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RESEARCH ARTICLE

# THE EFFECT OF DIURNAL VARIATION ON LABORATORY TESTS

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## ABSTRACT

**Purpose:** Commonly used biochemical tests in blood samples may be measured at any time of day. This study investigated the existence and clinical significance of diurnal variations in some of routine parameters to facilitate accurate and reliable decision-making in diagnosis and follow-up.
**Material and Methods:** Blood samples were collected from 17 healthy volunteers who were 18-50 years of age (11 men, 6 women) on the same day at 9.00 am, 12.00 am, 3.00 pm, 6.00 pm, and 12.00 pm. Samples collected at 9.00 am were regarded as baseline. The results of 19 biochemical parameters in blood samples obtained at 12.00 am, 3.00 pm, 6.00 pm and 12.00 pm were statistically and clinically compared with the results at 9.00 am baseline sample.
**Results:** Total protein, creatinine, aspartate transaminase, alanine transaminase, alkaline phosphatase and gamma glutamyl transferase showed no clinically significant variation within the day, but clinically significant changes were observed in levels of glucose, total cholesterol, HDL-cholesterol, triglyceride, total bilirubin (TBIL), direct bilirubin (DBIL), albumin, blood urea nitrogen, uric acid, sodium, potassium, chloride and amylase. Especially, BUN changed by maximum 20-30%, TBIL, DBIL and triglyceride maximum 40-50% within the day.
**Conclusion:** The results of our study suggest that clinicians should consider the timing of blood sampling and the diurnal variations in BUN, TBIL, DBIL and triglyceride parameters during diagnosis and treatment follow-up. Sampling throughout the day seems to pose no problem for other tests with limited diurnal variation.

**Keywords:** Bilirubin, blood urea nitrogen, diurnal rhythm, triglyceride

## INTRODUCTION

Clinicians make approximately 70% of diagnosis and treatment decisions based on laboratory tests (1). For this reason, the accuracy and reliability of laboratory tests are important. Analyzing patient samples in clinical laboratories is a complex process, and 46.0-68.2% of errors occur in the preanalytical process (2,3). One of the factors involved in the preanalytical process is diurnal variation. Test results may change with the release or metabolism of certain analytes within the day. Some parameters, including thyroid stimulating hormone, adrenocorticotropic hormone, cortisol, iron (Fe), and unsaturated iron binding capacity (UIBC), are known to fluctuate by up to 50% during the day (4-6). However, the diurnal rhythm is uncertain for some biochemical tests. Organisms' behavior and physiology can also exhibit rhythmic variations, especially over the 24-hour light–dark cycle. These diurnal changes are regulated with a biological timing system by the suprachiasmatic nuclei of the hypothalamus, as well as by the peripheral tissues (7).

Generally, clinicians prefer to have fasting blood from patients in the morning for laboratory tests. The Clinical Laboratory Standards Institute (CLSI) document H3-A6 reported that some test results vary

depending on the blood sampling time (8). However, CLSI document H3-A6 suggest that each laboratory make its own decision for blood sampling and fasting time. The Working Group on the Preanalytical Phase in the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM WG-PA) reported that all blood sampling for routine laboratory tests should be conducted between 7.00 and 9.00 am (9). However, blood samples can be collected at all hours, especially in inpatient and emergency departments.

Regardless of blood collection time, the same reference ranges are used for all samples and the potential influence of diurnal variation is ignored when evaluating the test results. Using the same reference ranges throughout the day, may mislead clinicians about the diagnosis and treatment of diseases.

Although some laboratory tests are known to show diurnal variation, the literature data on this subject are insufficient. The aim of our study was to evaluate whether 19 commonly used biochemical parameters show diurnal variation and to determine the clinical significance of these changes. Thus, it is to draw attention to the importance of the preanalytical stage when evaluating test results at the clinical decision point.

