Vitamin D deficiency is associated with thyroid diseases

S Jubair1*, A S Nsaif2, A H Abdullah3 and I H Dhefer4

1 Department of Pharmaceutical Chemistry, College of Pharmacy, University of Kerbala, Kerbala, Iraq.
2 Diabetes Centre, Mustansiriyah University, Baghdad, Iraq
3 Department of Chemistry, College of Science, Mustansiriyah University, Baghdad, Iraq
4 Department of Nursing, AL-Suwaira Technical Institute, Middle Technical University, Baghdad, Iraq.

*Corresponding: Suzanne.j@uokerbala.edu.iq, Telp: +9647802725293,

Abstract. Background The deficiency of vitamin D3 (VD) is a universal health issue, its role in different kind of diseases is being studied recently. However, its role in thyroid diseases is not well established yet. This study aims to determine the impact of VD in the pathogenesis of hypothyroidism and hyperthyroidism. Method. Three hundred Iraqi females with age ranged between 30 and 55 years participate in this research, 100 of them were hypothyroid patients, 100 females were hyperthyroid patients and the other 100 females were healthy volunteers served as controls. Thyroid hormones, VD, liver function parameters, and kidney function parameters were determined using different analysis techniques. Results. The levels of VD were significantly decreased in both hypothyroid and hyperthyroid patients (19.644 ± 10.524 for hypothyroid patients and 22.712 ± 11.249 for hyperthyroid patients vs. 30.880 ± 2.587 for controls, p <0.0001). Liver function parameters were within the normal ranges in all the patients. Creatinine and uric acid were within the normal ranges, while urea was significantly increased and out of the normal clinical range in both hypothyroid and hyperthyroid patients (39.560 ± 9.912 for hypothyroidism patients and 42.460 ±7.171 for hyperthyroid patients vs. 26.920 ± 5.033 for controls, p <0.0001). Conclusion. Vitamin D and kidney function tests must be included in the differential detection of thyroid diseases. Still, further investigations are needed to understand the underlying mechanism by which VD affects thyroid hormone regulation.

Keywords: Hypothyroidism, Hyperthyroidism, Vitamin D, Creatinine, Urea, Kidney function.

1. Introduction

Despite the abundance of sunlight in Iraq, the sunshine vitamin is reported to be deficient in most of the Iraqi population [1]. VD is a steroidal biomolecule synthesized into the skin via a reaction catalyzed by the UV light, then it is hydroxylated twice to produce its active 1, 25 (OH)2 D3 form. These hydroxylations occur in the liver and kidney, respectively [2,3]. Vitamin D3 performs its biological effects through binding to its nuclear receptor (VDR) [4]. The expression of VDR is widely distributed through the human body organs and tissues. Its expression in skin tissue, adipocytes, pancreatic β-cells, nonparenchymal cells of the liver, and immune cells indicates that VD has distinguished physiological roles other than bone and mineral homeostasis [5]. Importantly, a low level of VD was reported to be associated with Graves’ disease and Hashimoto’s thyroiditis, which are the most abundant autoimmune thyroid diseases. Furthermore, thyroid tumorigenesis reported to
be encouraged by impaired VD signaling [2]. Thyroid diseases, on the other hand, are assessed to be one of the most common endocrine abnormalities in the Iraqi population and the world [6,7]. Hypothyroidism and hyperthyroidism are associated with many serious clinical changes in kidney function, that’s why thyroid hormones are required in kidney assessment [8]. In another clinical point of view, the metabolism of thyroid hormones lies greatly on the liver, as it is the major organ responsible for the conversion of thyroxin (T4) to triiodothyronine (T3) by the enzyme Type 1 deiodinase [9]. Liver is also the major organ that synthesizes the thyroid binding proteins, hence it regulates thyroid hormones conjugation and excretion.

Although there are many previous studies investigated thyroid diseases and VD deficiency, there are still some limitations. This study was conducted to assess the extent of the association between these two clinical cases. Besides, assessing the impact of the liver and the kidney as organs included in the synthesis and metabolism of both VD and thyroid hormones.

