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Effect of Light on Serum Vitamin B_{12} and Folate Levels

[Işığın Serum Vitamin B_{12} ve Folat Düzeyleri Üzerine Etkisi]

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ABSTRACT

There is few information on the effect of light on serum vitamin B_{12} and folate measurements in the literature. Additionally, different kits and instrument systems for the measurement of these analytes can be affected by light in a different way. In this study, we aimed to investigate the effect of light on these analytes. Blood specimens were collected from 11 healthy volunteers in 16*100 mm Vacutainer® SST tubes. Sera were separated and aliquoted in two groups of plain polypropilen tubes capped and stored in dark (Group 1) and light (Group 2) at room temperature. Duplicate vitamin B_{12} and folate measurements were performed immediately (0 h, initial point), and after 8 h and 24 h from the initial point. The measurements were performed in an Immulite 2000 analyzer with reagents from the manufacturer. The significance of differences between and within groups were analyzed by repeated-measures ANOVA. When the 0 h specimens were considered reference, vitamin B_{12} measurements were not significantly affected by light up to 24 hours ($p>0.05$). Whereas folate measurements were significantly affected by light at 24th h after drawing. In conclusion, there is no need to store the samples in dark for vitamin B_{12} and folate measurements which performed in the same working day of drawing.

Key Words: Folate, Vitamin B_{12}, Interference, Immunoassay

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ÖZET

Literatürde serum vitamin B_{12} ve folat ölçümlerine ışığın etkisi hakkında yeterli bilgi yoktur. Ayrıca, bu analitlerin ölçümlerinde kullanılan farklı kit ve cihaz sistemleri ışıktan farklı şekilde etkilenebilir. Bu çalışmada ışığın bu analitler üzerine etkisini araştırmak amaçlanmıştır. Kan örnekleri 11 sağlıklı gönüllüden 16*100 mm'lik Vacutainer® SST tüplere alındı. Serumlar ayrıldıktan sonra düz polipropilen tüplere bölündü ve tüplerin bir bölümü karanlıkta (Grup 1), geri kalanı ışık altında (Grup 2) oda sıcaklığında kapakları kapalı olarak bekletildi. Örneklerde işlem tamamlandıktan hemen sonra (0. saat) ve bundan 8 ve 24 saaat sonra çift olarak vitamin B_{12} ve folat ölçümleri yapıldı. Ölçümler Immulite 2000 analizöründe üretici firmanın ayıraçları ile yapıldı. Gruplar arasındaki farkın önemi "tekrarlanan ölçümler ANOVA" ile yapıldı. Başlangıç değerleri esas alındığında vitamin B_{12} ölçümlerinin 24 saate kadar ışıktan etkilenmediği görüldü ($p>0.05$). Folat ölçümleri ise kan alımından sonraki 24. saatte ışıktan etkilendi. Sonuç olarak, vitamin B_{12} ve folat ölçümleri kan örneğinin alındığı işgünü yapılacaksa, örneklerin karanlıkta saklanmasına gerek yoktur.

Anahtar Kelimeler: Folat, Vitamin B_{12}, Interferans, Immunoassay

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INTRODUCTION

Vitamin B_{12} and folic acid are two coenzymes that have interdependent functions in intermediary metabolism. Vitamin B_{12}, a cyanocobalamin water-soluble hematopoietic vitamin, is required for the maturation of red blood cells. The generic term vitamin B_{12} refers to a group of physiologically active substances chemically classified as cobalamins or corrinoids. They are composed of tetrapyrrole rings surrounding central cobalt atoms and nucleotide side chains attached to the cobalt (1). Folic acid derives from pteroic acid to which one or more molecules of p-aminobenzoic acid and glutamic acid are attached. Folic acid is involved as a coenzyme in one-carbon metabolism together with vitamin B_{12} and they are essential nutrients for hematopoesis (1,2). Therefore, measurements of serum concentrations of vitamin B_{12} and folate are needed for diagnosis and monitoring of anemias, in particular megaloblastic anemias.

Knowledge of the stability of the specimens is important to obtain correct results for vitamin B_{12} and folate. Recent clinical laboratory textbooks contain conflicting statements about whether vitamin B_{12} and folate require protection from light (1,3-6). Mastropaolo and Wilson (7) and Komaromy-Hiller et al. (8) reported that light protection is necessary if the sample cannot be analyzed immediately for vitamin B_{12}. According to these authors, folate is more stable than vitamin B_{12} and it can be measured directly. Although these analytes are relatively special tests, they are increasingly measured daily in many laboratories. In general, vitamin B_{12} and folate are transported and processed without light protection in routine laboratory practice. Sometimes, these analytes cannot be studied immediately because of increased workload or technical problems. Additionally, some laboratories do not perform routine vitamin B_{12} and folate measurements daily and the specimens are generally sended to other laboratories or studied every other day as a more cost-efficient way. Therefore, transportation to the laboratory, timely processing and appropriate storing of the specimens may be critical. From this point of view, in the present study we aimed to investigate the effect of light during storing on folate and vitamin B_{12} levels under conditions simulating routine laboratory practice.

