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EFFECTS OF VITAMIN-D AND SUNLIGHT ON THE HEMATO-BIOCHEMICAL PARAMETERS IN MICE

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ABSTRACT

Moderate exposure to sunlight increases vitamin-D status in the body and vitamin-D helps in absorption of calcium from intestine. This study was aimed to investigate the efficacy of vitamin-D and sunlight on blood parameters and serum biochemistry in mice. For this purpose, 50 adult Swiss albino mice were collected and randomly divided into five equal groups (Group A, B, C, D and E). Group A was considered as control and fed only on balanced normal feed. Group B was supplied with 15% butter in feed as a semi-synthetic source of vitamin-D, group C was exposed to direct sunlight (1.5 hours daily) and group D and E were supplied with oral synthetic form of vitamin-D @ 50 IU and 100 IU daily, respectively. After 120 days, blood and serum samples were collected for analysis. Our results showed that total erythrocytes count (TEC) and total leukocytes count (TLC) were found higher in group D but insignificant. Lymphocytes were significantly ($P<0.001$) higher in group D but neutrophils was significantly ($P<0.01$) higher in group B. The aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (AP) levels were also raised very sharply with increased level of vitamin-D in blood. AST and AP level were found significantly ($P<0.001$) higher in group B but ALT level was significantly ($P<0.001$) higher in group E. The lipid profile such as total cholesterol (TC), high density lipoprotein (HDL) and triglyceride (TG) were also analyzed. TC level was slightly higher in group C but significantly ($P<0.01$) lower in group E HDL was significantly increases with increased level of vitamin-D in treatment groups ($P<0.01$). TG level was somewhat higher in group D but not significant. From our results it can be concluded that sunlight and butter are the potential sources of vitamin-D. Supplementation of vitamin-D at certain level improves health status but excessive supplementation cause harmful effects on the body systems.

Key Words: Sunlight, Vitamin-D, Blood, Biochemical analysis

INTRODUCTION

Vitamin-D is needed for bone growth and bone remodeling from osteoblasts and osteoclasts (Cranney*et al.*, 2007). The main function of vitamin-D is maintaining calcium homeostasis and low levels of vitamin-D result in lower absorption of calcium from intestine (Lane, 2010). Without vitamin-D, the body cannot absorb calcium and phosphorus adequately, the skeleton loses mineral content (secondary osteoporosis) and new bone is not adequately mineralized (rickets or osteomalacia). It is recommended that dietary allowance for human is 600 IU in a day for ages 1-70 years for this vitamin (Aloia, 2011; Abrams, 2011; Gallagher *et al*., 2014). Calcium and bone metabolism in adults depend heavily on concentration of vitamin-D (Silver, 2011). According to Bikle (1994), vitamin-D treatment is safe and probably most efficacious in populations with marginal vitamin-D intake or limited sunlight exposure and does not need high doses. Vitamin-D is present in many foods, including fishes, eggs, fortified milk, and cod liver oil. In this experiment butter is used as vitamin-D source. Although milk is normally low in vitamin-D but butter contains high fat and is rich in vitamin-D (Schmid and Walther, 2013). Regular receiving of butter through diet may also be helpful for immunity development (Cope *et al.*, 1996). Shankar *et al.* (2002), reported that moderate level of butter has some unique potential benefits on health, particularly in relationship to its vitamin-K and vitamin-D content. As per experiment of Astrup, (2014), consumption of yogurt and other dairy products like butter reduced risk of weight gain and obesity as well as of cardiovascular disease. Sunlight is an electromagnetic radiation and gives off different types of lights and rays having different wave length. They

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produce either positive or negative response in living cells. Moderate sun exposure, physical activity and normal-weight maintenance are modifiable factors, for improving vitamin-D status (Touvier*et al.*, 2014; Bener*et al.*, 2009). The ultraviolet radiation has both positive and negative health effects, as it is source of both vitamin-D_3 and a mutagen (Osborne and Hutchinson, 2002; Ohnaka, 1993). Cholesterol under the skin surface called provitamin-D_3 reacts with the ultraviolet-B rays to form vitamin-D_3. From there, it first goes to the liver and then through the kidneys, converting it into the form of active vitamin-D that the body needs (National Institutes of Health). Lack of sun exposure and vitamin-D deficiency has been linked to serious cardiovascular problems and cancers (Holick, 2008).