2. Materials and methods

2.1. Study subjects

This study adheres to the principles of the Helsinki Declaration; it is approved by the Ethics Committee of Department of Chemistry, College of Science, Mustansiriyah University, Baghdad, Iraq. Blood samples were collected only after gaining written consent from all the study participants. Three hundred Iraqi females aged 30-55 years participated in this study, 100 of them were hypothyroid patients, 100 females were hyperthyroid patients and the other 100 females were healthy volunteers served as controls. All the patients attended the National Diabetes Center. All the study subjects underwent a physical examination and biochemical screening. None of the patients was receiving steroids, a beta-adrenergic blocking agent, aspirin, or phenytoin sodium and no one had received radiographic contrast within two weeks before measurement of thyroid hormone levels. None of the patients presented signs or symptoms of gout, either previously or at the moment of hospitalization. All the participants were on a normal purine diet.

2.2. Samples and methods

Samples were obtained following overnight fasting and sera were separated and used to measure the parameters in studied groups. Thyroxin, T3, and 25(OH)D3 were determined using Minividas kits obtained from BioMeriux-France. Thyroid-stimulating hormone (TSH) was determined using enzyme-linked fluorescent assay (ELFA) from BioMeriux-France. Serum glutamic oxalo acetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), alkaline phosphatase (ALP), urea, uric acid, and creatinine were assessed using colorimetric commercial kits from Boehringer-Mannheim (Mannheim, Germany).

2.3. Statistical analysis

Program of Statistical package, for social sciences SPSS version 20 used for the statistical analysis of the biochemical factors. The data were represented as means ± SD, one-way ANOVA test was used to compare between means. The p-value of less than 0.05 considered as statistically significant.

3. Result

Some of the demographic factors such as age, BMI, and sun exposure showed no significant variation between hypothyroid patients, hyperthyroid patients, and controls. However, the fasting blood
glucose of the three groups was significantly different, still they all showed values of the normal range (Table1).

**Table 1:** Main comparisons of age, BMI, fasting blood glucose and sun exposure among the study groups.

| Parameters          | Hypothyroidism (n=100) | Hyperthyroidism (n=100) | Control (n=100) | p-Value |
|---------------------|-------------------------|-------------------------|----------------|---------|
| Age (year)          | 40.2 ±6.1               | 39.6 ±6.6               | 39.0± 6.2      | 0.60    |
| BMI                 | 25.50±0.91              | 25.90±2.22              | 25.70±1.83     | 0.667   |
| FBG (mg/dL)         | 91.32±38.910            | 95.08±36.712            | 86.52±8.968    | 0.001*  |
| Sun exposure (min/day) | 15.11 ± 3.32         | 14.21± 2.39             | 16.15± 1.22    | 0.640   |

BMI: body mass index, FBG: fasting blood glucose. The values are represented as Mean± SD,* Significant at p<0.05.

The hypothyroid patients exhibited no significant decreasing of T3, highly significant decreasing of T4 (p<0.0001), and highly significant increase of TSH (p<0.0001). While, the hyperthyroid patients showed a highly significant elevation of T3 (p<0.0001), a highly significant elevation of T4 (p<0.0001), and a highly significant decrease of TSH(p<0.0001). Furthermore, both hypothyroid and hyperthyroid patients exhibited a statistically high significant decrease of 25(OH) D₃ (p<0.0001) (Table 2). Table 2 also shows a non-significant elevation of GOT (p>0.05) in patients compared to controls, while GPT is significantly elevated (p<0.05) in both hypothyroid and hyperthyroid patients. ALP showed a significant increase in both hypothyroid and hyperthyroid patients (p<0.0001). Regarding kidney function parameters, both hypothyroid and hyperthyroid patients exhibited a highly significant elevation of urea (p<0.0001) and significant elevation of creatinine (p<0.001), while both the patient groups showed non-significant elevation of uric acid compared to controls (Table 2).

