Comparison of Vitamin B_{12} Levels in Light-Protected and Normal Tubes

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ABSTRACT

Objective: Vitamin B_{12} (cyanocobalamin) is a water soluble hematopoietic vitamin that functions in somatic cell metabolism. Vitamin B_{12} is commonly measured by direct chemiluminescence immunoassay methods in routine biochemistry laboratories. Serum samples to be tested for vitamin B_{12} are recommended to be kept protected from light. On the basis of this information, we aimed to evaluate the effect of light and waiting time till analysis on vitamin B_{12} tests.

Methods: In total, 52 volunteers (27 women and 25 men) were included in our study. To prevent the effect of light, we prepared darkened tubes for blood collection. Simultaneously, two tubes of blood samples (one darkened and one normal) were drawn from each patient. The measurements were performed on the 0th and 12th hours using the Roche Hitachi Modular E 170 autoanalyser. The Mann-Whitney U test and Wilcoxon test were used, and $p < 0.05$ was considered significant.

Results: There were no significant differences in vitamin B_{12} levels between darkened and normal tubes on the 0th and 12th hours ($p > 0.05$). In comparison with the baseline (0th hour), the percentage difference in darkened tubes were found to be significantly decreased on the 12th hour ($p < 0.05$).

Conclusion: Our findings indicate that the need for the darkened tubes may be ignored when cost-effectiveness is considered. *(JAREM 2015; 5: 14-6)*

Keywords: Vitamin B_{12}, immunoassay, preanalytic interference

INTRODUCTION

Vitamin B_{12} (cyanocobalamin) is a water-soluble hematopoietic vitamin that is necessary for the maturation of erythrocytes. Vitamin B_{12} consists of tetrapyrrole rings surrounding central cobalt atoms and nucleotide side chains attached to cobalt atoms. The tetrapyrrole ring, including cobalt and other side chains, is called corin, and all compounds, including this corin nucleus, are coronoid. The cobalt-corin complex is called cobamide. Cobalamin differs depending on the characteristic nature of the side groups attached to cobalt atoms (for instance, methylcobalamin, deoxyadenosylcobalamin, hydroxycobalamin, cyanocobalamin). Cyanocobalamin is the reference compound used in serum cobalamin method calibration. Its molecular weight is 1355 Da, and it gradually degrades on exposure to light (1).

Vitamin B_{12} is produced with microbial synthesis. The essential nutritional sources for vitamin B_{12} are meat and meat products, cereal products, milk and milk products, and fish and fishery products. Vitamin B_{12} combines with the intrinsic factor (IF) released from the parietal cells of the stomach. It is attached to the receptor on the surface of distal ileum mucosa cells and taken into the cell. It separates from IF in the epithelial cell and passes into the mucosal capillaries and the portal vein. Vitamin B_{12}, which is taken by hepatocytes, is stored in the liver. If an adequate amount of vitamin B_{12} is not taken in the diet or impairment occurs in the absorption mechanisms, vitamin B_{12} deficiency is observed. The most common cause of vitamin B_{12} deficiency is pernicious anemia, which is an autoimmune disease characterized by chronic atrophic gastritis as a result of autoantibody production for IF and gastric parietal cells. Other causes include malabsorption, vegetarian diet, and some drugs (phenytoin, dihydrofolate reductase inhibitors, metformin, proton pump inhibitors). In vitamin B_{12} deficiency, megaloblastic anemia and neuropathy (which can be irreversible unless it is treated) can be observed (1, 2). Therefore, measurement of the level of vitamin B_{12} in serum is one of the most frequently ordered routine tests.

Many physical and chemical factors generally affect vitamin B_{12} stability in a negative manner. Vitamins that dissolve in water in solution are inclined to get degraded, particularly when they are exposed to light (3). The guidelines of laboratory tests and kit prospectuses include warnings about protecting sampling tubes, which are to be used for analyzing vitamin B_{12} levels, from light in the pre-analytic period (4). In the blood withdrawal units of our hospital, no special precaution is taken for preventing the exposure of the sampling tubes of patients, who are requested to be evaluated for vitamin B_{12}, to light. Accordingly, in our study, it was aimed to investigate the effects of the exposure of sampling tubes to light and the waiting time on vitamin B_{12} levels.

METHODS

A total of 52 volunteer patients who were admitted to the outpatient clinics of the Gaziosmanpaşa Taksim Training and Research Hospital were randomly selected (27 female and 25 male). The mean age of patients was found to be 41 ± 11 years. An approval was received from the Ethics Committee of the Gaziosmanpaşa Taksim Training and Research Hospital (03.04.2013, Decision No: 17). For this study, darkened tubes were previously prepared by wrapping them with black tape for protection from light. After obtaining written informed consents from patients, their fasting

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blood samples were collected into darkened and transparent normal 8-mL dry gel tubes (Beckton Dickinson, Plymouth, UK) simultaneously (2 tubes for each volunteer). They were centrifuged at 4000 rpm for 10 min. The vitamin B$_{12}$ levels of these samples were studied in the Roche Hitachi Modular E 170 autoanalyser using the electro-chemiluminescence immunoassay (ECLIA) method with the vitamin B$_{12}$ kit (Roche Diagnostics, Mannheim, Germany) at the 0th and 12th hours (the caps of the tubes were kept closed to prevent vaporization at room temperature).

Statistical Analysis

For the statistical analysis of data obtained in this study, the Statistical Package for the Social Sciences (SPSS Inc.; Chicago, IL, USA) version 11.0 software was used. Mann–Whitney U test was employed for comparing dual groups, and Wilcoxon test was used for evaluating the values at the 0th and 12th hours. The results were assessed in accordance with the significance level of p<0.05.