METHODOLOGY

Animals: From seventy Swiss albino mice (*Musmusculus*), a total of 50 mice were randomly selected after acclimation for 7 days. Their weight was approximately 22-27 gm/mouse and the age was between 40 and 45 days which were divided into five equal groups.

Treatment: One group was fed on balanced pellet only and was considered as control group (group A). The remaining groups were considered as treated groups (B, C, D and E). Among the treated groups, only group B was fed with butter supplemented diet (15% of solid feed) and others were maintained with balanced diet. Group C was directly exposed to sunlight for 1.5 hrs daily and group D was supplied with additional vitamin-D @ 50 I.U and group-E with vitamin-D @ 100 I.U orally. This treatment regime was continued for 120 days without any changes.

Sample Collection: Samples were collected from mice by sacrificing them for hematological and serological study. For hematological test, blood was stored in test tubes containing anticoagulant (3.8% sodium citrate) except for DLC (Differential Leukocyte Count). For serum collection, blood without anticoagulant was kept in slanting position and serum supernatant was collected after 24 hours and being centrifuged. A drop of blood directly placed on slide and made a thin smear for DLC. For blood and serum analysis, we collected 5 samples from each group.

Blood Analysis: Hematological parameters were total erythrocytes count (TEC), estimation of hemoglobin by acid-hematin method, determination of erythrocyte sedimentation rate (ESR) by Wintrobe's method, determination of packed cell volume (PCV) or hematocrit (Hct) value, total leukocytes count (TLC), differential leucocyte count (DLC). All measurements were performed in the hematological laboratory, Department of Physiology, Bangladesh Agricultural University, Mymensingh, as described by Ghai (2008)

Serum Biochemistry: Alanine transaminase (ALT/GOT) and aspartate transaminase (AST/GPT), alkaline phosphatase, triglyceride (TG), total cholesterol (TC), high density lipoprotein (HDL) were analyzed to know the blood and liver response to additional supplement of vitamin-D. For serological analysis end point method was used for AST, ALT and AP and kinetic method was used for TC, HDL and TG (Human Humalyzer-3000, Germany).

Statistical analysis: The result was analyzed statistically by paired *t-test* and compared significance level at 95%, 99% and 99.99% by using SPSS software (Version 16.00, IBM Corporation). In this analysis, we compared each treatment group with control group separately.

RESULT

Hematological Tests

Table 1 and figure 1 shows the effects of vitamin-D and sunlight on blood parameters. The application of butter, sunlight and synthetic vitamin-D caused significant increase of blood cell counts in treated groups.

Table 1: Effects of butter, vitamin-D and sunlight on TEC, Hb, PCV, TLC and DLC-

Groups		Group-A (Control)	Group-B (Butter)	Group-C (Sun-light)	Group-D (Vit-D @ 50 IU)	Group-E (Vit D @ 100 IU)
TEC (million/µl)		6.43±0.25	7.58±0.19**	8.09±0.11***	8.90±0.06***	7.88±0.12**
Hbgm/dl		6.34± 0.22	7.28±0.12***	7.14±0.027*	7.84±0.082**	7.62±0.17**
PCV %		32.20± 1.32	36.20±0.862**	41.60±0.98***	44.20±0.80***	40.60±0.24**
TLC (10^3/µl)		7.21±0.14	7.75±0.12*	7.62±0.20	7.96±0.13***	7.47±0.21
DLC	Lympho (10^3/µl)	4.788±0.20	5.115±0.33	5.319 ± 0.39*	5.604±0.17***	5.110±0.41
	Mono (10^3/µl)	0.303±0.06	0.341±0.064	0.214±0.1	0.160±0.06*	0.135±0.1*
	Neutro (10^3/µl)	1.933±0.17	2.108±0.09	1.951±0.14	2.118±0.16*	2.032±0.16
	Eosino (10^3/µl)	0.231±0.13	0.186±0.12	0.138±0.11	0.080±0.08	0.195±0.08

Values above represent mean ± standard error of 5 samples per group (n=5)
* Significant at P<0.05, ** significant at P<0.01 and *** significant at P<0.001