**Table 2:** Mean comparisons of thyroid function tests, 25(OH)D, liver function parameters and kidney function parameters among the study groups.

| Parameters       | Hypothyroidism (n=100) | Hyperthyroidism (n=100) | Control (n=100) | p- Value |
|------------------|-------------------------|-------------------------|----------------|---------|
| T3 (nmol/L)      | 1.184±0.451             | 2.936±0.357             | 1.104±0.259    | 0.000** |
| T4 (nmol/L)      | 38.933 ±15.596          | 198.36 ±27.057          | 102.880 ±21.505| 0.000** |
| TSH(µmol/L)      | 18.512±8.878            | 0.062±0.101             | 1.990 ±1.353   | 0.000** |
| 25(OH)D₃ (ng/mL) | 19.644±10.524           | 22.712±11.249           | 30.880±2.587   | 0.000** |
| GOT (U/L)        | 19.360 ±5.329           | 19.400 ±5.462           | 17.240 ±3.961  | 0.219   |
| GPT (U/L)        | 29.000 ±10.132          | 28.440 ±10.137          | 22.200 ±6.035  | 0.015*  |
| ALP (U/L)        | 267.520 ±95.413         | 194.040 ±49.295         | 184.440 ±40.888| 0.000** |
| Urea (mg/dL)     | 39.560 ± 9.912          | 42.460 ±7.171           | 26.920 ± 5.033 | 0.000** |
| Creatinine (mg/dL) | 1.316 ± 0.416          | 1.232 ± 0.414           | 0.944 ± 0.183  | 0.001*  |
| Uric acid (mg/dL)| 6.236 ± 2.240          | 6.132 ± 1.563           | 5.160 ± 1.708  | 0.086   |
T₃: triiodothyronine, T₄: thyroxin, TSH: thyroid stimulating hormone, GOT: glutamic oxaloacetic transaminase, GPT: glutamic pyruvic transaminase, ALP: alkaline phosphatase. The values are represented as Mean± SD, * Significant at p<0.05, ** highly Significant at p<0.0001.

All the biochemical parameters were tested for possible correlation; the only strong obtained correlation was between urea and VD. Table 3 elucidates a significant negative correlation between urea and VD and a negative non-significant correlation between urea and each of T₃ and T₄.

**Table 3:** The correlation between urea and each of VD, T₃ and T₄ levels in hypothyroid and hyperthyroid patients.

| Correlation          | Hypothyroid Pearson Correlation | Significant (2-tailed) | Hyperthyroid Pearson Correlation | Significant (2-tailed) |
|----------------------|---------------------------------|------------------------|---------------------------------|------------------------|
| Urea and VD          | -0.265                          | 0.020*                 | -0.301                          | 0.030*                 |
| Urea and T₃          | -0.187                          | 0.164                  | -0.115                          | 0.301                  |
| Urea and T₄          | -0.115                          | 0.593                  | -0.158                          | 0.452                  |

VD: vitamin D3, T₃: triiodothyronine, T₄: thyroxin, *: Significant (2-tailed) at ≤ 0.05.

**Discussion**

Vitamin D has a great impact on the homeostasis of minerals in the bones. Its deficiency was reported to be involved in many clinical cases such as osteoporosis, cardiovascular disease, cancer, and infection [10] Remarkably, many studies reported that VD regulates the immune response and has potent roles in the incidence of thyroid diseases [11]. The best marker of VD status is the concentration of serum 25(OH)D₃. Serum 25(OH)D₃ with half-life of 15 days reflects the level of VD that synthesized inside the body and that obtained from food and supplements [2]. In contrast, serum 1,25(OH)₂D₃ because of having a short half-life of 15 hours cannot be a good marker of VD status, and also because its concentrations undergo a close regulation by parathyroid hormone, phosphate, and calcium [10]. The concentration of 1,25(OH)₂D₃ does not normally decrease until VD is severely deficient. Hence, in the current study, the concentration of 25(OH)D₃ was measured rather than measuring the concentration of 1,25(OH)₂D₃ to ensure getting more accurate results. The sun exposure periods were so close in all the study subjects (Table 1), this excludes this factor from being a cause for VD deficiency.

The association between the levels of VD and thyroid diseases was a subject for many studies [13,14]. In their study, Zhang, and co-workers indicated that high VD status is associated with low levels of circulating TSH, they hypothesized that VD may influence the thyrotrophs by acting on VDR, which are widely distributed through individual portions of the brain system [13]. While Jastrupand co-workers, in their research, did not find any significant alteration in VD levels in their hyperthyroid patients [15]. Other researchers demonstrated that VD deficiency is associated with Graves’ disease [16]. Meanwhile, many researchers have declared that patients with hypothyroidism and Hashimoto’s thyroiditis have low levels of VD [11,17,18]. Kmiec and his colleague, in their review paper, discussed an experimental data clarifying the role of VD in thyroid function, they revealed that diet deficient with VD resulted in a reduced level of TSH but not T4 in rodents and in VDR knock-out mice. The levels of TSH were low without alteration in thyroid function or morphology [18].