RESULTS

In the comparison of darkened tubes with transparent tubes, no statistically significant difference was observed in the vitamin B$_{12}$ values at the 0th and 12th hours (p>0.05) (Table 1).

The percentage values of the darkened tube group with respect to differences and changes at the 0th and 12th hours were found to be significantly lower than those of the transparent tube group (p<0.05) (Table 1). The mean B$_{12}$ values are shown in Figure 1 for darkened and transparent tubes according to time.

DISCUSSION

The development, acceleration, and extensive usability of automatized measurements in laboratory tests have caused an increase in the number of demands for serum vitamin B$_{12}$ and folate measurements. Currently, many clinicians request these tests to be performed for patients who are suspected to have vitamin B$_{12}$ deficiency, folate deficiency, or hyperhomocysteinemia (2).

Although competitive protein binding and immunometric methods are used for the direct quantitative determination of serum vitamin B$_{12}$ levels, IF is generally used as the binding protein. Vitamin B$_{12}$ is removed from the binding protein with alkaline medium or chemical reactions before measurement. Serum vitamin B$_{12}$ is converted to cyanocobalamin by potassium cyanide and then it is measured (1, 2).

Although the guidelines of laboratory tests and kit prospectuses include warnings about protecting sampling tubes, which are to be used for analyzing vitamin B$_{12}$ levels, from light in the pre-analytic period, there are a few studies about the effects of light and waiting time on vitamin B$_{12}$ measurement results in the literature. Kosem et al. (5) reported that different kits and device systems used in the measurement of vitamin B$_{12}$ and folate could be influenced by light in different ways. They separated serum of blood samples taken from 11 healthy volunteers and then divided them into two groups. The first group of samples was kept in the dark (group 1), and the other group (group 2) was kept under light with their caps closed. For all samples, vitamin B$_{12}$ and folate levels were measured twice at the 0th, 8th, and 24th hours. They observed that vitamin B$_{12}$ measurements were not affected by light until the 24th hour (p>0.05), but folate measurements were affected

Table 1. Comparison of B$_{12}$ values in darkened and transparent tubes

Time	Mean values	Darkened tube	Transparent tube	p value
0th hour	X±SD	393.24±168.26	387.05±161.81	0.884
	Median (IQR)	361.75 (246.08–510.88)	362.3 (248–483.13)	
12th hour	X±SD	403.82±173.99	387.41±162.18	0.635
	Median (IQR)	362.4 (251.4–528.7)	353.3 (249.7–496)	
Difference	X±SD	−4.73±26.07	5.25±17.77	0.047
	Median (IQR)	−0.55 (−12.15–9.6)	2 (−3.48–14.25)	
Change %	X±SD	−1.71±7.72	1.12±3.61	0.039
	Median (IQR)	−0.19 (−4.34–2.42)	0.85 (−1.37–4.06)	

X±SD: Arithmetic mean±standard deviation
IQR: Interquartile range
p<0.05; Statistically significant
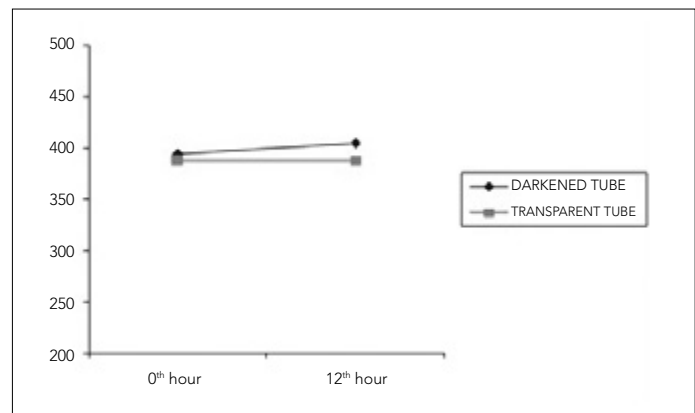
Figure 1. Change of vitamin B$_{12}$ values (Mean±Standard Deviation) according to time

(p<0.05). They reported that it was not necessary to keep the samples in the dark for B$_{12}$ and folate measurements if they were performed on the same day.

In the study of İnal et al. (6), blood samples were collected from 33 volunteers, and vitamin B$_{12}$ levels were measured at the 0th hour. Then, each specimen was divided into two tubes, and they were kept at room temperature under light (group 1) and in the dark (group 2) with their caps closed, and vitamin B$_{12}$ levels were measured at the 8th and 24th hours.

In this study, the effects of exposure to daylight and waiting time before analyzing sample tubes on the measurement results of vitamin B$_{12}$ were investigated. As a result, no statistically significant difference was observed in vitamin B$_{12}$ values at the 0th and 12th hours in the comparison of darkened and transparent tubes. This result is consistent with the result of the study conducted by Kosem et al (5). The percentage values of differences and changes at the 0th and 12th hours were found to be statistically

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significantly lower in the darkened tube group than in the transparent tube group.

CONCLUSION

At the peak hours in the blood withdrawal units of our hospital, although blood samples collected from patients are evaluated on the same day, some time passes before evaluation. The use of darkened tubes in the measurements of vitamin B$_{12}$ levels in the samples that will be waited can be recommended with respect to the statistical significance of difference and change percentages. However, when the cost-effectiveness ratio is considered, the effects of waiting time and exposure to light on vitamin B$_{12}$ values can be ignored.

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