MATERIALS AND METHODS

Blood specimens from 11 healthy volunteers were collected in standard but light protected tubes (Vacutainer® SST; 16*100 mm; Beckton-Dickinson Diagnostics, Belliver Industrial Estate, Playmouth, UK). All specimens were allowed to clot for 30 min at room temperature before centrifugation under light protected conditions. Serum specimens were separated and aliquoted in two groups of plain polypropilen tubes capped and stored in dark (Group 1) and light (Group 2). The light protected specimens were immediately covered with aluminum foil (Group 1). The other tubes were placed at a distance of 0.50 m to and under 72 W white fluorescent light (Group 2). Vitamin B_{12} and folate in fresh serum were measured in duplicate immediately (in max. 45 min after drawing, 0 h) and the other specimens were studied in duplicate after 8 h and 24 h from the initial point (0 h) on an Immulite 2000 immunoassay analyzer with reagents from the manufacturer (Diagnostic Products Corporation, Los Angeles, CA, US).

Data analysis

We used arithmetic means of the duplicates for statistical analysis. The significance of differences between and within groups were analyzed by repeated – measures ANOVA. The significance of differences between baseline analyte means of the groups were assessed by the Student's paired t-test. A p value of <0.05 was considered statistically significant. Data analysis is performed by a SPSS software program (SPSS for Windows version 11.0, SPSS Inc., Headquarters, Chicago, Illinois, US).

RESULTS

The results are presented in Table 1. The concentration ranges for vitamin B_{12} and folate in baseline specimens were 144 – 419 pg/mL and 4.20 – 13.0 ng/mL, respectively. Percent differences from baseline vitamin B_{12} (A) and folate (B) results at 8th and 24th h are seen in Fig. 1. Repeated - measures ANOVA for vitamin B_{12} showed a significant time-effect (F= 4.208; p= 0.022) but an insignificant group-effect (F= 0.047; p= 0.831). Statistically significant differences were found at the 8th h specimens but no difference was observed between the initial and 24th h specimens. Group-time interaction was also insignificant (F= 1.625; p= 0.210). There was a significant time effect (F= 74.239; p<0,001), but an insignificant group effect (F<0.001; p= 0.988) for folate. Group-time interaction was found to be significant (F= 3.796; p= 0.031). Folate values at 24th h were decreased significantly as compared with initial values. Paired t-test showed a significant difference between vitamin B_{12} results at initial vs dark 8th h (p= 0.015); and between folate results at initial vs. light 8th h (p<0.001), light 24th h (p<0.001) and dark 24th (p<0.001); and dark 24th h vs light 24th h (p= 0.002).

DISCUSSION

There are conflicting reports on the stability of vitamin B_{12} and folate and on storing conditions of specimens for these analytes in the literature. Mastropaolo and Wilson concluded that testing of specimens for vitamin B_{12} and folate is acceptable for specimens stored for < 8 h in light; however, if folate specimens are exposed to light for > 8 h, a new specimen should be drawn (7). Komaromy-Hiller et al. (8) showed that serum vitamin B_{12} is very unstable. If frozen and analyzed within 4 hours, specimens collected for vitamin B_{12} analysis do not need to be light protected and the frozen, light

Table 1. Vitamin B_{12} and folate values in light and dark

Time Points	Mean ± SD and difference vs. baseline at time points				
	0th h	8th h	Difference from 0th h (%)	24th h	Difference from 0th h (%)
Vitamin B_{12}, pg/mL (n=11)					
Dark	267 ± 91	284 ± 98 [a]	107 ± 6.3	278 ± 93	105 ± 10
Light	267 ± 91	273 ± 94	103 ± 10.6	263 ± 97	98 ± 9.8
Folate, ng/mL (n=11)					
Dark	7.8 ± 2.9	7.9 ± 3.0	102 ± 6.2	7.2 ± 2.6 [c]	93 ± 4.2 [d]
Light	7.8 ± 2.9	8.2 ± 3.0 [b]	105 ± 3.6	6.9 ± 2.7 [c]	89 ± 4.5 [d]

Vitamin B_{12} and folate changes in dark and light vs. baseline concentrations of each analyte. Percent difference from baseline concentrations at 8th and 24th hours are also presented.
Difference from baseline value for each analyte: *a: p≤0.05; b: p≤0.005; c: p≤0.001.*
Difference between dark and light groups: *d: p≤0.05.*

protected specimens are stable up to a week. They proved that folate is more stable than was expected for literature sources although folate is light sensitive in pure form or in aqueous solution. They show that both vitamin B_{12} and folate increase after 0 h. In concordance, we found higher vitamin B_{12} and folate levels at 8th h specimens, but 24th variables showed a decrease following the 8th. The cause of this increase remains unclear in the literature.