The results of the current study showed that VD concentrations were lower in a significant manner in hypothyroid and hyperthyroid patients. These results are compatible with previous studies [11, 16-18]. Regarding the decreased function of the thyroid gland associated with hypothyroidism, two mechanisms may be proposed to explain the low levels of VD in hypothyroid patients and they are either poor absorption of VD from the intestine or poor activation of VD inside the body. On the other hand, low levels of VD in our hyperthyroid patients may be caused by enhanced metabolism of VD. Furthermore, the interaction between VD and the thyroid gland is believed to be reciprocal because both VD and thyroid hormones bind to similar receptors called steroid hormone receptors.
Also, the polymorphisms in the VDR gene and VD binding protein gene were found to be associated with thyroid diseases [16].

The liver, on the other side, plays an essential role in the metabolism of thyroid hormone since it is the manufacturer of thyroid shormone-binding proteins, such as thyroxine-binding globulin and albumin. It is also the major site of peripheral metabolism of thyroid hormone and is involved in its conjugation, renal excretion, oxidative deamination, and the extra-thyroidal deiodination of T4 to T3 [19]. Reciprocally, thyroid hormones have a crucial role in normal hepatic function and bilirubin metabolism. Feasibly, the disorders of these two organs would interact or influence each other [20]. Al-Tonsi and his colleagues, in their research, declared unaltered levels of the liver function parameters (GOT, GPT, and ALP) indicating a normal liver function in thyroid patients [19].

Other researchers reported an elevated serum ALP with the unchanged concentration of calcium and phosphate in patients with Grave's disease [21]. Thompson and his co-worker established that hyperthyroid patients have at least one of liver function parameters is elevated and GOT, GPT and ALP abnormalities returned toward normal as the euthyroid state is restored [22]. Despite the significant alteration of liver function parameters (GOT and GPT) in the current study, GOT and GPT are still in the normal values from a clinical point of view. This excludes the liver from being involved in the thyroid diseases in our thyroid patients. Regarding this fact, the elevated levels of ALP may indicate the change in the rate of bone turnover in hypothyroid and hyperthyroid patients. This conclusion can be confirmed when noticing Table 2, by which it can be gathered that the hypothyroid and hyperthyroid patients who have a deficiency in VD, have elevated levels of ALP. This finding confirms the effect of VD deficiency on bone resorption in our thyroid patients.

On the other point of view, thyroid hormones have an important impact on the growth, differentiation, and development of the kidney, they also regulate the maintenance of water and menials homeostasis [8, 23]. In reciprocal, the kidney affects the metabolism and elimination of thyroid hormones. It is well established that both hypothyroidism and hyperthyroidism are associated with significant alterations in the metabolism and homeostasis of water and electrolytes [24]. Thyroid dysfunction also affects the purine nucleotide metabolism that may increase uric acid concentration, which is the end-product of purine metabolism [25].

In their study, Ford and co-workers illustrated that hyperthyroid patients have lower serum creatinine levels and this reflects the increased glomerular filtration rate, which is associated with increased T4 levels. They also found that the increased serum uric acid in hyperthyroid patients reflects an increase in the turnover of tissue nucleic acids [26]. Other researchers found that decreased serum uric acid levels of the hyperthyroid state were significantly higher than of controls and euthyroid state [27]. Verhelst and his colleagues reported that increased clearance and a decreased production result in decreased concentration of creatinine in hyperthyroid patients. In contrast, hypothyroidism increases the concentration of creatinine due to increased production and decreased clearance of creatinine. They also declared that the increased catalobism of peripheral protein leads to increased serum urea levels [28]. Conversely, Rhee in his review paper demonstrated that patients with chronic kidney disease (CKD) could be at higher risk of thyroid dysfunction via several suggested pathways. Iodine retention, due to impaired kidney excretion, has been hypothesized as a possible mechanism for hypothyroid and hyperthyroid patients in CKD via the Wolff-Chaikoff effect and Jod-Basedow phenomenon, respectively [23].