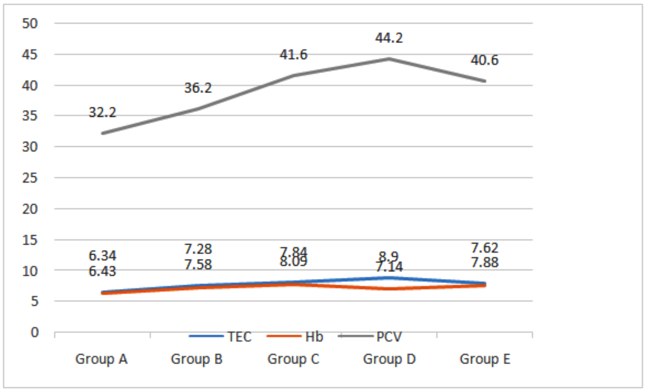

Figure 1: The status of TEC, Hb and PCV in different groups of mice

Hemoglobin level in experimental mice was gradually increased with increased level of vitamin-D supplementation (Figure-1).It was the highest in group-D (7.84±0.082 gm/dl) followed by group E (7.62±0.17 gm/dl) in comparison to control group (6.34± 0.22 gm/dl). Its value was 7.28±0.12 gm/dl in group B and 7.14±0.027 gm/dl in group C. PCV was found significantly higher (44.20±0.80 %, P<0.001) in group D and group C (41.60±0.98%, P<0.01) but non-significant in group E and group B. Figure 1 shows that PCV level dramatically increases with treatment at certain level and then suddenly decrease with excessive supplementation (group E).

TLC (illustrated in table1) was 7.21±0.14 thousand/µl in group, 7.75±0.12 thousand/µl in group B, 7.62±0.20 thousand/µl in group C in 7.96±0.13 thousand/µl in group D, and 7.47±0.21 thousand/µl in group E. TLC was highest in group D and lowest in group A (control group).

In DLC, lymphocytes were significantly higher in vitamin-D @ 50 IU (5604 /µl, P< 0.001) but when dose increased to 100 IU lymphocytes decreased to 5110/µl. Table-1 shows that monocyte number decreases among the treatment except butter receiving mice. Monocyte number was higher in butter treated mice which was 0.341 thousand/µl (P< 0.001) than the control group (0.303 thousand/µl). Among the experimental mice it was lowest in group E (0.135 thousand/µl) which was significant statistically (P<0.05). Neutrophil number was higher in groupD treated mice (2.118 thousand/µl, P< 0.05) but insignificantly lowest in counting eosinophil in sunlight exposed group. In butter, sunlight and vitamin-D @ 100 IU receiving mice neutrophil number was 2.108 thousand/µl, 1.951 thousand/µl and 2.032 thousand/µl, respectively. Neutrophils were significant at P<0.001 (group D). Conversely when eosinophil number increases, the neutrophil number decreases in experimental mice.

Biochemical Tests

To know internal body functions, liver function tests and lipid profile tests were conducted. Liver functions test includes alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (AP) and lipid profile tests includes High Density Lipoprotein (HDL), Total Cholesterol (TC) and Triglyceride (TG). The biochemical reports of different groups of mice are presented in table 2. The graphical presentation of liver function tests and lipid profile tests are shown in figure 2 and 3, respectively.

Test result shows that AST level is steadily increase with increase vitamin-D in group B, C, D and E than control group (49.11±8.82U/L). AST level reaches peak 84.88±25.17 U/L (P<0.001) in vitamin-D @50 IU treated group. Significantly highest (P<0.001) level also observed in sunlight group, C (78.21±23.78U/L) followed by butter treated group, B (75.77±6.39U/L). Suddenly, AST level was the lowest) in group E (64.21±15.93U/L, P<0.001) supplemented with very high doses of vitamin-D (@ 100 IU).

Table 2: Effects of vitamin-D and sunlight on AST, ALT, AP in experimental mice.

Groups	Group-A (Control)	Group-B (Butter)	Group-C (Sunlight)	Group-D (Vit D @ 50 IU)	Group-E (Vit D @ 100 IU)
AST U/L	49.11±8.82	75.77±6.39**	78.21±23.78	84.88±25.17	64.21±15.93
ALT U/L	20.21±3.47	21.77±2.21	44.66±4.34**	33.11±4.84	55.77±14.05*
Alkaline Phosphatase U/L	52.52±10.61	61.56±7.95	70.70±9.14	87.53±9.17*	21.00±4.38*
Total Cholesterol mg/dl	149.80± 9.13	138.49±5.36	158.68± 5.45	152.07± 5.21	117.55± 5.71**
HDL mg/dl	46.79± 3.26	57.92±1.68*	70.77 ± 3.30**	71.15± 2.53***	67.18 ± 3.22**
Triglyceride mg/dl	89.40±2.77	83.34± 6.49	84.08 ± 7.54	92.38 ± 4.20	90.77 ± 6.13

Values above represent mean ± standard error of 5 samples per group (n=5)
** Significant at P<0.01 and *** significant at P<0.001.