We measured serum vitamin B_{12} and folate levels by an automated competitive displacement assay. Our results showed insignificant differences between the vitamin B_{12} values of light-protected and light-exposed specimens (insignificant group effect). However, vitamin B_{12} values changed significantly with time (significant time effect). Increased 8th h values may play a role for this significant time effect. This finding is in concordance with the results presented by Komaromy-Hiller et al (8). However, the reason of this increase is unclear in the literature. In vitamin B_{12} measurements, the binding protein, intrinsic factor, may bind not only metabolically active vitamin B_{12} but related metabolically inactive compounds that may be present in the specimen and so may give artificially elevated vitamin B_{12} results (2). Additionally, during storing of specimens in first 8 h, it is possible that some metabolites or analogues of vitamin B_{12} in serum specimens may have a larger response in the chemiluminescence assay procedure of Immulite 2000. Another possible cause may be gradually increased free vitamin B_{12} with time during first 8 h due to release of the vitamin from binding proteins. The following decrease of vitamin B_{12} at 24th h may be due to degradation of the molecule by light.

Folate measurements were also insignificantly affected by light at 8th h. Like vitamin B_{12}, slightly increased folate results at 8th h may result from different signals of different folate forms in the reaction mixture. Multiple

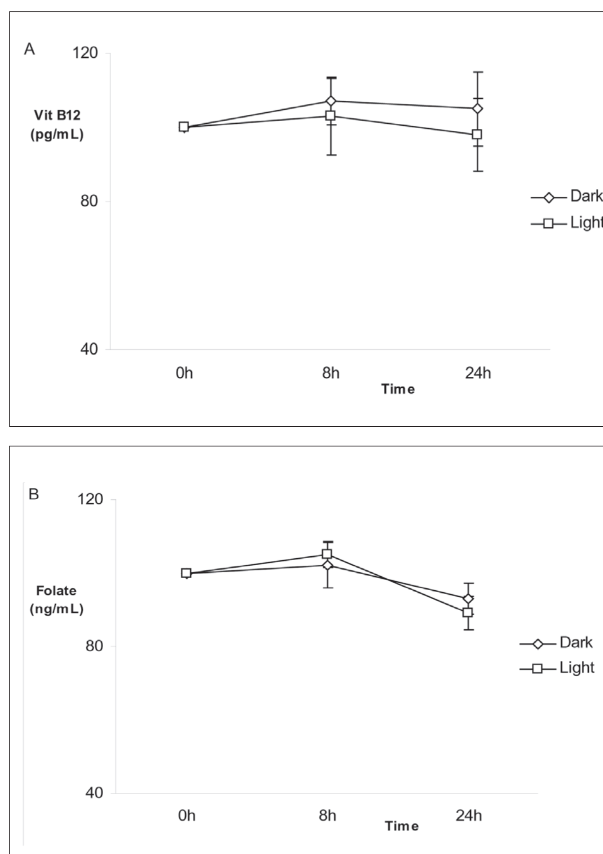

Figure 1. Percent difference (mean ± SD) of vitamin B12 (A) and folate (B) concentrations from baseline in dark (◊) and light (□).

forms of folate are present in human serum and folate immunoassays measure a composite "blend" of these forms (9). Additionally, release of folate from binding proteins may increase during the first 8 h of storing. In contrast to the 8th h results, we found significantly decreased folate values at 24th h specimens ($p<0.001$). Sig-

nificant group-time interaction shows an important light effect on folate measurements in our study. The most important finding of the study is significantly decreased folate values in light-protected and light - exposure conditions at 24th h measurements. Although folate is light sensitive in pure form (7) decreased folate results at 24th h are seem to be not only result from light exposure but also length of storing period in our study.

In conclusion, the difference from baseline concentrations of vitamin B_{12} and folate are not clinically significant. On the contrary of previously reported data, there is no need to store the samples in dark for vitamin B_{12} and folate measurements which were performed on the same working day of drawing. Storing conditions may be more important than light exposure of the specimens during routine studies.

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