Our results showed a non-significant increase in serum uric acid indicating a normal purine metabolism. Creatinine levels were significantly increased both in hypothyroid and hyperthyroid patients, but it is still in the clinically normal range, while the significantly increased serum urea level which is clinically out of the normal range may indicate a kidney dysfunction both in hypothyroid and hyperthyroid patients. It is reported that the altered kidney function leads to altered synthesis, secretion, metabolism, and elimination of thyroid hormones [23, 24, 27, 28].

When discerning Table 3, it can be concluded that there is no net effect of the increased urea on the levels of both T3 and T4 in the two groups of our patients; this may indicate that the dysfunctional
kidney is not the primary reason for the altered levels T3 and T4 in our study. At the same time, observing table 3 lead to perceive that the elevated levels of urea are associated with reduced levels of VD in both hypothyroid and hyperthyroid patients, which may indicate a direct association between dysfunctional kidney and the deficiency of VD. This result is compatible with the results illustrated by Gal-Moscovici and Sprague who established in their report that VD deficiency develops very early in the course of CKD. In the progress of kidney disease, there will be a decreased functional renal mass and a decreased activity of renal 1α-hydroxylase, which converts 25(OH)D₃ to 1,25(OH)₂D₃, thus decreased renal production of calcitriol at the early stages of CKD [29, 30]. As VD alters the synthesis and regulation of thyroid hormones by different mechanisms, its deficiency could be a primary cause of thyroid diseases [23,27]. The results obtained from the current study appointed that kidneys could participate in thyroid diseases by an alternative pathway. The results showed that kidney dysfunction, which is denoted by the increased urea levels is strongly associated with VD deficiency. VD deficiency in turn is well known to be concerned with thyroid diseases. Such a pathway may be a possible mechanism for the pathogenesis of thyroid diseases in our patients.

4. Conclusion
Vitamin D is deficient in both hypothyroid and hyperthyroid patients. The very high levels of serum urea in thyroid patients indicate a dysfunctional kidney, which alters the synthesis of the active form of VD that in turn alters the regulation of thyroid hormone secretion. VD and kidney function tests are included in the differential diagnose of thyroid diseases. More studies are necessary to assess the underlying mechanism by which VD affects thyroid hormones.

5. References
[1] Fields J, Trivedi NJ, Horton E, Mechanick JI. Vitamin D in the Persian Gulf: integrative physiology and socioeconomic factors. Curr. Osteoporos. Rep. 2011;9(4):243-250.
[2] Kim D. The role of vitamin D in thyroid diseases. Int J Mol Sci. 2017;18(9):1949-1968.
[3] Barchetta I, Angelico F, Del Ben M, Baroni MG, Pozzilli P, Morini S, et al. Strong association between nonalcoholic fatty liver disease (NAFLD) and low 25 (OH) vitamin D levels in an adult population with normal serum liver enzymes. BMC med. 2011;9(1):85-92.
[4] Lim LY, Chalasani N. Vitamin d deficiency in patients with chronic liver disease and cirrhosis. Curr. Gastroenterol. Rep. 2012;14(1):67-73.
[5] Sharifi N, Amani R, Hajiani E, Cheraghian B. Does vitamin D improve liver enzymes, oxidative stress, and inflammatory biomarkers in adults with non-alcoholic fatty liver disease? A randomized clinical trial. Endocrine. 2014;47(1):70-80.
[6] Mohamedali M, Reddy Maddika S, Vyas A, Iyer V, Cheriyath P. Thyroid disorders and chronic kidney disease. Int. J. Nephrol. 2014;2014, 6 pages.
[7] Kreisman SH, Hennessey JV. Consistent reversible elevations of serum creatinine levels in severe hypothyroidism. Arch Intern Med. 1999;159(1):79-82.
[8] Mariani LH, Berns JS. The renal manifestations of thyroid disease. J Am SocNephrol. 2012;23(1):22-26.
[9] Punekar P, Sharma AK, Jain A. A study of thyroid dysfunction in cirrhosis of liver and correlation with severity of liver disease. Indian J EndocrinolMetab. 2018;22(5):645-650.
[10] Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357(3):266-281.
[11] Kivity S, Agmon-Levin N, Zisappl M, Shapira Y, Nagy EV, Dankó K, et al. Vitamin D and autoimmune thyroid diseases. Cell MolImmunol. 2011;8(3):243-247.
[12] Yetley EA, Brulé D, Cheney MC, Davis CD, Esslinger KA, Fischer PW, et al. Dietary reference intakes for vitamin D: justification for a review of the 1997 values. Am J ClinNutr. 2009;89(3):719-727.
[13] Zhang Q, Wang Z, Sun M, Cao M, Zhu Z, Fu Q, et al. Association of high vitamin D status with low circulating thyroid-stimulating hormone independent of thyroid hormone levels in middle-aged and elderly males. Int J Endocrinol. 2014.

