

## Research Article

# Ischemia-modified albumin level in vitamin D deficiency

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### Abstract

**Objectives:** Vitamin D has been associated with extra-skeletal pathologies through mechanisms involving inflammatory and oxidative stress processes. Ischemia-modified albumin (IMA) is one of the earliest indicators of ischemia, and is regarded as a marker of oxidative stress. In the present study, the IMA level in serum samples with various 25-OH vitamin D [25(OH)D] concentrations was examined for signs of oxidative stress as a result of vitamin D deficiency.

**Methods:** A total of 80 serum samples requested by clinicians for 25(OH)D testing and analysis were randomly selected and divided into 4 groups (n=20 in each group) according to the 25(OH)D concentration. Group 1:  $\leq 10$  ng/mL (severe deficiency), Group 2: 10-20 ng/mL (deficiency), Group 3: 20-30 ng/mL (insufficiency), and Group 4:  $\geq 30$  ng/mL (sufficiency) were formed. Serum IMA was measured spectrophotometrically, and the results were expressed in absorbance units (ABSU).

**Results:** The IMA level in Group 1 through Group 4 was  $0.541 \pm 0.082$  ABSU,  $0.515 \pm 0.059$  ABSU,  $0.438 \pm 0.085$  ABSU, and  $0.467 \pm 0.102$  ABSU, respectively. The IMA level was found to be significantly different in comparisons between Groups 1 and 3, Groups 1 and 4 and Groups 2 and 3 ( $p=0.001$ ,  $p=0.032$ ,  $p=0.022$ , respectively); no significant difference was found in other comparisons of the groups. There was a weak negative correlation between serum 25(OH)D and IMA level ( $r = -0.346$ ;  $p=0.002$ ).

**Conclusion:** The serum IMA level is elevated in severe vitamin D deficiency and vitamin D insufficiency due to increased oxidative stress resulting from the inadequate antioxidant function of vitamin D. The IMA level may have been higher in the vitamin D sufficiency group compared with the insufficiency group due to a possible pro-oxidant effect of vitamin D as its level rises. If this hypothesis is confirmed with future studies, it may be appropriate to consider a serum 25(OH)D level of between 20 and 30 ng/mL sufficient.

**Keywords:** Ischemia modified albumin, oxidative stress, vitamin D

Vitamin D is a lipid-soluble vitamin, and its main function is to facilitate calcium absorption from the intestines. Through acting on calcium and phosphorus metabolism, vitamin D also regulates bone metabolism [1, 2]. Following dietary intake or its synthesis in the skin upon exposure to sunlight, vitamin D is transported to the liver. The first hydroxylation step occurs in the liver at the carbon atom 25 position, forming 25(OH)D. This is the main circulating metabolite of vitamin D, and its serum concentration is regarded as an indicator of the vitamin D status of the body [3].

Although the main function of vitamin D is the regulation of calcium and phosphorus metabolism and balancing bone metab-

olism, recent studies have suggested that vitamin D has other previously unknown roles as well. Among these new roles, providing beneficial effects to cardiovascular functions through acting on the vascular smooth muscle cells, myocardium, and endothelium is quite significant [2]. A reduced level of vitamin D is associated with increased cardiovascular mortality [4]. Furthermore, vitamin D plays a key role in the prevention of some cancer types, including colon, prostate, and lung cancer; autoimmune diseases, like multiple sclerosis, type 1 diabetes mellitus, and Crohn's disease; metabolic syndrome; and infectious diseases, such as tuberculosis [5, 6].

The association between vitamin D and extra-skeletal patholo-

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**Table 1. Distribution of age, gender and IMA values across the patients**

	<b>Group 1 severe vitamin D deficiency (≤10 ng/mL)</b>	<b>Group 2 vitamin D deficiency (10-20 ng/mL)</b>	<b>Group 3 vitamin D insufficiency (20-30 ng/mL)</b>	<b>Group 4 sufficient vitamin D (≥30 ng/mL)</b>	<b>P value</b>
Male/female (n)	7/13	7/13	4/16	7/13	0.666
Age (years)	44±26	44±26	52±19	44±26	0.688
IMA (ABSU)	0.541±0.082	0.515±0.059	0.438±0.085	0.467±0.102	0.001*

IMA: Ischemia-modified albumin.

