The Association between Glycemic Control with Oxidant Status Parameters in Type 2 Diabetic Patients

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Abstract. Purpose: Glycemic control is important in order to avoid LDLs increased susceptibility to oxidation in diabetic patients. This study assess the relationship between diabetes control with serum prooxidant-antioxidant balance (PAB), oxidized LDL cholesterol (oxLDLc), homocysteine and vitamin D levels in patients with type 2 diabetes. Material and methods: This was a cross-sectional study on three groups including 80 subjects as well (WGC) and poor (PGC) glycemic control and 40 healthy subjects. Presence of nephropathy and retinopathy were determined using IDF criteria. HbA1c level was determined with columnar chromatography using BioSystems kit. Serum PAB, homocysteine, oxLDLc and vitamin D levels were measured by the standard tests. Results: There was a significant association between PAB with PGC (P< 0.001), diabetic retinopathy (P< 0.01) and nephropathy (P< 0.01) in type 2 diabetic patients. Moreover, the results showed that vitamin D serum levels was significantly lower in PGC patients (P< 0.01), and diabetic patients with retinopathy (P< 0.01). Multiple linear regression analysis revealed that the vitamin D deficiency can predict the HbA1c variations by 77.7% (β= -0.775) in subjects with type 2 diabetes mellitus (P<0.001). Conclusions: There was a significant association between prooxidant-antioxidant balance and vitamin D serum levels with diabetic complications. (www.actabiomedica.it)

Keyword: Type 2 diabetes mellitus; Prooxidant-antioxidant balance; Homocysteine; Oxidized LDL; Vitamin D

Introduction

Diabetes mellitus can be considered as one of the serious and costly multifactorial diseases with micro and macrovascular complications (1). According to the International Diabetes Federation (IDF), 285 million people worldwide are currently living with diabetes and 90-95% have type 2 diabetes (2). This number is expected to reach over 300 million by the year 2025 (3).
It was shown increased risk of cardiovascular disease (CVD) can explain the mortality in type 2 diabetes. Lipid peroxidation is high in type 2 diabetic patients and could be due to hyperglycemia, hyperhomocysteinemia and probably decreased antioxidant enzymes activity (4). It has been shown that hyperglycaemia has an important role in LDL susceptibility to oxidation (5). Elevated levels of oxidized LDL cholesterol (oxLDLc), which consider as a biochemical risk marker for CVD, have found in diabetic patients (6). Disruption of the prooxidant-antioxidant balance (PAB) can cause oxidative stress (7) which is well-documented in groups of patients with type 2 diabetes (8). Many studies reported conflicting results regarding the LDL cholesterol oxidation (9,10) and homocysteine level (11,12) in patients with type 2 diabetes. Epidemiological evidence supports the link between hypovitaminosis D and increased risk of mortality due to diabetes (13). It has been suggested that vitamin D exerts its anti-oxidant features by activating the MEKs/ERKs/SirT-1 axis (14).

Diabetes microvascular complications including retinopathy and nephropathy were associated with higher levels of HbA1c (15). The major cause of type 2 diabetes mellitus complications has not yet been clarified. Understanding the role of various nutritional or other modifiable risk factors that may contribute to the pathogenesis of diabetes, is important in the effort to combat the rising tide of diabetes and CVD worldwide (16). This study was conducted to assess the relationship between diabetes control, serum PAB, oxidized LDL cholesterol, homocysteine and vitamin D levels and microvascular diabetic complications in patients with type 2 diabetes mellitus in Iran.

Material and Methods

Study population

This cross-sectional study was approved by Ethics Committee of Mashhad University of Medical Sciences, Mashhad, Iran. One hundred and twenty volunteers were included (80 with and 40 without type 2 diabetes). Questionnaires containing past medical history, social and family history were obtained and written informed consent was obtained from all individuals.

Venous blood samples (8-10 ml of whole blood) were obtained after a 10-h overnight fasting. EDTA was used as anticoagulant. HbA1c was determined in whole blood samples with columnar chromatography using BioSystems kit (Barcelona, Spain). Volunteers were divided into three age- and sex-matched groups including 40 subjects as well glycemic control: (HbA1c < 8%), 40 subjects as poor glycemic control, (HbA1c >10%) and 40 healthy subjects.

