Effect of the addition of the antioxidant taurine on the complete blood count of whole blood stored at room temperature and at 4°C for up to 3 days

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Abstract

Background: The complete blood count is one of the most common routine tests. This study aimed to evaluate possible effects of the antioxidant taurine on the complete blood count of whole blood stored at room temperature and at 4°C over three days.

Methods: Venous blood samples of 15 healthy males were distributed into two sets of tubes with each set of three tubes containing 50 µL of solutions with zero, 5 g/L, 10 g/L taurine. The tubes were kept at room temperature or at 4°C. Complete blood counts were performed on three successive days. The mean percentage changes [Δ = (mean value - mean baseline value) / mean baseline value x 100] were calculated and compared.

Results: Complete blood count parameters exhibited different patterns of behaviour which were affected by the storage temperature, time and taurine concentration. Taurine at room temperature significantly enhanced the stability of: the platelet count over three days (Δ3 at 5 and 10 g/L taurine were 6.18, and 2.53 x 10^9 cells/L, respectively); the red blood cell count over three days (Δ3 at 5 and 10 g/L taurine were 2.59, and 1.39 x 10^{12} cells/L, respectively); mean corpuscular haemoglobin over three days (Δ3 at 5 and 10 g/L taurine were,-0.62 and -0.52 fl respectively); and red cell distribution width over two days (Δ2 at 5 and 10 g/L taurine were 1.30% and -0.1%, respectively). No additional stabilizing effects of taurine were reported for the mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, haematocrit and haemoglobin, while it negatively affected the white blood cell stability.

Conclusion: Complete blood count parameters exhibited variable stability patterns in respect to temperature, time and taurine concentration.

Keywords: Blood preservation; Taurine/blood; Platelet count; Antioxidants; Cold temperature

Introduction

The complete blood count (CBC) is one of the most common routine laboratory tests requested as the first step to diagnose an illness or clinical presentation. With the development of automated haematological analysers, the CBC has become an easy, quick, and reliable test that can give valuable information to physicians, leading to provisional diagnosis and to direct further testing. Currently, most clinical laboratories are equipped with modern automated analysers that are capable of processing a large number of haematological tests in an efficient and timely manner (1,2). However, for reliable results, it is essential that specimens are collected properly and in the right anticoagulant and then examined in a calibrated analyser within a specified time

frame according to the manufacturer instructions (3-6).

The recent trends towards large centralized laboratories, and changes in laboratory organizations, have brought a new perspective to redistribution. Laboratories now test specimens that have been dispatched over long distances; as a result, testing is often delayed by 12–24 hours or more after venepuncture. Moreover, at weekends, this interval may exceed 36 hours. Therefore, when such specimens arrive in the laboratory, technicians must decide on whether to accept or refuse the processing of the specimens (6). Although laboratories should maintain the consistency of results, disproportionate delays in processing might affect the reliability of results (7).

Taurine (2-aminoethanesulfonic acid - $NH_2CH_2CH_2SO_3H$), which is a naturally-occurring ß-sulfonated amino acid, is one of the most abundant free amino acids in the human body (8); a 70 kg person can have up to 70 g of taurine (9). Taurine has been demonstrated to function as a direct or indirect antioxidant, inhibiting lipid peroxidation, and stabilizing bio-membrane structures and function by preventing any increase in membrane permeability due to the effect of oxidants, maintaining intracellular ion homeostasis, and inhibiting membrane protein phosphorylation (8-10).

Different studies have revealed the stability of whole blood samples when stored at a low temperature or when certain anticoagulants are used (2,5,11-13), however, to the best of our knowledge no published studies have investigated or considered the effect of antioxidants on the stability of CBC parameters. Therefore, due to the aforementioned effects of taurine, the present work was designed to evaluate any possible effects of the antioxidant taurine on the reliability of the CBC of whole blood specimens stored *in vitro* at room temperature and at 4^{o}C over three days. The complete blood count is one of the most common routine tests. This study aimed to evaluate possible effects of the antioxidant taurine on the complete blood count of whole blood stored at room temperature and at 4°C over three days.