Age and IMA values are expressed as mean±SD.

\*Significance of differences between the groups was tested with one-way analysis of variance and a chi-square test. P value <0.05 was considered significant.

gies may involve inflammation and changes in oxidative stress. There are studies in the literature related to the antioxidant properties of vitamin D [7]. Nonetheless, the effect of vitamin D supplementation on the oxidant-antioxidant balance is not clearly understood. The anticancer effect of vitamin D has been associated with its antioxidant property [8].

Among recently discovered cardiac markers, ischemia modified albumin (IMA) stands out as one of the earliest indicators of ischemia. Free radicals that form during the ischemic state cause alterations in the serum albumin. This altered form of albumin, which loses its ability to bind to metals such as cobalt, is called IMA [9]. The concentration of this temporarily altered molecule can be measured using a method based on an assessment of cobalt-binding capacity [10]. Initially proposed as a promising marker of myocardial ischemia, IMA has also been accepted as a marker of oxidative stress in non-cardiac pathologies as well, since it is also affected by pathologies of other organ systems [11].

In the present study, the IMA concentration in samples with various levels of vitamin D were examined to determine the antioxidant properties of vitamin D and possible oxidative stress development in vitamin D deficiency.

## Materials and Methods

A total of 80 serum samples requested by clinicians and analyzed by the laboratory for the level of 25(OH)D were randomly selected and separated according to the concentration of 25(OH)D. The IMA level in the remainder of the serum sample was analyzed. There were 4 groups according to the serum 25(OH)D vitamin level: Group 1 comprised patients with severe vitamin D deficiency [25(OH)D level ≤10 ng/mL], Group 2 consisted of patients with vitamin D deficiency [25(OH)D level 10-20 ng/mL], Group 3 was made up of patients with vitamin D insufficiency [25(OH)D level 20-30 ng/mL], and patients with sufficient vitamin D [25(OH)D level ≥30 ng/mL] were assigned to Group 4 [12, 13]. Each group consisted of 20 patient samples. The patients' clinical and descriptive information was retrieved retrospectively from the hospital information system. Patients who used any medication or antioxidant supplement or who

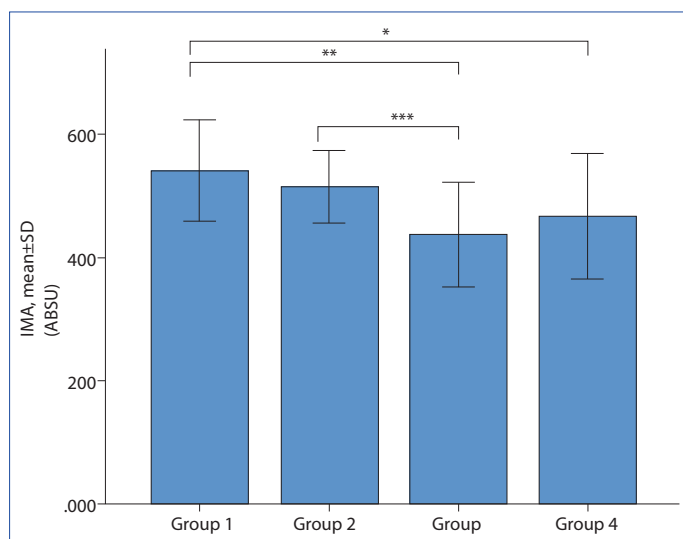
had any disease that could affect oxidative status were excluded from the study.

The blood samples were processed into clot-activator tubes containing gel (BD Vacutainer SST II Advance, 5 mL, 13x100mm, catalogue number 367955; Becton Dickinson and Co., Franklin Lakes, NJ, USA) and centrifuged at 1500 g for 10 minutes to separate the serum from the blood clot. Serum 25(OH) D measurements were performed using a chemiluminescence immunoassay on an Advia Centaur XP analyzer (Siemens Healthineers, Erlangen, Germany). The intra-assay and inter-assay coefficients of variation (CV) for the 25(OH)D test were below 8% and 12%, respectively. The left-over serum samples were stored at -20°C. The serum IMA level was analyzed spectrophotometrically at 470 nm using the cobalt-binding assay described by Bar-Or [14] within a week. The results were expressed in absorbance units (ABSU). The intra-assay CV for 2 different levels (mean±SD: 0.484±0.006 and 0.647±0.006) was 1.23% and 0.92%, respectively [15].

IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA) was used for all statistical analyses. Normality assessment in each group was performed using the Shapiro-Wilks test. Since all data fit into normal distribution, statistical analyses were done using parametric tests. The significance of differences in numerical parameters (age, IMA level) between groups was tested with one-way analysis of variance and the Tukey test was applied as a post hoc test, since the variations were homogeneously distributed. The significance of differences in a categorical parameter (gender) was analyzed with a chi-square test. The correlation between serum 25(OH)D and IMA level in the whole study sample (n=80) was analyzed with Pearson's correlation test. P values less than 0.05 were considered significant.

## Results

Table 1 summarizes the age, gender, and IMA level of the patients. There was no significant difference between groups in respect to the distributions of age and gender (p=0.688 and p=0.666, respectively). With regard to the serum IMA level, there was a statistically significant difference in the comparisons of Groups 1 and 3, Groups 1 and 4, and Groups 2 and 3 (p=0.001,

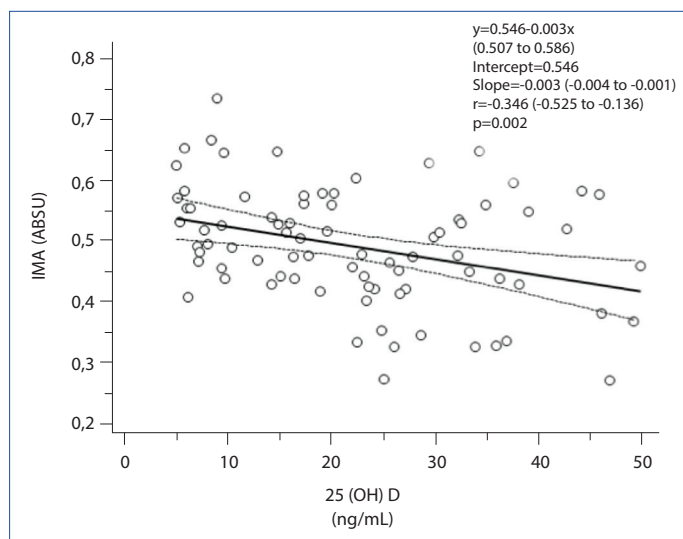


**Figure 1.** IMA levels across the groups. Significant differences between the IMA levels of the groups were tested with the Tukey test as a post hoc test. IMA: Ischemia-modified albumin.

\* $P$  value=0.032 for Group 1 vs. Group 4

\*\* $P$  value=0.001 for Group 1 vs. Group 3

\*\*\* $P$  value=0.022 for Group 2 vs. Group 3



**Figure 2.** Correlation between IMA and 25-OH vitamin D. The solid and dashed lines in regression graphs represent the regression line and its confidence intervals, respectively.

25(OH)D: 25-OH vitamin D; IMA: Ischemia-modified albumin.

$p = 0.032$ , and  $p = 0.022$ , respectively). No significant difference was found in other comparisons of the groups (Fig. 1). There was a weak negative correlation between serum 25(OH)D and IMA level ( $r = -0.346$ ;  $p = 0.002$ ) (Fig. 2).

## Discussion

Currently available methods for 25(OH)D measurement include immunoassays, high performance liquid chromatogra-

phy (HPLC), and liquid chromatography-mass spectrometry (LC-MS). LC-MS is regarded as the gold standard. Although HPLC appears superior due to its ability to distinguish vitamin D derivatives as well as the wide range of detection and lower cost per test, it requires more complex sample preparation steps and trained personnel. Therefore, immunoassays are most often used for routine measurements despite providing lower values and the inability to distinguish vitamin D derivatives. Immunoassay test results can be affected by the properties of different antibodies used by manufacturers [16].

Although a consensus has been achieved to measure the 25(OH)D level in order to determine vitamin D status in the body, there is still controversy about what level should be accepted as sufficient. For bone health, a 25(OH)D level of 20 ng/mL or greater is thought to be sufficient and a level less than 20 ng/mL is considered to reflect vitamin D deficiency. However, when considering the extra skeletal functions of vitamin D through receptors on various organs other than bone, 30 ng/mL is the threshold for sufficient vitamin D [17]. A level of 25(OH)D below 30 ng/mL has been associated with reduced intestinal calcium absorption and an increased PTH release that is inversely correlated with the reduced calcium absorption. Therefore, a vitamin D level between 20 ng/mL and 30 ng/mL has been defined as relative deficiency or partial sufficiency, whereas 30 ng/mL or higher has been deemed a sufficient level [18].