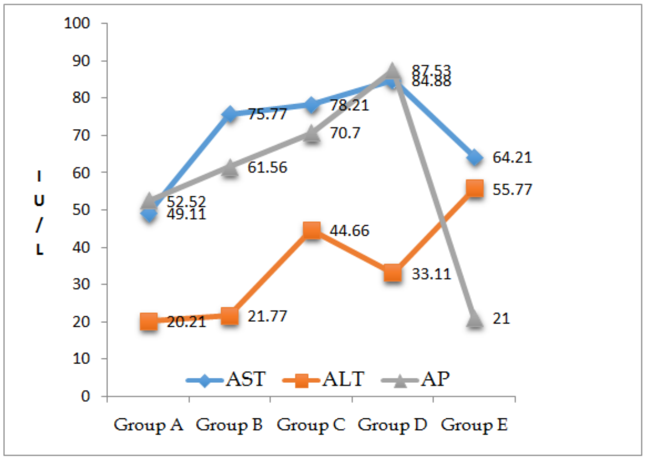

Figure 2: Alteration style of liver activities with different doses of vitamin-D in mice -

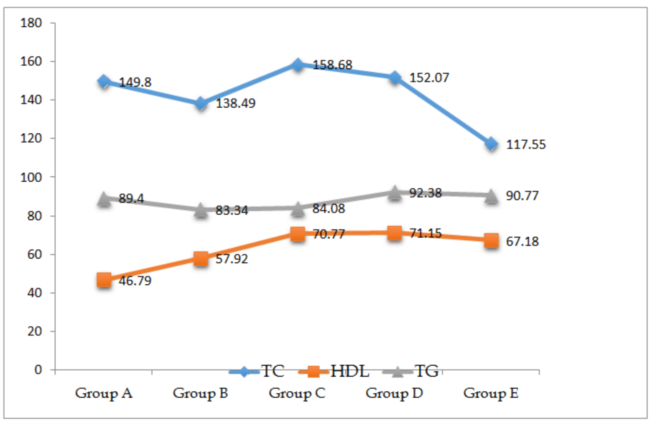

Figure 3: Lipid profile trends of Responses against sunlight and vitamin-D in mice.

There is significant fluctuation of ALT level in experimental mice. This parameter is higher in group E (55.77±14.05 IU, P<0.01), followed by group C (44.66±4.34 IU). In group D, ALT level was lowest (33.11±4.84 IU) among the treated groups. But in butter treated group, ALT was (21.77±2.21 IU which was close to the control group 20.21±3.47 IU). All the values are statistically significant.

AP is one of the major parameters to determine the activities of liver as well as bone restoration and formation. According to Figure-2, AP level gradually increased up to group-D, then it dramatically dropped down in group-E (21.00 U/L). AP level is highest (P< 0.001) in vitamin treated group, group D (87.53±9.17 U/L). Significantly higher (P< 0.001) level was found in sun-light group, 70.70±9.14 U/L. Butter treated group showed AP level was medium (61.56±7.95 U/L P<0.001). Among the treatment groups, group E was the lowest (21.00±4.38 U/L).

Lipid Profile of Experimental Mice

In our study, cholesterol level was near similar to the proportional level of triglyceride (Table 2 and figure 3) which was not steadily influenced by vitamin-D concentration. Cholesterol level was significant (P<0.01) in butter supplemented mice (138.49±5.36 mg/dl) and. However, it was 158.68 ± 5.45 mg/dl in sunlight exposed group, 152.07 ± 5.21 mg/dl in vitamin-D @ 50 IU and 117.55 ± 5.71 mg/dl in vitamin-D @ 100 IU treated groups, respectively.

Table 2 represents HDL level significantly increased (P<0.001) in group D (71.15± 2.53 mg/dl). Second highest was 70.77 ± 3.30 mg/dl in group C which was gradually increased with vitamin-D concentration in blood. In control group HDL was 46.79± 3.26 mg/dl, 57.92±1.68 mg/dl in group B and 67.18 ± 3.22 mg/dl in group E.