## MATERIAL AND METHODS

### Participants

Seventeen volunteers between the ages of 18 and 50 years were included in the study. Patients with acute infection, chronic disease (diabetes mellitus, hypertension, rheumatoid disease etc.), medical treatment, supplement intake, history of malignancy, body mass index greater than 30 and pregnant or breastfeeding women were not included in the study. Because the study focused on diurnal changes, the menstrual cycles of the women in the study were not taken into consideration. The detailed information about the study was given to volunteers and the signed consent forms were obtained from each volunteer. The study was conducted in accordance with the Declaration of Helsinki. Ethics approval was obtained from the Izmir Atatürk Training and Research Hospital Clinical Research Ethics Committee (Approval date: 19.01.2017; Approval number: 7).

### Collection of Blood Samples and Working Plan

Blood samples were collected from the volunteers into gel vacuum tubes (BD Vacutainer ® SST II Advance tube, 5 mL, 13x100 mm, NJ, USA), at 9.00 am, 12.00 am, 3.00 pm, 6.00 pm and 12.00 pm. The samples were centrifuged at 1500 x g for 10 minutes, and the separated serum samples were stored at -20°C until analysis.

Because it is recommended the clinically evaluation for many tests are performed from fasting blood sample given in the morning, the blood samples taken at 9.00 am were considered "baseline". Blood samples taken at 12.00 am, 3.00 pm, 6.00 pm and 12.00 pm were compared with the baseline sample to evaluate variations. The participants had a standard breakfast, lunch and dinner after blood collection at 9.00 am, 12.00 am and 6.00 pm, respectively.

### Biochemical Parameters

The participants' serum samples were analyzed for the following biochemical parameters: glucose (Glu), total cholesterol (CHOL), high density lipoprotein cholesterol (HDL-C), triglyceride, total bilirubin (TBIL), direct bilirubin (DBIL), total protein (TP), albumin (Alb), blood urea nitrogen (BUN), uric acid (UA), creatinine (Crea), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT) and amylase were analyzed by using spectrophotometric method; sodium ($Na^+$), potassium ($K^+$) and chloride ($Cl^-$) parameters were analyzed by ion selective electrode method on the autoanalyzer (Architect C1600, Abbott Laboratories, IL, USA).

### Sample Size Calculation

Sample size was calculated using GPower Sample Size Calculator assuming an effect size of 0.802 and 0.935 for the diurnal rhythm of serum potassium and sodium. A total of at least 12-15 subjects had to be recruited based on a statistical power of 80% and an alpha error of 5% (10).

### Statistical Analysis

Statistical analysis was performed by Statistical Package for the Social Sciences (SPSS) version 15 (SPSS Inc. Chicago, IL). The normal distribution of data was analyzed using Shapiro-Wilk test. The blood sample taken at 9.00 am was accepted as baseline and blood samples taken at other 4 different times were compared with the baseline sample. Paired-samples t test was used for normally distributed data and Wilcoxon test for non-normally distributed data.

**Table 1.** Mean (±standard deviation) or median (25th-75th percentile) values, bias, p values for Glu, CHOL, HDL-C, TG, TBIL and DBIL according to times.