Vitamin D deficiency has been shown to cause oxidative stress, and vitamin D supplementation has been associated with improvement in both glucose homeostasis and oxidative stress [19]. Vitamin D level lower than 30 ng/mL has also been associated with reduced velocity of coronary blood flow, endothelial dysfunction, and subclinical atherosclerosis [20]. Vitamin D level lower than 20 ng/mL was detected in obese children with increased oxidative/nitrosative stress, markers of inflammation, and endothelial activation [21]. Similar studies have indicated that perhaps vitamin D deficiency contributes to oxidative imbalance by inducing inflammatory processes [22]. It has been emphasized that vitamin D demonstrates an antioxidant effect by inhibiting the expression of nicotinamide adenine dinucleotide phosphate oxidase and by inducing the synthesis of various molecules in the antioxidant defense system, such as glutathione, glutathione peroxidase, and superoxide dismutase, in the prevention of chronic diseases [7]. It has been suggested that the antioxidant effect of vitamin D may be partially due to binding to the vitamin D receptor, which is a nuclear receptor [23, 24]. In the literature, it has been demonstrated that vitamin D in mature erythrocytes had an important antioxidant role [25], and that it could be like a direct antioxidant via stabilizing and protecting the membrane from lipid peroxidation through the hydrophobic portions of vitamin D [26]. The antioxidant effect of vitamin D was even stronger than that of vitamin E, beta-estradiol, and melatonin in an in vitro experiment [27].

Chandrashekar et al. [28] measured the IMA level to evaluate

oxidative stress in patients with psoriasis, and they observed that the vitamin D level decreased with an advancing inflammatory state, while the level of high-sensitivity C-reactive protein and IMA increased. Baser et al. [2] reported that the total antioxidant level decreased as IMA, total oxidant status, and fibrinogen levels increased in patients with a vitamin D deficiency (<20 ng/mL) in comparison with healthy individuals (>30 ng/mL).

We observed similar results in the IMA level in individuals categorized into 4 groups based on the 25(OH)D levels. The mean IMA level in the group with severe vitamin D deficiency was  $0.541 \pm 0.082$  ABSU. This is quite a bit higher than  $0.339 \pm 0.093$  ABSU, the reference value recommended for the Turkish population [29]. The mean IMA was also quite high in the group with vitamin D deficiency, at  $0.515 \pm 0.059$  ABSU [29]. The mean IMA level in both Group 1 and Group 2, which were defined as severe vitamin D deficiency and vitamin D deficiency, respectively, was found to be significantly higher than that of Group 3, which was defined as insufficiency (Group 1:  $0.541 \pm 0.082$  ABSU, Group 2:  $0.515 \pm 0.059$  ABSU, Group 3:  $0.438 \pm 0.085$  ABSU). This suggests that a vitamin D level below 20 ng/mL is associated with oxidative stress, with a quite pronounced state of oxidative stress at levels below 10 ng/mL. The fact that there was a weak negative correlation between 25(OH)D and IMA supports the hypothesis that vitamin D is protective against oxidative stress. Similarly, Baser et al. [2] observed a negative correlation between vitamin D level and IMA ( $r = -0.500$ ;  $p < 0.001$ ).

Our study also yielded another result that we did not encounter in previous studies. In Group 4, the vitamin D sufficiency group, the IMA level was higher compared with Group 3, the insufficiency group ( $0.467 \pm 0.102$  vs.  $0.438 \pm 0.085$ ), although the difference was not statistically significant. Moreover, the reduction in the IMA level in Group 4 was not statistically significant when compared with the deficiency group (Group 2). However, when compared with patients with severe vitamin D deficiency (Group 1), the reduced IMA level in Group 4 was statistically significant. Based on these results, Group 3, the vitamin D insufficiency group, appears to be in better condition than Group 4, the vitamin D sufficient group, in terms of protection against oxidative stress through the action of vitamin D. Although vitamin D is thought to be protective against oxidative stress, the fact that the IMA level showed a tendency to increase with a higher level of vitamin D prompts an inquiry about whether higher levels of vitamin D have a pro-oxidant effect or not. A possible explanation may be that increased levels can trigger inflammation through overstimulation of the receptors. If this is the case, ideal levels of vitamin D need to be investigated, and it should be ascertained whether oversupply of this vitamin would be hazardous rather than having a protective effect. Due to the sensitivity and importance of vitamin D level, cut-off values should be verified.