Material and Methods

Pre-research planning: Before the start of research, a pre-research planning was done in which all the aspects were considered. It included selection of research site, target population, sample size, self-designed proforma, sampling method, research methodology, organizational issues and work plan. Logistics and ethical implications were thoroughly discussed with the supervisor at Department of Pathology, King Edward Medical University Lahore.

Study design: This was a cross-sectional study.

Study setting: Study was conducted at the Pathology laboratory of King Edward Medical University.

Sample size: Sample size of 25 patients is estimated by using 95% confidence level, 3% absolute precision with expected %age of patients as 87.6%.

$$n = \frac{Z^2_{1-a/2} \cdot p.q}{d^2}$$

$Z^2_{1\text{-}a/2}$ = Confidence level 95% = 1.96

p=Prevalence 40.8%

q = 1 - P

d = Absolute precision

Twenty-five healthy non-smoking male/female university students (18-25 years old)

Sampling technique: Venous blood samples (20 mL each) were collected in K3-ethylenediaminetetraacetic acid (EDTA) tubes**.**

Sample inclusion criteria: Non-smoker healthy individuals with age 18-25 years

Exclusion Criteria: Those with active infections & with any disease.

Data collection procedure: Venous blood samples of 15 healthy males were distributed into two sets of tubes with each set of three tubes containing 50 µL of solutions with zero, 5 g/L, 10 g/L taurine. The tubes were kept at room temperature or at 4°C. Complete blood counts were performed on three successive days. The mean percentage changes [Δ = (mean value - mean baseline value) / mean baseline value x 100] were calculated and compared. After the approval of the study by both the Pathology Department and the Institutional Review Board (IRB) of King Edward Medical University, Lahore, apparently healthy non-smoking male university students (18-24 years old) were informed about the objectives and scope of the study and invited to participate. Twenty-five students freely accepted, and all signed the consent form of the study thus accepting enrolment and the drawing of venous blood.

Venous blood samples (20 mL each) were collected in K3- ethylenediaminetetraacetic acid (EDTA) tubes and distributed almost equally into two groups. One set of tubes was kept at the ambient room temperature (23 ± 2^{o}C) while the other set was kept refrigerated at 4^{o}C. Each set contained four K3-EDTA tubes, and each tube represented a study subgroup. Therefore, three study subgroups (one control and two with different taurine concentrations) were included in each set for the present study.

For each set, CBC measurements [white blood cell (WBC), red blood cell (RBC), haemoglobin (Hb), haematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), and platelets (PLT)] were performed at the time of collection and then daily for the following three days using a Sysmex KX-21 Haematology Analyser.

Study subgroups

The three subgroups at each storage temperature were as follows:

Subgroup I:

2.5 mL of venous blood was collected in K_3-EDTA and 50 µL of normal saline solution, no addition of taurine (Control Group).

Subgroup II:

2.5 ML of venous blood was collected in K_3-EDTA and 50 µL of 5 g/L taurine solution (ten times the normal plasma taurine concentration) was added.

Subgroup III :

2.5 mL of venous blood was collected in K_3-EDTA and 50 µL of 10 g/L taurine solution (twenty times the normal plasma taurine concentration) was added.

Statistical analysis

The IBM SPSS program (version21, IBM Corporation, Somers, NY) was used for all statistical analyses in the study. Comparisons between the mean values of the haematological parameters were made using the t-test. One-way analysis of variance (ANOVA) was used to compare the means of

more than two groups. Any two-tailed p-value < 0.05 was considered to be statistically significant. In addition, for each parameter the mean percentage change was calculated and compared, where i represents the day during the storage period.

$$\Delta_i = \left[\frac{mean\ value\ on\ day\ _I - mean\ basline\ value}{mean\ base\ line\ value\ X\ 100}\right]$$

Results:

Baseline values and reliability of complete blood count parameters: There were no significant differences between the baseline means of all CBC parameters of samples stored at room temperature and at 4°C either when compared to the control group or between different taurine subgroups (p-value > 0.05). The reliability of different CBC parameters was evaluated in terms of stability from baseline values. Non-significant differences or changes from baseline values indicate stability and hence reliability. Moreover, for each parameter the mean percentage change (Δ_i) was calculated over the three days compared to baseline values, which could be used to describe the level of precision of the readings compared to baseline values. So, when a precision level of 5% is acceptable this is reflected by $\Delta_i \leq \pm$ 5%.

