin results bears resemblance with the literature (23, 24). At that point, blood gas analyzers provide many advantages in terms of usage. Blood gas analyzers can analyze whole blood, using direct ion-selective electrode. They have short processing time, no centrifuge step. Their results are not affected by high protein levels (25, 26). Furthermore, in our laboratory, only 95 µl blood is sufficient for the ABL 735 blood gas analyzer in pediatric samples. Therefore, the requirement for sample preparation makes blood gas analyzers a more convenient choice. In this study, a statistically significant relationship was showed in ABL 735 blood gas analyzer and the Roche Cobas C8000 chemical analyzer as in the study by Peake et al. (27).

In the literature, many researchers have suggested that there is not a difference between the blood gas analyzer and the routine biochemistry analyzer. For example, Rolinski et al. concluded that the measurement of TB with the ABL 735 blood gas analyzer from whole blood samples was a promising method (28). Peake et al. compared bilirubin results obtained on the Radiometer ABL 735 blood gas analyzer with Roche diazo method for Hitachi analyzers concluded that the Radiometer ABL 735 bilirubin assay was suitable for assessment of neonatal jaundice using whole blood, thus eliminating the need for sample centrifugation (27).

There are many national and international studies comparing blood gas analyzer and biochemical autoanalyzer results, in a different source of fluid and electrolyte balance are searched (29, 30). Apart from these ones, there are also studies showing that different parameters are evaluated and the results of the blood gas analyzer are not compatible (31, 32). Nevertheless, in the literature, analyzer studies comparing bilirubin values which are very important for the newborn and childhood are rarely encountered.

In one study, lactate, hemoglobin F, and neonatal bilirubin measurements were determined with Vitros 950, ABL 735 and Unistat and the mean values ± Standard deviations were found 12.08 ± 7.55mg/dL, 11.12 ± 7.33mg/dL and 12.37 ± 8.32 mg/dL respectively (33). The results show that there is an acceptable correlation between the different analyzers. Grohmann et al. (2006) compared 9 frequently used methods for bili-

rubin determination in newborns under routine conditions and they found a good correlation coefficient between ABL 735 and other analyzers (20) and they recommended that bilirubin concentration results from blood gas analyzers that exceed 250 μmol/L (14.62 mg/dL) should be confirmed with standard laboratory methods. In our study, 407 patients were evaluated and these results are in parallel with our findings. In addition to these studies, we also found a very good correlation coefficient (r = 0.949) for the results obtained on ABL 735 analyzer and Roche Cobas C8000 chemistry analyzer in all groups. Especially, in the 2-15 days old neonates groups, TB values are within the confidence interval, but their SD values increased.

In 2016 Toshihiko Nambara et al. published a research which demonstrated resemblance to our findings, which are; concordance correlation coefficient analysis showed a strong relationship between the bilirubin levels obtained by gas analyzer and autoanalyzer with a Pearson's coefficient of 0.97. Bland-Altman difference plots demonstrated that, on average, bloodgas analyzer inclined to undervalue the chemistry analyzer, with a mean (95% confidence interval) bias of -0.7 (-0.6 to -0.8) mg/dL (34).

Rolinsky et al. 2001 mentioned that the presence of lipemia or hemolysis in the plasma samples, as measured by spectral analysis, did not interfere with TB determination by the 2,5-dichlorophenyldiazonium (DPD) method. The age or the number of patient samples did not affect the results of the correlation between TB concentrations measured on the Hitachi 917 and the ABL 735 (28).

Our study and similar studies in the literature show that the measurement of TB by blood gas analyzer is consistent with the automated reference method. However, when the TB levels exceed of 14.6mg/dL, those are incompatible with each other.

Using bilirubin values which are measured by blood gas analyzer cannot be suggested as a thorough alternative for determining and assessment of bilirubinemia especially in newborns, in whom rare conditions of bilirubin can arise high levels than accuracy performance of POCT device.

Study Limitation

Blood sample result data were selected randomly and retrospectively. No exclusion criteria except for measurement not being carried out simultaneously, patient age range. Results of metabolic parameters obtained by blood gas analyzer which is given with question mark is thought to be insufficient and inappropriate sample (coagulated or waited sample).

Protection of humans rights and data confidentiality

The authors declare that the procedures were followed according to the regulations established by the Clinical Research and Ethics Committee and to the Helsinki Declaration of the World Medical Association.

The authors declare having followed the protocols in use at their working center regarding patients' data publication.

Authors' contribution

EA (Investigation; Methodology; Writing – original draft; Writing – review & editing)
FH (Formal analysis; Methodology; Software; Writing – original draft)
TK (Resources)
FCE (Supervision)
HMK (Data curation; Supervision)
CB (Formal analysis)

Conflicts of Interest

There is no conflict of interest in this study.

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