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Published online 2014 February 30. **Brief Report**

Biochemical Interfering Factors and Blood Cells Indices

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Background: The presence of interfering substances is the most common preanalytic factor that can affect the precision and accuracy of Blood Cell Count results. These clinical laboratory interferences are difficult to determine and are largely underestimated.
Objectives: This study evaluated the effects of biochemical Interfering factors include hyperglycemia, hyperlipemia, and uremia on blood cells indices including different RBC indices, Plt and WBC count. Therefore, this study was conducted to investigate the potential impact of the increased levels of blood lipids, glucose, and urea on different RBC indices, Plt, and WBC count by automated impedance cell counters.
Materials and Methods: This Analytical study performed on 366 individuals with hyperglycemia, hyperlipemia, or uremia and 120 randomly selected healthy participants referring to Imam Hospital of Minoodasht city from February to June 2012. Initially, glucose, triglyceride, and urea levels were measured by Biochemical autoanalyzer and then complete blood cell count was done for each participant. Eventually statistical analysis and comparison between case and control groups were done by SPSS v.16 software.
Results: Comparison between case and control groups revealed that uremia caused significant increase in MCV and RDW levels ($P < 0.05$) and had no effect on MCH level ($P > 0.05$). Both hyperglycemia and hyperlipemia caused increased in MCV level ($P < 0.05$). In addition, hyperlipemia increased Hb, Hct, and MCH levels ($P < 0.05$).
Conclusions: According to the results of this study, effect of biochemical interfering factors on test results of impedance cell counter must be considered in clinical laboratories.

Keywords: Uremia; Hyperglycemia; Hyperlipidemias; Blood Cell Count

1. Background

A complete blood count measures several components and features of blood cells and gives important information about them. In most clinical laboratories, there are three types of automated machines used for blood counts including electrical impedance counter, optical method counters, and flowcytometry (1, 2). These automated instruments can estimate the different red blood cells (RBC) indices, platelets (Plt), and white blood cells (WBC) counts (3). Impedance offers a simple method for counting blood cells (4). Due to the principle used in this method, different factors in analytical and preanalytical stages can affect the results of test. The presence of interfering substances is the most common preanalytic factor that can affect the precision and accuracy of the results produced by impedance cell counter. These substances are classified as endogenous and exogenous interferences (5, 6).

Endogenous interferences consist of some important factors such as hemolysis, bilirubinemia, lipemia, hyperglycemia, and paraproteinemia. Exogenous interferences include drugs and chemical additives that are used in some devices (3, 5, 6). Hyperlipidemia as an endogenous interference manifests as abnormally increased lipids or lipoproteins in the blood. It is classified in two types: primary and secondary (7). Hyperlipidemia causes increase in hemoglobin and MCHC especially in cases with higher serum turbidity. Hyperlipidemia can also increase the WBC count (3, 8, 9). Alteration of plasma lipids leads to an enhanced RBC aggregation (10).

Hyperglycemia (high blood sugar) occurs when an ex-

Implication for health policy/practice/research/medical education:
Biochemical interfering factors such as hyperglycemia, hyperlipemia and uremia can alter results of blood cell indices and led to misdiagnosis. This manuscript determined the interfere level of these factors with blood cell indices on automated impedance cell counters.

cessive glucose circulate in plasma (11). In automated cell counters, severe hyperglycemia results in falsely high mean corpuscular volume (MCV). This effect seems to be secondary to the osmotic imbalance between RBCs and the diluents used in cell counters. The diversity of cell sizes depend on the temperature, glucose concentration, and the type of cell counter (3, 12, 13). Uremia affects RBCs mechanical properties such as deformability and fragility that results in morphologically deformed erythroid cells (echinocytes) (14). The frequency of clinical laboratory interferences are difficult to determine; hence, they are largely underestimated (6, 15).

2. Objectives

This study evaluates the effects of biochemical Interfering factors include hyperglycemia, hyperlipemia, and uremia on blood cells indices like the different RBC indices, Plt, and WBC count.