Platelet count and mean platelet volume: The PLT count changed significantly and was affected by storage temperature and time (Table1). However, storage with taurine at room temperature considerably enhanced the stability of the PLT count over three days, with no significant differences compared to the baseline. In terms of mean percentage changes, all the values were within ± 10% except for the 3rd day. While storage of blood in taurine at 4°C showed no remarkable effect toward the stability of the PLT count. Neither storage temperature nor the addition of taurine at the different concentrations stabilized mean platelet volume (MPV). The ANOVA statistical test showed significant changes of MPV over the three days of storage, with a trend towards an increase in MPV. The mean percentage change in MPV increased considerably with time and by the 3rd day it reached values of 24.97% and 28.60% at room temperature and at 4°C, respectively.

Red blood cell count and related indices: Table 2 illustrates the instability of the RBC count over time with significantly different values from baseline (p-value < 0.05). However, taurine at 10 g/L tends to exert some stabilizing effect at room temperature over the first three days of storage. While the mean percentage changes in the RBC count at day three were almost 2.79% and 2.97% at room temperature and at 4°C. At all investigated concentrations, taurine did not enhance the stability of MCV at room temperature or at 4°C. The MCV was stable for two days both at room temperature and at 4°C (Table 3). However mean percentage changes of MCV increased over time but these changes were lower at 4°C with a value of about 10% by day three compared to 3.8% at room temperature. MCH values were found to be unstable over the three days both at room temperature and at 4°C (Table 4). However, the addition of taurine increased the stability of MCH at both temperatures. Mean percentage changes in MCH were very small with a maximum change of 2.25%. Additionally, MCHC values were not stable at both temperatures even with the addition of taurine. The values of MCHC decreased

significantly with time. However, mean changes in MCHC values were less than 10% for three days at room temperature without showing any significant effect of taurine. Hct at room temperature and at 4ºC showed significant increases over the 3rd days of storage. Taurine did not stabilize Hct at both temperatures, however, the mean percentage changes in Hct values were less than 10% over three days at room temperature whether taurine was added or not. The Hb concentration remained stable over the three days of storage compared to baseline values at room temperature and at 4ºC, and thus there was no advantage on the stability of Hb with the addition of taurine (Table 5). The mean percentage change was almost 1% in the Hb concentration over the three days without any significant effect of taurine. At room temperature, the RDW changed significantly over time starting from the second day. However, taurine at the different concentrations stabilized RDW readings for two days after which taurine had no stabilizing effect and all RDW readings after the second day were significantly different from the baseline values. While at 4ºC, the RDW values were stable for two days with and without taurine. Moreover, storage with taurine at 5 g/L enhanced the stability of RDW value for an additional 24 hours. The mean changes in RDW were less than 10% at room temperature for the first three days and less than 10% at 4ºC for 5 days.

White blood cell count: WBC count was stable with non-significant changes from the baseline value when preserved at room temperature over three days (Table 6). The addition of taurine at 2.5 g/L did not alter the stability, however, increasing the concentration of taurine to 5 and 10 g/L, negatively affected the stability of WBC counts. On the other hand, at 4ºC the WBC counts were stable only for three days compared to baseline values. The changes in the WBC counts at room temperature were less than 4% over the three days, while the changes reached almost 20% when the sample was kept at 4ºC.