In conclusion, the IMA level was elevated in severe vitamin D deficiency and vitamin D insufficiency due to increased oxida-

tive stress resulting from inadequate anti-oxidant function of vitamin D. On the other hand, unexpectedly, the IMA level in the vitamin D sufficient group was higher than that seen in the insufficient group. We think a possible explanation for this result may be that as vitamin D levels rise, it has a pro-oxidant effect, or triggers inflammation and causes oxidative stress. If this hypothesis is confirmed with future studies, it may be appropriate to reconsider sufficient levels of 25(OH)D, and perhaps appraise serum 25(OH)D levels between 20 and 30 ng/mL as sufficient. In that case, it may be necessary to avoid high dose vitamin D supplementation, and bring acceptable levels of vitamin D to much lower than the toxic levels.

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## References

1. Akelma Z, Abacı A, Çelik A, Özdemir O, Avcı Z, Razi CH. Three cases of nutritional vitamin D deficiency: rickets is still a public health problem. *Türk Aile Hek Derg* 2010;14:104–8. [CrossRef]
2. Baser H, Can U, Baser S, Hidayetoglu BT, Aslan U, Buyuktorun I, et al. Serum total oxidant/anti-oxidant status, ischemia-modified albumin and oxidized-low density lipoprotein levels in patients with vitamin D deficiency. *Arch Endocrinol Metab* 2015;59:318–24. [CrossRef]
3. Nicolaidou P, Hatzistamatiou Z, Papadopoulou A, Kaleyias J, Floropoulou E, Lagona E, et al. Low vitamin D status in mother-newborn pairs in Greece. *Calcif Tissue Int* 2006;78:337–42.
4. Dobnig H, Pilz S, Scharnagl H, Renner W, Seelhorst U, Wellnitz B, et al. Independent association of low serum 25-hydroxyvitamin d and 1,25-dihydroxyvitamin d levels with all-cause and cardiovascular mortality. *Arch Intern Med* 2008;168:1340–9.
5. Ongen B, Kabaroglu C, Parıldar Z. Biochemical and Laboratory Evaluation of Vitamin D. *Türk Klinik Biyokimya Derg* 2008;6:23–31.
6. Janner M, Ballinari P, Mullis PE, Flück CE. High prevalence of vitamin D deficiency in children and adolescents with type 1 diabetes. *Swiss Med Wkly* 2010;140:w13091. [CrossRef]
7. Mokhtari Z, Poustchi H, Eslamparast T, Hekmatdoost A. Egg consumption and risk of non-alcoholic fatty liver disease. *World J Hepatol* 2017;9:503–9. [CrossRef]
8. Deeb KK, Trump DL, Johnson CS. Vitamin D signalling pathways in cancer: potential for anticancer therapeutics. *Nat Rev Cancer* 2007;7:684–700. [CrossRef]
9. Duman C, Eşsizoğlu E, Demircan E, Kırtay Tütüncüler F, Bakar C, Kurtulmuş Y. Relationship Between Ischemia Modified Albumin and Inflammatory Parameters. *Türk Klinik Biyokimya Derg* 2013;11:113–7.