| Analytes | Basal | 12.00 am | 03.00 pm | 06.00 pm | 12.00 pm | $Bias_d$,% |
|---|---|---|---|---|---|---|
| **Glu (mg/dL)** | 88.8±6.5 | 88.3±8.9 | 93.6±12.1 | 84.2±8.8 | 95.9±10.6 | |
| **%** | | -0.53 | **5.43** | **-5.17** | **8.08** | **2.34** |
| ***p*** | | *0.841* | *0.100* | *0.083* | *0.020* | |
| **CHOL (mg/dL)** | 196.1±49.1 | 190.2±46.9 | 192.1±43.8 | 194.4±48.0 | 191.2±45.2 | |
| **%** | | -3.03 | -2.07 | -0.87 | -2.49 | **4.10** |
| ***p*** | | *0.035* | *0.285* | *0.687* | *0.184* | |
| **HDL-C (mg/dL)** | 44.2±9.0 | 42.9±9.9 | 42.2±10.0 | 43.5±9.8 | 43.7±9.4 | |
| **%** | | -2.97 | -4.68 | -1.58 | -1.25 | **5.61** |
| ***p*** | | *0.064* | *0.022* | *0.640* | *0.530* | |
| **TG (mg/dL)** | 92.0 (73.0-153.0) | 134.0 (87.0-236.0) | 126.0 (96.5-247.0) | 99.0 (74.5-204.0) | 138.0 (84.0-205.5) | |
| **%** | | **45.65** | **36.96** | 7.61 | **50.00** | **9.57** |
| ***p*** | | ***0.002*** | ***0.003*** | *0.037* | ***0.003*** | |
| **TBIL (mg/dL)** | 0.57 (0.36-0.80) | 0.45 (0.29-0.58) | 0.39 (0.27-0.60) | 0.38 (0.23-0.45) | 0.34 (0.25-0.47) | |
| **%** | | **-21.05** | **-31.58** | **-33.33** | **-40.35** | **8.95** |
| ***p*** | | ***<0.001*** | ***0.001*** | ***0.001*** | ***0.001*** | |
| **DBIL (mg/dL)** | 0.19 (0.13-0.25) | 0.14 (0.11-0.17) | 0.10 (0.10-0.15) | 0.11 (0.10-0.17) | 0.11 (0.10-0.18) | |
| **%** | | **-26.32** | **-47.37** | **-42.11** | **-42.11** | **14.92** |
| ***p*** | | ***0.002*** | ***0.001*** | ***0.001*** | ***0.003*** | |

$Bias_d$, desirable bias; Glu, glucose; CHOL, cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; TBIL, Bilirubin total; DBIL, Bilirubin direct. Bonferroni correction was applied and p value less than 0.0125 was considered statistically significant and written in bold and italic letters. If the calculated bias value was more than desirable bias value, it was considered clinically significance and written in bold letters

Normally distributed data were expressed as mean ± standard deviation, non-normally distributed data as median and interquartile range (IQR; 25–75th percentiles). Because samples collected at 4 different time points were compared with baseline levels, Bonferroni correction was applied and p values less than 0.0125 (0.05/4=0.0125) were considered statistically significant (11).

In addition, blood samples collected at 12.00 am, 3.00 pm, 6.00 pm and 12.00 pm were compared with baseline and bias (%) values were calculated using the formula ([mean or median value of the parameter at the different time point – mean or median baseline value]/mean or median value of the baseline level x 100). The calculated bias value was compared with desirable bias values to evaluate clinical significance (12, 13).

## RESULTS

Of the 17 participants in the study, 11 (64.7%) were men and 6 (35.3%) were women. Their mean age was 34.2±6.6 years and their body mass index were 25.0±3.0.

While there was no statistically significant diurnal change in Glu, there was clinically significant elevation (5.43% and 8.08%, respectively) at 3.00 pm and 12.00 pm and a significant decrease at 6.00 pm (-5.17%) compared to baseline. Although CHOL and HDL-C levels decreased during the day compared to baseline, no clinically significant differences were observed. Both clinically and statistically significant increases in Trig levels during the day, but only increase at 6.00 pm is not significant. The decreases in TBIL and DBIL levels at 12.00 am to 12.00 pm were both statistically and clinically significant compared to baseline levels. These differences ranged in magnitude from 19.30% to 47.37% (Table 1).

Alb levels showed a clinically significant decrease (-4.55%) only at 12.00 am compared to baseline, but there were no statistically significant differences. TP had no diurnal rhythm. Statistically and clinically significant decreases in UA were observed at 3.00 pm and 6.00 pm (-7.49% and -9.90%, respectively).