Discussion: The CBC or hemogram is a routine laboratory test that evaluates number, size, morphology and related indices of the blood: RBC, WBC, and PLT. Significant time-and temperature-dependent morphological changes can occur with the prolonged storage of blood (6,7,13,15,16). The CBC as well as blood smears should be processed as quickly as possible after collection to avoid normal degenerative changes in blood cell morphology, which are mainly due to weakness in controlling cell membrane integrity, and consequently changing cell volume and related indices. Most importantly, blood cells can rupture after swelling (17). Different studies have been performed to evaluate the effect of storage time and temperature on the stability of CBC parameters and indices. Most of such studies define stability in terms of mean percentage changes and authors usually considered parameters stable when the mean percentage change is within 5% of baseline values (7,15,16,18). However, other studies defined stability differently and used the ANOVA or t-test to identify significant differences compared to baseline values over the study period (13,19,20). In the current work both approaches were used and presented, however, parameters or indices were considered stable when there were no

significant differences in the mean values compared to baseline values according to the ANOVA or t-test. Furthermore, it is worth mentioning that none of the published studies investigated the effect of antioxidants on the stability of CBC parameters. Thus, to our best knowledge, this work may be considered the first work where the possible effects of antioxidants are investigated and discussed.

Baseline values were not significantly different between the two sets of tubes and subgroups which shows the homogeneity of the data. It is worth mentioning that all investigated blood samples were drawn from apparently healthy never-smoker males which minimizes any interfering or confounding factors related to physiological and haematological differences between males and females and also between smokers and non-smokers (21).

The PLT count was found to be considerably changed over time with no stabilizing effect of storage temperature. These changes could be attributed to alterations in PLT morphology, movement and aggregation during storage (13). However, taurine significantly enhanced PLT stability at room temperature, but not at 4^{o}C where fluctuating and dissentious stabilities are observed. Therefore, for the PLT count, the preservation of samples at room temperature with taurine, even at low concentrations such as 5 g/L, could enhance stability and provide reliable results for at least three days. The instability of the PLT count over time, whether kept at room temperature or refrigerated at 4^{o}C, are concomitant to the findings of other works (15,16,20) although one study in 2008 reported a better stability of the PLT count at room temperature (18).

These stabilizing effects of taurine on the PLT count may be attributed to different mechanisms including the stabilization of the PLT membrane, re-supplementing PLT with taurine *in vitro* (9), an antithrombotic effect (22,23) and decreased PLT aggregation (24).

The RBC count was found to be instable over time as indicated by the significant differences between means. While, when defining stability in terms of mean percentage changes, the RBC count could be considered stable within a precision of ± 5%, which is comparable to the findings of other researchers (7,16,18). The stabilizing effect of taurine on RBC might be attributed to its antioxidant role by scavenging free radicals that stimulate membrane lipid peroxidation and hence stabilize the RBC membrane (7,9,10). Therefore, the addition of taurine could be suggested for late-arriving EDTA blood samples when the evaluation of RBC count is of central importance.

Although, taurine was found to exert stabilizing effects on PLT and RBC counts, it did not stabilize the mean volumes of these blood components, the MPV and MCV, respectively, as have been observed elsewhere (6,7,15,16). This may be attributed to the preservation of structural integrity of their membranes by taurine but not the functional transport across the membrane which seems to be independent of the action of taurine.

It is worth mentioning that the addition of taurine was found to exert some stability on the MCH values both at room temperature and at 4^{o}C for three days, respectively, but at different concentrations. The instability of MCHC values at different temperatures and despite the addition of taurine may be

attributed to the increasing MCV over time and consequently increases in Hct which is the denominator for calculating MCHC. On the other hand, haemoglobin concentration was found to be stable over the three days of the study at both temperatures, which is concomitant to other findings (7,16,19). Storage with taurine did not enhance haemoglobin stability. Therefore, haemoglobin concentration can be considered reliable for one week whether the blood is kept at room temperature or at 4^{o}C. Effectively, taurine exerted a stabilizing function on the RDW values both at room temperature and at 4^{o}C. However, it exerted a negative effect on the stability of the WBC count, therefore, WBC counts of blood preserved in EDTA at room temperature without taurine are more reliable as these conditions will provide laboratories with reliable results for three days.