[14] Salama M, El-Sakka A, Anatoliotaki M, Tsilimigaki A, Tsekoura T, Schinaki A, et al. The vitamin D questions: How much do you need and how should you get it?. Pak J BiolSci. 2003;13(9):389-91.

[15] Jastrup B, Mosekilde L, Melsen F, Lund BI, Lund BJ, Sørensen OH. Serum levels of vitamin D metabolites and bone remodelling in hyperthyroidism. Metabolism. 1982;31(2):126-132.

[16] Yasuda T, Okamoto Y, Hamada N, Miyashita K, Takahara M, Sakamoto F, et al. Serum vitamin D levels are decreased and associated with thyroid volume in female patients with newly onset Graves’ disease. Endocrine. 2012;42(3):373-741.

[17] Muscogiuri G, Mitri J, Mathieu C, Badenhoop K, Tamer G, Orio F, et al. Mechanisms in endocrinology: vitamin D as a potential contributor in endocrine health and disease. Eur J Endocrinol. 2014;171(3):R101-110.

[18] Kmieć P, Sworczak K. Vitamin D in thyroid disorders. ExpClinEndocrinol Diabetes. 2015;123(07):386-393.

[19] Al-Tonsi AA, Abdel-Gayoum AA, Saad M. The secondary dyslipidemia and deranged serum phosphate concentration in thyroid disorders. ExpMolPathol. 2004;76(2):182-187.

[20] Huang MJ, Liaw YF. Clinical associations between thyroid and liver diseases. J Gastroenterol Hepatol. 1995;10(3):344-350.

[21] Jodar E, Martinez-Diaz-Guerra G, Azriel S, Hawkins F. Bone mineral density in male patients with L-thyroxine suppressive therapy and Graves disease. Calcif. Tissue Int. 2001;69(2):84-87.

[22] Thompson Jr P, Strum D, Boehm T, Wortofsky L. Abnormalities of liver function tests in thyrotoxicosis. Mil Med. 1978;143(8):548-551.

[23] Rhee CM. The interaction between thyroid and kidney disease: an overview of the evidence. CurrOpinEndocrinol Diabetes Obes. 2016;23(5):407-415.

[24] Iglesias P, Diez JJ. Thyroid dysfunction and kidney disease. Eur J Endocrinol. 2009;160(4):503-515.

[25] Rodrigues SL, Baldo MP, Capingana P, Magalhães P, Dantas EM, Molina MD, et al. Gender distribution of serum uric acid and cardiovascular risk factors: population based study. Arq Bras Cardiol. 2012;98(1):13-21.

[26] Ford HC, Lim WC, Chisnall WN, Pearce JM. Renal function and electrolyte levels in hyperthyroidism: urinary protein excretion and the plasma concentrations of urea, creatinine, uric acid, hydrogen ion and electrolytes. ClinEndocrinol. 1989;30(3):293-301.

[27] Yazar A, Döven O, Atus S, Gen R, Pata C, Yazar EE, et al. Systolic pulmonary artery pressure and serum uric acid levels in patients with hyperthyroidism. Arch Med Res. 2003;34(1):35-40.

[28] Verhelst J, Berwaerts J, Maescau B, Abs R, Neels H, Mahler C, et al. Serum creatine, creatatine, and other guanidino compounds in patients with thyroid dysfunction. Metabolism. 1997;46(9):1063-1067.

[29] Gal-Moscovici A, Sprague SM. Use of vitamin D in chronic kidney disease patients. Kidney Int. 2010;78(2):146-151.

[30] AlSheikh MH, Almubayadh SI. Effect of Vitamin D Supplementation on Insulin, Fasting Blood Glucose, and Waist-Hip Ratio in Young Females with Pre-existing Vitamin D Deficiency. The Indonesian Biomed. J. 2019;11(1):42-7.