Biochemical measurements

The whole blood samples were centrifuged at 3000 rpm for 10 min, and the serum aliquots were separated and stored at -20°C for the subsequent measurement of PAB, homocysteine, oxidized LDL cholesterol and vitamin D levels.

The PAB assay is based on 3,3',5,5'-tetramethylbenzidine (TMB) and its cation (17), and measure the balance of oxidants and antioxidants simultaneously in one experiment. It uses two different kinds of reactions: one is an enzymatic reaction where the chromogen TMB is oxidized to a color cation by peroxides and the second is a chemical reaction where the TMB cation is reduced to a colorless compound by antioxidants. The photometric absorbance is then compared with the absorbances given by a series of standard solutions (17). The standard solutions were prepared by mixing varying proportions (0–100%) of 250 mM hydrogen peroxide with 3mM uric acid (in 10mM NaOH). A low PAB value means that antioxidants are present at greater concentration than oxidants, while a high PAB value means more oxidants are present than antioxidants (18).

Serum homocysteine level were measured using immunoturbidometry based on assays to co-substrate conversion product (Demeditec Diagnostic GmbH, Lise-Meitner-strabe 2, D-24145 Kiel Germany). Serum homocysteine level was determined with an intra-assay precision CV of 5.08%.

Oxidized LDL cholesterol levels were measured using a specific immunometric assay for human ox-LDLc using an enzyme-linked immunosorbent assay kit (Mercodia Oxidized LDL ELIZA; Mercodia AB,
Uppsala, Sweden) and results were multiplied by dilution factor (6561) for this factor. Intra-assay precision CV was 6.2% for measuring serum oxidized LDL cholesterol levels.

Serum 25-hydroxy vitamin D (25OHD) levels were measured using a competitive electroluminescence protein binding assay (Roche Diagnostics vitamin D total assay kit; Roche Diagnostics, Mannheim, Germany) on a Cobas e411 analyzer. Serum 25OHD was measured with an intra-assay precision CV of 3.07%. Vitamin D insufficiency and deficiency were defined as a serum 25OHD of 50-75 nmol/L and <50 nmol/L, respectively (19). Presence of nephropathy and retinopathy were determined using the IDF criteria (20).

Statistical analysis

All data were analyzed using the Statistical Package for Social Sciences (SPSS version 11.5). The normality of distribution was assessed by the Kolmogorov Smirnov test. Categorical variables were compared using Chi-square test. Quantitative data were expressed as the mean ± SD for normally distributed variables or the median and IQR for not normally distributed variables. Analysis of normally distributed variables is included independent-samples T tests, One-Way ANOVA tests. Mann-Whitney U test and Kruskal-Wallis one-way analysis of variance used for not normal distribute variables. In order to assess the independent effects of vitamin D, homocysteine, PAB, oxidized LDL cholesterol, smoking, sex and age on measures of HbA1c, multiple regression analyses was performed separately in each group. A P-value <0.05 is considered as level of statistical significance for all tests.

Results

Basic characteristics

There were three groups in this study including 40 subjects as well glycemic control, (HbA1c <8%, comprised 11 male and 29 female, aged 50.27±8.00 years), 40 subjects as poor glycemic control, (HbA1c >10%, included 15 male and 25 female, aged 48.5±11.00 years) and 40 healthy subjects comprised 11 male and 29 female (aged 46.02±7.71 years). There was no significant difference in gender distribution between the groups (P= 0.535). Demographic and some clinical characteristics in each group of study are shown in Table 1. Data indicate significant differences in clinical factors such as cardiovascular disease, retinopathy and nephropathy between three groups. Poor control diabetic patients had a significantly higher CVD, diabetic retinopathy and nephropathy (P <0.001, for all), (Table 1).

Biochemical factors

Level of biochemical factors in the three groups of the study are shown in Table 1. Poor control diabetic patients had a significantly lower vitamin D concentration and higher PAB, homocysteine and oxidized LDL cholesterol levels (P <0.001, for all), (Table 1). There was a significant difference in frequency of vitamin D deficiency between the study groups. The frequency of vitamin D deficiency was 100% and 95% in subjects with poor glycemic control and healthy subjects, respectively. The frequency of vitamin D insufficiency was 100% in well control glycemic subjects (Table 1).