Triglyceride level in different groups was different and fluctuated irregularly with vitamin-D concentration (Table 2). Triglyceride level in control group was 89.40±2.77 mg/dl whereas it was 83.34 ± 6.49 mg/dl in butter supplemented mice. The level of triglyceride was 84.08 ± 7.54 mg/dl in sunlight exposed group (group C) and 92.38 ± 4.20 mg/dl and 90.77 ± 6.13 mg/dl in vitamin-D @50 IU (group D) and 100 IU treated group (group E), respectively. All values were statistically significant (P< 0.001).

DISCUSSION

Hematology:

Application of excess vitamin-D than daily requirement is effective to improve RBC in peripheral blood and stimulates hemopoietic organs. On the other hand, sunlight produces good amount of vitamin-D (www.infinitheism.com), increases RBC level by acting on bone, liver and kidney. Besides that, butter may also act as a good source of vitamin-D but had lower strength to reveal effects on TEC values. At the same time vitamin-D in the diet is thought to invigorate the liver function that indirectly enhances erythropoiesis (Guyton and Hall, 2006). The table 1 revealed that stimulation in hemopoetic organs like bone and liver at adequate level may ameliorate Hb formation in comparison with control group (6.34± 0.22gm/dl). From this finding it can be assumed that vitamin-D has positive effect on hemoglobin concentration. This finding is closely related to the findings of others (Jennifer *et al,*. 2013; Shu*et al,*.2011).When body exposed to excess vitamin-D, hemopoietic organ produce more blood cells and thus the level of PCVgrown up gradually. Although vitamin-D level was increases in treated mice but the There was no relation with vitamin-D concentration among the groups for counting WBC. The fluctuation was not equal in all cases. This may indicate that there was no similarity in increasing leukocytes which is also reported by

Ashraf *et al.* (2012). This experiment reveals that vitamin-D could be responsible for raising leucocytes number from normal condition. This is also described by Marwah*et al.* (2012). Increase number of leukocyte may be an indication of vitamin-D action on cell formation from bone by the activation of stem cells in the body (Cynthia, 2011). According to Charles *et al.* (2007) hepatic injury increases peripheral lymphocytes in blood which is similar to this experiment. Increase number of lymphocyte may increase the recruitment of cells in the inflammatory hepatocyte to minimize the lesion and improving healing process (Geoffrey *et al.*, 2002; Patricia *et al.*, 2002).

Beside lymphocytes, the number of neutrophils significantly increases in treated groups. In vitamin-D @ 100 IU and butter treated mice this number was comparatively higher than others (P<0.01) which was also reviewed by some scientists. The increase numbers of polymorphs indicate that there may be presence of inflammation and have possibility of hepatocyte degeneration (Jaeschke and Hasegawa,2006; Ramaiah and Jaeschke,2007).

Monocyte number significantly decreases with increase vitamin-D in blood (Table 1). Although in butter supplemented group it is higher (4.40±0.40%, P<0.001) than other groups but it decreases with increase vitamin-D level in blood. The causes of reduction in number may be associated with chronic inflammatory problem in liverin where subset of monocyte may be happened for fibrogenesis (Liaskou*et al.,* 2013; Frank, 2012).

Eosinophil number was significantly (P<0.001) higher in treated groups (3.20±0.80%) in compare with control group (Table 1). However they were statistically significant. A low number of eosinophils in the blood (eosinopenia) could occur in stress condition caused by excess vitamin-D but does not usually cause problems (Merck Manual, 2013). There may be another reason of gradual decreasing eosinophil number was the infiltration of this cell in liver and it is an unusual condition (Hyun *et al.,* 2012).

Liver Function Tests

The excess and sudden low level of AST indicates that there may have both positive and negative effects of vitamin-D on liver enzyme production and very high dose may causes toxicity (Khaled *et al.*, 2009). According to Holmes and Kummerow (1983) and Glenville (2008) vitamin-D is toxic compound and excessive amount causes calcification of soft-tissues. This toxicity and calcification may affect both liver and muscle and secrete more AST in blood serum. Nature like sunlight may not have enough effect to produce AST but increase level indicates that regular exposure to sunlight may create hepatocellular disease and raises transaminase activities (Beran and Ulker, 2006).