3. Materials and Methods

3.1. Study Design

This Analytical study was conducted on 366 individuals referring to Imam Hospital of Minoodasht city from February to June 2012. Samples from 120 healthy individual with normal level of blood glucose, triglyceride, cholesterol, urea, and creatinine were obtained as controls. The study was conducted after achieving Ethic Committee approval and all the participants signed a written consent form.

3.2. Data Collection

Inclusion criteria were 366 individuals with increased plasma glucose (120), lipids (123), or uremia (123) and exclusion criteria were patients with anemia, special drugs, and hemolyzed samples. Initially, two samples from each patient, one in EDTA added tube and another in a tube without anticoagulant were obtained. For the measurement of serum triglyceride (PARS AZMON lot no. 90013), cholesterol (PARS AZMON lot no. 90005), fast blood sugar (PARS AZMON lot no. 91008), creatinine (PARS AZMON lot no. 90008), urea (PARS AZMON lot no. 91002), serum was separated and analyzed by Mindray chemistry analyzer (BS-200 China). complete blood cell count (CBC) and determination of blood cell indices were done by using EDTA added samples with Sysmex (kx 21 japan) cell counter. This device work is based on impedance technology. This procedure was repeated for an additional 120 randomly selected individuals (for each parameter) as control group.

3.3. Data Analysis

Statistical analysis was done by SPSS version 16 statistical software (SPSS Inc., Chicago, Illinois, USA). We used t-independent test for statistical comparison of two groups and $P < 0.05$ was considered significant.

4. Results

In the case group of uremia, there were 198 (54%) men and 168 (45%) women; in hyperglycemia group there were 192 (52%) men and 174 (48%) women; there were 178 (49%) men and 188 (51%) women in hyperlipidemia group. The average age of participants was 49.48 ± 3.2, 43.67 ± 2.8, and 45.89 ± 1.9 respectively. The mean age of control group was 46.32 ± 2.7 and this group consisted of 58 48% men and 62 52% women. In addition, the average level of urea, glucose, triglyceride, and cholesterol in patients' plasma were 92 ± 2.1, 217 ± 1.9, 346 ± 2.1, and 241 ± 2.5, respectively. The levels of these parameters in control group were 25, 81, 105, and 165, respectively. Statistical analysis of these biochemical parameters and their comparison with the control group were shown in separate tables. In case of uremia, there was a statistically significant different between case and control groups with regard to Hb, RBC count, and Hct ($P < 0.05$) (Table 1).

With regard to hyperlipidemia, comparison between patient and control groups revealed a significant difference between patient and control groups in the levels of Hb, Hct, and MCV ($P < 0.05$).

Table 1. Statistical Characteristics of Individuals With Uremia and Healthy Participants [a]

	Groups	Number	Mean ± SD	P Value
Hb	case	123	12.21 ± 2.06	0.0001
	control	120	13.33 ± 1.12	
RBC	case	123	4.28 ± 0.74	0.0001
	control	120	4.70 ± 0.44	
HCt	case	123	34.70 ± 9.12	0.0001
	control	120	39.39 ± 4.61	
MCV	case	123	86.75 ± 7.69	0.008
	control	120	84.45 ± 4.36	
MCH	case	123	28.55 ± 3.14	0.661
	control	120	28.39 ± 2.07	
MCHC	case	123	32.82 ± 1.65	0.001
	control	120	33.58 ± 1.57	
WBC	case	123	8.35 ± 3.01	0.0001
	control	120	6.98 ± 1.67	
Plt	case	123	229.60 ± 76.6	0.063
	control	120	246.70 ± 55.22	
RDW	case	123	13.96 ± 1.52	0.0001
	control	120	13.06 ± 0.76	

[a] Abbreviations: Hb, Hemoglobin; HCt, Hematocrit; MCH, Mean Cell Hemoglobin; MCHC, Mean Cell Hemoglobin Concentration; MCV, Mean Cell Volume; Plt, Platelet; RDW, Red Cell Distribution Width RBC, Red Blood Cell; WBC, White Blood Cell.

Statistical analysis of patients with hyperglycemia revealed that MCV and Hct have significant different ($P < 0.05$) in these two groups; however, there was no significant different in the levels of RDW, MCH, and MCHC ($P > 0.05$).