10. Dusek J, Tichý M, Stásek J, Bis J, Gregor J, Pudil R, et al. Ischemia-modified albumin: new marker of myocardial ischemia? *Cas Lek Cesk* 2005;144:295–7.
11. Reddy VS, Perugu B, Garg MK. Ischemia-modified albumin must be evaluated as an oxidative stress marker together with albumin and bilirubin in individuals with acute appendicitis. *Clinics (Sao Paulo)* 2015;70:531–2. [\[CrossRef\]](#)
12. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2011;96:1911–30. [\[CrossRef\]](#)
13. Kennel KA, Drake MT, Hurley DL. Vitamin D deficiency in adults: when to test and how to treat. *Mayo Clin Proc* 2010;85:752–7.
14. Bar-Or D, Lau E, Winkler JV. A novel assay for cobalt-albumin binding and its potential as a marker for myocardial ischemia—a preliminary report. *J Emerg Med* 2000;19:311–5. [\[CrossRef\]](#)
15. Ellidag HY, Bulbulla N, Eren E, Abusoglu S, Akgol E, Cetiner M, et al. Ischemia-modified albumin: could it be a new oxidative stress biomarker for colorectal carcinoma? *Gut Liver* 2013;7:675–80. [\[CrossRef\]](#)
16. Bekmez M, Alataş O. Comparison of vitamin D levels measured by HPLC and Immunoassay. *Türk Klinik Biyokimya Derg* 2016;14:46–50.
17. Houston DK. Vitamin D and Age-Related Health Outcomes: Movement, Mood, and Memory. *Curr Nutr Rep* 2015;4:185–200. [\[CrossRef\]](#)
18. Sözen T. D Hormonu: Güncel Gelişmeler. *Hacettepe Tıp Dergisi* 2011;42:14–7.
19. Sepehrmanesh Z, Kolahdooz F, Abedi F, Mazroii N, Assarian A, Asemi Z, et al. Vitamin D Supplementation Affects the Beck Depression Inventory, Insulin Resistance, and Biomarkers of Oxidative Stress in Patients with Major Depressive Disorder: A Randomized, Controlled Clinical Trial. *J Nutr* 2016;146:243–8.
20. Oz F, Cizgici AY, Oflaz H, Elitok A, Karaayvaz EB, Mercanoglu F, et al. Impact of vitamin D insufficiency on the epicardial coronary flow velocity and endothelial function. *Coron Artery Dis* 2013;24:392–7. [\[CrossRef\]](#)
21. Codoñer-Franch P, Tavárez-Alonso S, Simó-Jordá R, Laporta-Martín P, Carratalá-Calvo A, Alonso-Iglesias E. Vitamin D status is linked to biomarkers of oxidative stress, inflammation, and endothelial activation in obese children. *J Pediatr* 2012;161:848–54. [\[CrossRef\]](#)
22. de Almeida JP, Liberatti LS, Barros FE, Kallaur AP, Lozovoy MA, Scavuzzi BM, et al. Profile of oxidative stress markers is dependent on vitamin D levels in patients with chronic hepatitis C. *Nutrition* 2016;32:362–7. [\[CrossRef\]](#)
23. Labudzynski DO, Zaitseva OV, Latyshko NV, Gudkova OO, Veliky MM. Vitamin d3 contribution to the regulation of oxidative metabolism in the liver of diabetic mice. *Ukr Biochem J* 2015;87:75–90. [\[CrossRef\]](#)
24. Kanikarla-Marie P, Jain SK. 1,25(OH)2D3 inhibits oxidative stress and monocyte adhesion by mediating the upregulation of GCLC and GSH in endothelial cells treated with acetoacetate (ketosis). *J Steroid Biochem Mol Biol* 2016;159:94–101.
25. Wolden-Kirk H, Gysemans C, Verstuyf A, Mathieu C. Extraskeletal effects of vitamin D. *Endocrinol Metab Clin North Am* 2012;41:571–94. [\[CrossRef\]](#)
26. Wiseman H. Vitamin D is a membrane antioxidant. Ability to inhibit iron-dependent lipid peroxidation in liposomes compared to cholesterol, ergosterol and tamoxifen and relevance to anticancer action. *FEBS Lett* 1993;326:285–8. [\[CrossRef\]](#)
27. Lin AM, Chen KB, Chao PL. Antioxidative effect of vitamin D3 on zinc-induced oxidative stress in CNS. *Ann N Y Acad Sci* 2005;1053:319–29. [\[CrossRef\]](#)
28. Chandrashekar L, Kumarit GR, Rajappa M, Revathy G, Munisamy M, Thappa DM. 25-hydroxy vitamin D and ischaemia-modified albumin levels in psoriasis and their association with disease severity. *Br J Biomed Sci* 2015;72:56–60. [\[CrossRef\]](#)
29. Duman C, Bakar C, Essizoglu E, Tutunculer FK, Demircan E. The Reference Values Of Ischemia Modified Albumin Levels In Healthy People Admitted To A University Hospital. *Turk J Biochem* 2014;39:132–9. [\[CrossRef\]](#)