**Table 2.** Mean (±standard deviation) or median (25th-75th percentile) values, bias, p values for TP, Alb, BUN, UA, Crea, $Na^+$, $K^+$, $Cl^-$ according to times

| Analytes | Basal | 12.00 am | 03.00 pm | 06.00 pm | 12.00 pm | $Bias_d$,% |
|---|---|---|---|---|---|---|
| **TP (g/dL)** | 6.86±0.39 | 6.80±0.27 | 6.79±0.49 | 6.87±0.52 | 6.81±0.36 | |
| **%** | | 0.94 | -1.03 | 0.09 | -0.86 | **1.36** |
| ***p*** | | *0.530* | *0.656* | *0.973* | *0.680* | |
| **Alb (g/dL)** | 4.40 (4.30-4.60) | 4.20 (4.20-4.10) | 4.40 (4.10-4.55) | 4.40 (4.20-4.65) | 4.40 (4.15-4.60) | |
| **%** | | **-4.55** | 0 | 0 | 0 | **1.43** |
| ***p*** | | *0.295* | *0.556* | *0.977* | *0.819* | |
| **BUN (mg/dL)** | 13.0±3.5 | 14.3±3.7 | 14.6±3.2 | 14.6±3.5 | 16.3±3.2 | |
| **%** | | **10.09** | **12.58** | **12.31** | **25.02** | **5.57** |
| ***p*** | | ***0.001*** | ***0.001*** | ***0.005*** | ***0.001*** | |
| **UA (mg/dL)** | 4.65±1.35 | 4.42±1.23 | 4.29±1.12 | 4.18±1.12 | 4.59±1.14 | |
| **%** | | -4.57 | **-7.49** | **-9.90** | -1.02 | **4.87** |
| **P** | | *0.022* | ***0.004*** | ***0.004*** | *0.677* | |
| **Crea (mg/dL)** | 0.81±0.14 | 0.79±0.14 | 0.79±0.14 | 0.78±0.14 | 0.83±0.13 | |
| **%** | | -2.47 | -2.47 | -3.7 | 2.47 | **3.96** |
| ***p*** | | *0.056* | *0.429* | *0.240* | *0.094* | |
| **$Na^+$ (mmol/L)** | 137.0 (136.0-139.0) | 136.0 (135-138) | 137.0 (136-138.5) | 138.0 (137.5-139) | 138.0 (137-139) | |
| **%** | | **-0.72** | 0 | **0.72** | **0.72** | **0.23** |
| ***p*** | | *0.324* | *0.841* | *0.234* | *0.265* | |
| **$K^+$ (mmol/L)** | 4.4 (4.1-4.5) | 4.2 (4.0-4.4) | 4.2 (4.1-4.4) | 4.0 (4.0-4.5) | 4.1 (4.1-4.2) | |
| **%** | | **-4.55** | **-4.55** | **-9.09** | **-6.82** | **1.81** |
| ***p*** | | *0.153* | *0.086* | *0.110* | ***0.005*** | |
| **$Cl^-$ (mmol/L)** | 105.0 (104.0-106.0) | 105.0 (103.5-106.5) | 105.0 (103.0-106.5) | 105.0 (104.0-106.0) | 106.0 (102.5-107.0) | |
| **%** | | 0 | 0 | 0 | **0.95** | **0.50** |
| ***p*** | | *0.799* | *0.720* | *0.685* | *0.841* | |

$Bias_d$, desirable bias; BUN, blood urea nitrogen, UA, uric acid; Crea, Creatinine; TP, total protein; Alb, Albumin; $Na^+$, sodium; $K^+$, potassium; $Cl^-$, chloride. Bonferroni correction was applied and p value less than 0.0125 was considered statistically significant and written in bold and italic letters. If the calculated bias value was more than desirable bias value, it was considered clinically significance and written in bold letters.