Table 2. Statistical Characteristics of Individuals With Hyperlipemia and Healthy Participants [a]

	Groups	Number	Mean ± SD	P Value
Hb	case	123	13.89 ± 1.67	0.005
	control	120	13.33 ± 1.12	
RBC	case	123	4.79 ± 0.51	0.169
	control	120	4.70 ± 0.44	
HCt	case	123	41.26 ± 4.08	0.002
	control	120	39.39 ± 4.61	
MCV	case	123	86.36 ± 5.87	0.007
	control	120	84.45 ± 4.36	
MCH	case	123	29.03 ± 2.60	0.047
	control	120	28.39 ± 2.07	
MCHC	case	123	35.50 ± 2.92	0.361
	control	120	33.58 ± 1.57	
WBC	case	123	7.86 ± 2.33	0.002
	control	120	6.98 ± 1.67	
Plt	case	123	274.05 ± 72.46	0.002
	control	120	246.70 ± 55.22	
RDW	case	123	12.98 ± 0.83	0.479
	control	120	13.06 ± 0.76	

[a] For abbreviations please refer to table 1.

Table 3. Statistical Characteristics of Individuals With Hyper Glycemia and Healthy Persons [a]

	Groups	Number	Mean ± SD	P Value
Hb	case	120	13.55 ± 1.62	0.259
	control	120	13.33 ± 1.12	
RBC	case	120	4.88 ± 0.52	0.007
	control	120	4.70 ± 0.44	
HCt	case	120	40.61 ± 3.51	0.027
	control	120	39.39 ± 4.61	
MCV	case	120	86.62 ± 7.63	0.007
	control	120	84.45 ± 4.36	
MCH	case	120	28.94 ± 8.33	0.517
	control	120	28.39 ± 2.07	
MCHC	case	120	33.31 ± 1.82	0.250
	control	120	33.58 ± 1.57	
WBC	case	120	8.09 ± 1.95	0.0001
	control	120	6.98 ± 1.67	
Plt	case	120	267.09 ± 63.68	0.013
	control	120	246.70 ± 55.22	
RDW	case	120	13.33 ± 1.38	0.079
	control	120	13.06 ± 0.76	

[a] For abbreviations please refer to table 1.

5. Discussion

Different factors in preanalytical and analytical stages have influences on tests results produced by impedance cell counter. Some of these factors cause rejection of the samples and some of them interfere with the results of the tests (5, 6). In order to minimize these effects, the sample must be rejected if some problems were observed in it. These problems include improper collection, inadequately labeled sample, improper collection containers, and inappropriate storage temperature (5).

Hyperlipidemia is usually associated with increase of hemoglobin concentration, MCHC, and WBC count (3, 8, 9). In our study, hyperlipidemia with increased level of both triglyceride and cholesterol caused a significant increase in most blood cell count indices include Hb, Hct, MCV, MCH, and Plt count ($P < 0.05$); on the other hand, MCHC and RDW indices remained unchanged. Sadeghian et al. showed hypertriglyceridemia can falsely increase MCHC but cannot alter MCV or MCH and hypercholesterolemia does not alter the red cell indices (9). Sandberg et al. reported increased in hemoglobin concentration with elevated amounts of lipid in the samples in some cell counters (16).

Uremia is a condition resulting from the accumulation of organic waste products and causes true thrombocytopenia and spurious leukopenia. Although renal transplantation and dialysis are the treatment of uremia, because of short supply of kidney donors, the most effective treatment is dialysis (14). The result of our study showed that uremia caused an increased in the MCV and RDW ($P < 0.05$) and a decrease in RBC count, Hb, Hct, and MCHC ($P < 0.05$); on the other hand, it had no effect on platelet count and MCH ($P > 0.05$).

Hyperglycemia is a condition with high amount of glucose in blood stream and results in falsely high mean corpuscular volume (MCV) due to the osmotic imbalance between red blood cells and the diluents (3, 12, 13). Strauchen et al. added glucose to normal blood at the concentrations of 1000 and 2000 mg/dL that resulted in consistent elevation of the MCV and hematocrit and depression of the MCHC in contrast to control samples without such a hyperglycemic environment (13). Planas et al. showed that the spurious macrocytosis in electronic cell counters was due to hyperglycemia (12).