Haematological changes of whole blood collected for diagnosis and for transfusion purposes may be co-related. Therefore, we recommend that other researchers investigate the addition of taurine together with anticoagulant-preservative solutions such as acid-citrate dextrose (ACD) and citrate phosphate dextrose adenine (CPDA-1) on the physiological and biochemical properties of whole blood used for transfusion purposes. We expect promising effects of taurine due to its many important and beneficial effects and safety in the human body and on aforementioned cells.

Conclusion:

The different CBC parameters and their related indices exhibited variable stability patterns according to storage temperature, time of storage and the addition of taurine. Some parameters and indices are more stable at 4^{o}C than at room temperature (RDW, RBC), one is more stable at room temperature than at 4^{o}C (WBC), and others exhibited different levels of stability at the different temperatures (MCV, Hb). Several are limited or unstable at any temperature over time (PLT, MPV, MCH, MCHC, Hct) while for some, the stability could be partially or totally enhanced by the addition of taurine (PLT, RDW, MCH).

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Table 1 - Platelet count (x 10^9 cells/L) for samples stored at room temperature and at 4ºC

Parameter	Day1 Baseline Mean (SD)	Day 2 Baseline Mean (SD)	Δ2	Day 3 Baseline Mean (SD)	Δ3
Room Temperature Control	220.08 (51.20)	235.68 (57.79)	7.09	237.00 (55.49)	7.69
2.5 g/L taurine	228.20 (65.52)	231.80 (58.19)	6.18	228.16 (63.31)	6.18
10 g/L taurine	228.08 (54.66)	230.52 (65.06)	2.53	233.84 (64.16)	2.53
4ºC Control	222.96 (57.16)	232.28 (60.60)	4.18	226.52 (54.36)	1.60
5 g/L taurine	229.48 (62.09)	229.52 (58.14)	0.02	232.76 (56.86)	1.43
10 g/L taurine	236.12 (51.70)	244.92 (48.95)	3.73	244.68 (48.00)	3.63

Data presented as mean ± SD

Δ = mean percentage changes = [(mean value - mean baseline value) / mean baseline value] x100

* No significant changes were found compared to the baseline value

Table 2- Red blood cell count (x 10^{12} cells/L) for samples stored at room temperature and at 4ºC.

Parameter	Day1 Baseline Mean (SD)	Day 2 Baseline Mean (SD)	Δ2	Day 3 Baseline Mean (SD)	Δ3
Room Temperature Control	5.04 (0.70)	5.13 (0.70)	1.79	5.13 (0.73)	1.79
5 g/L taurine	5.01 (0.66)	5.10 (0.69)	1.80	5.14 (0.72)	2.59
10 g/L taurine	5.05 (0.67)	5.09* 0.72	0.79	5.12 (0.72)	1.39
4ºC Control	5.05 (0.75)	5.10 (0.74)	0.99	5.13 (0.75)	1.58
5 g/L taurine	5.10 (0.75)	5.17 (0.73)	1.37	5.16* (0.70)	1.18
10 g/L taurine	5.07 (0.75)	5.16 (0.73)	1.78	5.17 (0.72)	1.97

Data presented as mean ± SD

Δ = mean percentage changes = [(mean value - mean baseline value) / mean baseline value] x100

* No significant changes were found compared to the baseline value

Table 3 - Mean corpuscular volume (fl) for samples stored at room temperature and at 4ºC

Parameter	Day1 Baseline Mean (SD)	Day 2 Baseline Mean (SD)	Δ2	Day3 Baseline Mean (SD)	Δ3
Room temperature					
Control	75.10 (9.65)	76.36* (10.05)	1.68	78.00 (10.06)	3.86
5 g/L taurine	74.63 (9.54)	75.62 (9.51)	1.33	78.02 (9.88)	4.54
10 g/L taurine	74.80 (9.78)	76.21 (9.89)	1.89	78.02 (9.91)	4.30
4ºC					
Control	75.23 (9.85)	75.28 (10.11)	0.07	76.00 (10.01)	1.02
5 g/L taurine	74.41 (9.93)	74.50 (9.35)	0.90	75.49 (9.86)	1.45
10 g/L taurine	74.69 (9.70)	74.54 (9.58)	-0.20	75.24 (9.71)	0.74