**Table 3.** Mean (±standard deviation) or median (25th-75th percentile) values, bias, p values for AST, ALT, ALP, GGT and amylase according to times.

| Analytes | Basal | 12.00 am | 03.00 pm | 06.00 pm | 12.00 pm | $Bias_d$,% |
|---|---|---|---|---|---|---|
| **AST (U/L)** | 17.0 (14.0-21.0) | 16.0 (13.0-21.5) | 16 (14.0-21.0) | 17.0 (13.0-20.5) | 16.0 (13.5-19.5) | **6.54** |
| **%** | | -5.8 | -5.8 | 0 | -5.8 | |
| ***p*** | | *0.193* | *0.936* | *0.835* | *0.574* | |
| **ALT (U/L)** | 12.0 (9.5-17.0) | 12.0 (10.0-16.5) | 11.0 (10.0-16.0) | 12.0 (10.0-15.5) | 12.0 (10.0-17.0) | **11.48** |
| **%** | | 0 | -8.3 | 0 | 0 | |
| ***p*** | | *0.835* | *0.681* | *0.858* | *0.319* | |
| **ALP (U/L)** | 56.2±13.5 | 56.9±12.0 | 58.0±12.4 | 57.6±12.1 | 58.7±12.4 | **6.72** |
| **%** | | 1.15 | 3.24 | 2.41 | 4.39 | |
| ***p*** | | *0.550* | *0.195* | *0.508* | *0.263* | |
| **GGT (U/L)** | 16.9±8.0 | 16.6±7.1 | 16.8±7.3 | 16.9±7.1 | 16.6±7.1 | **11.06** |
| **%** | | -1.39 | -0.35 | 0 | -1.74 | |
| ***p*** | | *0.553* | *0.887* | *1.000* | *0.501* | |
| **Amylase (U/L)** | 62.7±14.2 | 63.8±16.1 | 65.1±16.4 | 65.7±16.1 | 67.7±16.1 | **7.40** |
| **%** | | 1.69 | 3.75 | 4.78 | **7.97** | |
| ***p*** | | *0.510* | *0.134* | *0.195* | ***0.007*** | |

$Bias_d$, desirable bias; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma glutamyl transferase. Bonferroni correction was applied and p value less than 0.0125 was considered statistically significant and written in bold and italic letters. If the calculated bias value was more than desirable bias value, it was considered clinically significance and written in bold letters.

There were statistically significant differences in BUN results at 12.00 am, 3.00 pm, 6.00 pm and 12.00 pm compared to baseline, as well as clinically significant increases at these times (10.09%, 12.58%, 12.31%, and 25.02%, respectively) (Table 2).

The decrease at 12.00 am (-0.72%) and increase at 6.00 pm and 12.00 pm (0.72%) in $Na^+$ levels compared to baseline were found to be clinically significant. However, these diurnal fluctuations were not statistically significant. $K^+$ levels at 12.00 am, 3.00 pm, 6.00 pm and 12.00 pm were found to be clinically significant lower than baseline (-4.55%, -4.55%, -9.09%, and -6.82%, respectively). Only the difference at 12.00 pm was statistically significant. $Cl^-$ showed a clinically significant increase at 12.00 pm (0.95%) compared to baseline, but none of the values showed a statistically significant diurnal fluctuation (Table 2).

The only change in amylase from baseline was a statistically and clinically significant (7.97%) increase at 12.00 pm. In terms of diurnal variation, there were no statistically or clinically significant differences in other enzymes at any time point compared to those obtained at 9.00 am (Table 3).

## DISCUSSION

Since the development of automated laboratory systems, tests can be analyzed throughout the day. This development leads clinicians to examine more frequent and more patients in hospitals and to take blood samples at various times of day. In turn, this has made it difficult to interpret the results of tests that show diurnal variation. Therefore, in the present study we evaluated changes in 19 of the commonly biochemical parameters within a day.