ALT level in mice was significantly changed with different treatment. This variation may depend on degree of liver damage (Amina *et al.*, 2010; Zhou-wen *et al.*, 2008). The ratio between AST and ALT in treatment group indicates that there is progressively high degeneration in hepatocytes (Raiza*et al.,* 2014). In addition, calcification of liver may responsible for increasing level of ALT in blood (Holmes and Kummerow, 1983; Glenville, 2008).

Alkaline Phosphatase level is increased with increasing vitamin-D concentration as linear motion than compared group (Figure 2). The experimental data (Table 2) may cause bone turnover that means mineralization of bone is happened due to rise of vitamin-D volume (Guyton and Hall, 2006). The low level of AP in group E was due to vitamin-D toxicity in liver and kidney, and causes excessive damage to hepatocyte. Higher level of AP in other treated groups also indicator of liver damage (Khaled *et al*., 2009) and further the elevated AP levels accompanied by increased bone and liver activity (Konstantinos *et al.,* 2002). This result could interfere with calcium metabolism and bony architecture either had positive or negative effect (http://labtestsonline.org).

Lipid Profile of Experimental mice

Table 2 shows that there is no improvement or no significant effect of cholesterol due to over doses of vitamin-D supplement.Similar type of study have been trialed in man (Ponda*et al.,* 2012; Asemi *et al.,* 2012). There is presence of abnormal cholesterol level in group E where each mouse receives 100 IU vitamin-D daily. It may be for liver damages, thus liver could not synthesize adequate cholesterol. Such kind of information also reported earlier (Jerome and Robert, 1970). This finding also supports that supplementation of diet with vitamin-D which enhances the beneficial effect of weight loss on plasma lipid and lipoprotein concentrations (Geneviève *et al.,* 2007).

Although vitamin-D is a fat soluble vitamin but increase of vitamin-D concentration improves HDL level. There may have possibility of increasing metabolism rate of liver and vitamin-D also increases blood circulation to the tissues. These two factors might be responsible for raising HDL in blood. The HDL level of this study is supported by Sun *et al.* (2014) and Jorde and Grimnes (2011). According to their findings, serum 25(OH)-D is positively related with high-density lipoprotein cholesterol (HDL-C) and is favorable to HDL-C and high-density lipoprotein cholesterol (LDL-C) ratio. Additional supplement of vitamin-D may improve lipid profile as well as serum HDL in aged people (Jungert*et al.,* 2014).

Triglyceride level fluctuates irregularly with different treatment. According to Ponda *et al.* (2012) vitamin-D could not improve triglyceride and has no direct effect on triglyceride levels (Figure 8). Vitamin-D supplementation can lower triglycerides (Jorde and Grimnes, 2011). It is a comparable matter that the vitamin-D concentration at certain level may improve triglyceride (group B and C) but excess vitamin-D may be harmful for liver and kidney and is responsible for elevated triglyceride (José *et al.,* 2009). Elevated triglyceride level is caused by medical conditions such as diabetes, hypothyroidism, kidney disease, or liver disease (Shaoqing*et al.,* 2010; James *et al.,* 2007). Vitamin-D treated group D and E shows slight high level of triglyceride compare to normal mice.

CONCLUSION

The purpose of this study was to evaluate the role of vitamin-D on the body and some special tissues and its side effects on body. In hematological tests, TEC and TLC level was higher in vitamin-D supplemented group. However, these values are statistically insignificant. Excess amount of vitamin-D would be harmful for blood cell production. One the other hand biochemical tests of serum may indicate the huge detoxifying activities of liver may produce cirrhosis and brings structural changes

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ABBREVIATION

ALT	=	Alanine Serum Transaminase
AP	=	Alkaline Phosphatase
AST	=	Aspartate Transaminase
DLC	=	Differential Leukocyte Count
EDTA	=	Ethylene DiamineTetrachloro Acetic Acid
ESR	=	Eryhthrocyte Sedimentation Rate
Hb	=	Hemoglobin
HCl	=	Hydrochloric Acid
HDL	=	High Density Lipoprotein
LDL	=	Low Density Lipoprotein
RBC	=	Red Blood Cell/Corpuscle
PCV	=	Packed Cell Volume
TC	=	Total Cholesterol
TEC	=	Total Erythrocyte Count
TG	=	Triglyceride
TLC	=	Total Leukocyte Count
U/L	=	Unit per Liter
WBC	=	White Blood Cell/Corpuscle

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