Data presented as mean ± SD

Δ = mean percentage changes = [(mean value - mean baseline value) / mean baseline value] x100

* No significant changes were found compared to the baseline value

Table 4 - Mean cell haemoglobin (pg) for samples stored at room temperature and at 4ºC

Parameter	Day1 Baseline Mean (SD)	Day 2 Baseline Mean (SD)	Δ2	Day 3 Baseline Mean (SD)	Δ3
Room temperature					
Control	27.61 (4.06)	27.40 (4.08)	-0.76	27.33 (4.14)	-1.01
5 g/L taurine	27.59 (3.95)	27.40 (4.02)	-0.69	27.42 (4.09)	-0.62
10 g/L taurine	27.42 (4.07)	27.48* (4.11)	0.22	27.27* (4.04)	0.52
4ºC					
Control	27.69 (4.24)	27.36 (4.13)	-1.19	27.33 (4.14)	-1.30
5 g/L taurine	27.43 (4.17)	27.23* (3.98)	-0.73	27.13 (4.00)	-1.09
10 g/L taurine	27.53 (4.08)	27.24 (4.06)	-1.05	27.16 (4.04)	-1.34

Data presented as mean ± SD

Δ = mean percentage changes = [(mean value - mean baseline value) / mean baseline value] x100

* No significant changes were found compared to the baseline value

Table 5 - Haemoglobin concentration (g/dL) for samples stored at room temperature and at 4ºC

Parameter	Day1 Baseline Mean (SD)	Day 2 Baseline Mean (SD)	Δ2	Day 3 Baseline Mean (SD)	Δ3
Room temperature					
Control	13.65 (0.98)	13.80* (1.06)	1.10	13.75* ((0.99)	0.73
5 g/L taurine	13.66 (1.04)	13.74 (1.03)	0.59	13.84 (1.05)	1.32
10 g/L taurine	13.62 (0.96)	13.72 (1.01)	0.73	13.70 (1.00)	0.58
4ºC					
Control	13.70 (1.03)	13.79 (1.06)	0.65	13.76 (1.04)	0.44
5 g/L taurine	13.70 (0.96)	13.84 (1.01)	1.02	13.76 (0.98)	0.44
10 g/L taurine	13.69 (1.00)	13.78 (0.98)	0.66	13.79 (1.00)	0.73

Data presented as mean ± SD

Δ = mean percentage changes = [(mean value - mean baseline value) / mean baseline value] x100

* No significant changes were found compared to the baseline value

Table 6 - White blood cell count (x 10^9 cells/L) for samples stored at room temperature and at 4ºC

Parameter	Day1 Baseline Mean (SD)	Day 2 Baseline Mean (SD)	Δ2	Day 3 Baseline Mean (SD)	Δ3
Room temperature					
Control	5.55 (1.44)	5.51* (1.30)	-0.72	5.53* (1.35)	-0.36
5 g/L taurine	5.52 (1.33)	5.52* (1.32)	0.00	5.63* (1.45)	1.99
10 g/L taurine	5.53 (1.31)	5.68 (1.38)	2.71	5.60* (1.40)	1.27
4ºC					
Control	5.73 (1.43)	5.89* (1.46)	2.79	5.78* (1.39)	0.87
5 g/L taurine	5.59 (1.40)	5.63* (1.38)	0.72	5.66* (1.34)	1.25
10 g/L taurine	5.63 (1.36)	5.77 (1.46)	2.49	5.70* (1.43)	1.24

Data presented as mean ± SD

Δ = mean percentage changes = [(mean value - mean baseline value) / mean baseline value] x100

* No significant changes were found compared to the baseline value