The main factor in blood glucose variation during the day is nutrition. Pocock et al. reported that glucose changes by about 7% during the day and suggested that this change may be related to nutrition and hunger (14). In our study, glucose levels were lower at the preprandial time points 9.00 am, 12.00 am and 6.00 pm compared to other time points. Only the decrease at 6.00 pm was clinically significant (-5.17%). Clinically significant increases over baseline were observed at 3.00 pm and 12.00 pm (5.43% and 8.08%, respectively). We attribute this to carbohydrate metabolism in the hunger–satiety cycle. Sennels et al. also determined that glucose showed diurnal variation (intra-individual change of 10.4% over 24 hours) with a peak at 11.30 pm (6.89%) (15). Similarly, the highest glucose level in our study was detected at 12.00 pm. This finding may be explained by the fact that at night there is a peak in growth hormone, which has a hyperglycemic effect (16), but a decrease in insulin, which has a hypoglycemic effect (15).

Postprandial increase in serum TG concentration as well as smaller changes in HDL-C and LDL-C concentrations have been reported (17). Sennels et al. found no significant changes in HDL-C during the day, while significant changes were detected in TG and CHOL (37.00% and 4.80%, respectively) (15). In the study conducted by Miettinen et al., HDL-C reached the lowest levels in the early morning and the highest levels after breakfast in the afternoon (18). Pocock et al. did not detect any diurnal variation in CHOL, whereas TG level increased progressively within the day and varied by 40.54% (14). In this study, no significant difference was found in CHOL and HDL-C levels. However, TG levels were found to be clinically and statistically significant higher at 12.00 am, 3.00 pm and 12.00 pm compared to baseline levels, with increases varying between 36.60% and 50.00%. This increase in TG levels were thought to be related to feeding. The lower TG level at 6.00 pm than other time points may be a result of the prolonged fasting period during the individuals' active period.

In the study by Sennels et al., bilirubin showed diurnal variation, decreasing after 10.30 am and reaching its lowest level around 12.00 pm, with a daily variation of 32.13% (19). Morrison et al. reported that bilirubin level was 17.3% lower at 4.30 pm compared to a sample collected at 8.30 am (20). Pocock et al. found that bilirubin levels decreased during the day from 12.00 am to 6.00 pm and the magnitude of this change was over 30% (14). Similarly, in our study, TBIL and DBIL levels during the day were found to be clinically significantly lower at 12.00 am, 3.00 pm, 6.00 pm and 12.00 pm compared to baseline, with these decreases reaching 40.35% and 47.37%, respectively. Barrett determined that bilirubin levels tripled after 2 days of fasting (21). The diurnal variation in bilirubin may be explained by an increase in bilirubin concentrations during the morning hours due to overnight fasting and a decrease in bilirubin levels with food intake over the course of the day. Another reason why bilirubin levels are high at 9.00 am and decrease during the day may be the degradation of bilirubin by sunlight.

When we examined the diurnal variation of TP and Alb, we obtained different results than those in the literature. Rejnmark et al. reported that Alb level was

highest at 4.00 pm and lowest at 4.00 am, changing significantly by 12.00% during the day (22). According to their study, Alb levels were higher in the daytime and evening hours and decreased after midnight. This was attributed to the hemoconcentration caused by fluid flow from the vascular compartment to the extravascular compartment while upright. Morrison et al. found that TP level was 2.05% and Alb level was 2.55% higher at 4.30 pm compared to 8.30 am and reported this difference to be statistically significant (20). In our study, while there was no diurnal variation in serum TP levels during the day, there was a clinically significant decrease in Alb level only at 12.00 am compared to baseline. It doesn't seem possible to attribute this situation to hemoconcentration as in other studies. In our study, we found diurnal variations between 9.00 am and 12.00 pm. However, this analysis excludes changes that may occur after midnight. Therefore, more comprehensive studies covering the full 24-hour day are needed.