According to the results of this study and other similar works, we recommend that the effect of interferences such as hyperlipidemia, hyperglycemia, and uremia on impedance cell counter test results must be considered in clinical laboratories and appropriate measures must be evaluated to correct these errors.

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We would like to dedicate this manuscript to the noble soul of Mr. Mohammad Mahmoodi.

Authors' Contribution

Hosnie Hosseini and Akbar Dorgalaleh were responsible for the study conception and design; Shadi Tabibian, Meysam Kashiri, Samira Esmaeili performed the data collection; Esmaeil Sanei Moghaddam analyzed the data ; Shaban Alizadeh and Taregh Bamedi were in charge of the drafting of the manuscript; Akbar Dorgalaleh supervised the study.

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References

1. Kickler TS. Clinical Analyzers. Advances in Automated Cell Counting. *Anal Chem.* 1999;**71**(12):363–5.
2. Weiss DJ. Application of flow cytometric techniques to veterinary clinical hematology. *Vet Clin Pathol.* 2002;**31**(2):72–82.
3. Zandecki M, Genevieve F, Gerard J, Godon A. Spurious counts and spurious results on haematology analysers: a review. Part II: white blood cells, red blood cells, haemoglobin, red cell indices and reticulocytes. *Int J Lab Hematol.* 2007;**29**(1):21–41.
4. DeNicola DB. Advances in hematology analyzers. *Top Companion Anim Med.* 2011;**26**(2):52–61.
5. Kazmierczak SC, Catrou PG. Analytical interference. More than just a laboratory problem. *Am J Clin Pathol.* 2000;**113**(1):9–11.
6. Kroll MH, Elin RJ. Interference with clinical laboratory analyses. *Clin Chem.* 1994;**40**(11 Pt 1):1996–2005.
7. Chait A, Brunzell JD. Acquired hyperlipidemia (secondary dyslipoproteinemias). *Endocrinol Metab Clin North Am.* 1990;**19**(2):259–78.
8. Kroll MH. Evaluating interference caused by lipemia. *Clin Chem.* 2004;**50**(11):1968–9.
9. Sadeghian MH. Correlation Between Hyperlipemia and Erythrocytes Indexes. *Int J Hematol Oncol.* 2008;**18**(3):150–4.
10. Cicha I, Suzuki Y, Tateishi N, Maeda N. Enhancement of red blood cell aggregation by plasma triglycerides. *Clin Hemorheol Microcirc.* 2001;**24**(4):247–55.
11. Giugliano D, Ceriello A, Esposito K. Glucose metabolism and hyperglycemia. *Am J Clin Nutr.* 2008;**87**(1):217S–22S.
12. Planas AT, Van Voolen GA, Kelly LA. Hyperglycemic macrocytosis in electronically determined mean corpuscular volume. Use of three different automatic cell counters. *Ann Clin Lab Sci.* 1985;**15**(4):286–91.
13. Strauchen JA, Alston W, Anderson J, Gustafson Z, Fajardo LF. Inaccuracy in automated measurement of hematocrit and corpuscular indices in the presence of severe hyperglycemia. *Blood.* 1981;**57**(6):1065–7.
14. Meyer TW, Hostetter TH. Uremia. *N Engl J Med.* 2007;**357**(13):1316–25.
15. Steen G, Vermeer HJ, Naus AJ, Goevaerts B, Agricola PT, Schoenmakers CH. Multicenter evaluation of the interference of hemoglobin, bilirubin and lipids on Synchron LX-20 assays. *Clin Chem Lab Med.* 2006;**44**(4):413–9.
16. Sandberg S, Sonstabo K, Christensen NG. Influence of lipid and leukocytes on the haemoglobin determination by Coulter Counter S Plus III, Technicon H 6000, Technicon H 1, LK 540, Reflotron and Hemocap. *Scand J Clin Lab Invest.* 1989;**49**(2):145–8.