BUN elevation can occur due to kidney-related diseases or increased protein intake. Pocock et al. found in their study that urea levels tended to increase substantially after 2.00 pm (14). Sennels et al. reported that BUN was low at 9.00 am, increased after 12.00 am, and peaked at midnight (19). In our study, BUN levels showed an increasing trend from 12.00 am to 12.00 pm compared to baseline. This may be related to the daily cycle of protein synthesis and degradation. In our study, UA levels were significantly lower at 3.00 pm and 6.00 pm (-7.49% and -9.90%, respectively) and showed a very similar diurnal variation to that reported by Sennels et al. (19). In addition, it was observed in Sennels' study that the UA peak (3.94%) occurred after midnight, between 4.00 am and 5.00 am (18).

Research of diurnal variation in Crea has yielded discrepant results. In the study conducted by Sennels et al., Crea did not show a significant change during the day (19). In our study, there was also no clinically significant change in serum Crea levels during the day. In contrast, Pocock et al. observed that Crea levels increased after 2.00 pm (14). However, this upward trend was of lesser magnitude than that of urea. They suggested that the increase in Crea may be a result of muscle activity and energy use throughout the day.

Previous studies have demonstrated diurnal variation in serum $Na^+$ levels, but with different peak times. Sennels et al. (18) reported peak $Na^+$ levels around 1.00 pm (0.79%) while Kanabrocki et al. (23) observed peaks at 7.00 pm (0.92%) and 11.00 pm (0.66%). Similar to the studies of Kanabrocki et al, we found that $Na^+$ increased by 0.72% at 6.00 pm and 12.00 pm. Statland et al. (25) reported that $Na^+$ levels changed by 1.00% and -0.36% at 11.00 am and 2.00 pm, respectively. Morrison et al. also observed a decrease of -0.35% in $Na^+$ at 12.30 pm but did not accept this change as significant (20). In some studies, $Na^+$ did not show any diurnal variation (25-27).

The studies on diurnal variation in $K^+$ have also showed different results. Sennels et al. found that there was significantly decrease (maximum -4.70%) in samples taken between 12.00 am and 12.00 pm, peaked at 10.00 am (4.61%) (19). We also found that serum $K^+$ level was lower at all time points during the day compared to baseline, with maximum decrease of -9.09% at 6.00 pm. However, $K^+$ did not show diurnal variation in several other studies (23, 27, 28).

There are fewer studies in the literature on diurnal variation in $Cl^-$. $Cl^-$ decreased during the day with a maximum difference of -0.87% at 12.30 pm, but it was not significant statistically (20). Böning et al. found that $Cl^-$ did not show any diurnal variation (29). In our study, a clinically significant increase (0.95%) in serum $Cl^-$ levels was observed only at 12.00 pm.

In our study, we observed no clinically or statistically significant diurnal changes in the activities of any enzymes except amylase. Amylase level increased from 12.00 am to midnight and it reached clinically significant peak with 7.97%. In the study conducted by Sennels et al., amylase levels peaked at 7.00 pm (2.99%) and varied up to 6.02% during the day. However, because this difference was not statistically significant, amylase was regarded as not showing diurnal variation (19). Keller et al. found that amylase release from the pancreas increased in the evening hours compared to the daytime (30). These findings suggest that the digestive secretion of enzymes may have variable sensitivities toward influences of the day-night and wake-sleep cycle.

## CONCLUSION

In conclusion, while there were no clinically significant diurnal changes in Crea, TP, ALT, AST, GGT and ALP levels; clinically significant changes were observed in Glu, BUN, UA, Alb, TBIL, DBIL, $Na^+$, $K^+$, $Cl^-$, amylase, CHOL, HDL-C, and TG levels. In particular, BUN varied by a maximum of 30% and TBIL, DBIL and TG varied by up to 40–50% over the

course of the day. These results indicate that diurnal variations in these parameters must be taken into consideration during diagnosis and treatment monitoring.

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