Prooxidants antioxidants levels

There was a significant higher PAB in poor glycemic control than well glycemic control (p <0.001). Also, positive retinopathy (p <0.01), and nephropathy (p <0.01) diabetic patients had higher serum PAB levels compared to without retinopathy and nephropathy subjects, (Table 2).

Homocysteine levels

Average homocysteine level in poor glycemic control patients was higher than other groups, (p <0.001), (Table 1). As shown in Table 2, poor glycemic control patients had a significantly higher serum homocysteine concentration than well glycemic control subjects, (p <0.01). Positive nephropathy patients had a higher serum homocysteine compared to without nephropathy diabetic subjects (p <0.05).
Oxidized LDL cholesterol levels

Average oxidized LDL cholesterol was higher in poor control subjects compared to well control group (p <0.05), (Table 2). As shown in Table 2, there was no difference in serum oxidized LDL cholesterol level between subjects with and without retinopathy, or nephropathy (p = 0.134 and p = 0.407, respectively).

Vitamin D

There was a significant difference in serum vitamin D levels between well glycemic control diabetic patients, poor glycemic control diabetic patients and control group (p <0.001), (Table 1). Serum vitamin D levels in well glycemic control patients were higher than poor glycemic control ones; this rate was 58.00 (56.79-59.73) and 19.57 (14.25-26.84) nmol/L, respectively (p <0.001). There was a significantly lower in serum vitamin D levels in positive retinopathy diabetic patients than no-retinopathy diabetic patients (p <0.001), (Table 2).

### Multiple regression analyses results

According to Table 3, efficient variables can explain the variation of HbA1c 73.8%, in diabetic patients (Adjuster R square = 0.738 ± 1.39, p = 0.0001). Multiple linear regression analysis showed that the serum vitamin D can predict the HbA1c variations by 77.7% (β = - 0.775) in type 2 diabetes (p <0.001), (Table 3).

PAB: prooxidant-antioxidant balance; Oxidized LDL cholesterol: oxidized low density lipoprotein cholesterol. In healthy subjects: Adjuster R square = -0.017 ± 0.758, p = 0.511, SE = Standard Error. In diabetic subjects: Adjuster R square = 0.738 ± 1.39, p = 0.0001, SE = Standard Error.

Discussion

In current study, some oxidant status parameters including serum pro-oxidant-antioxidant balance, oxidized LDL cholesterol, homocysteine and vitamin D levels determined in well and poor control diabetic patients.
patients and healthy subjects. Our results are largely confirmatory in a small number of patients. The most important finding of this study was the fact that the poor control diabetic patients had significantly higher serum PAB, homocysteine and oxidized LDL cholesterol levels compared to the well control diabetic patients. Additionally, the serum level of vitamin D was lower in poor control diabetic patients than the well control diabetic patients.

Another interesting finding was the high frequency of vitamin D deficiency in our subjects that has not been widely reported. The major reasons for low vitamin D levels have not been known in the Iranian population. Clothing due to cultural issues can decrease sun exposure in Iran (21). Daneshvar et al. have reported that the intake of vitamin D is lower than recommended dietary allowances in adults in Isfahan (22). Bonakdaran et al. have suggested that sedentary life style and sunscreen use are other reasons for high frequency of vitamin D deficiency in Iranian population. They have shown that the frequency of vitamin D deficiency was 80.7% in patients with metabolic syndrome in Iranian population (23).

In current study, there was a significant lower in serum vitamin D levels in positive retinopathy diabetic patients compared to no-retinopathy ones. Additionally, multiple linear regression analysis showed that the vitamin D deficiency can predict the HbA1c variations by 77.7%. A number of studies have reported that vitamin D supplementation has no significant effects on HbA1c level in type 2 diabetic patients (24, 25). Calvo-Romero et al. have found a small non-significant reduction in HbA1c in patients with type 2 diabetes mellitus after supplementation with vitamin D at least for 8 week (26). While, Ahmadieh et al. have reported hypovitaminosis D is an independent predictor of HbA1c, diabetic neuropathy and retinopathy in patients with type 2 diabetes (27). It seems that low vitamin D receptor signaling is the potential mechanism by which vitamin D deficiency can mediate risk of cardiovascular disease in type 2 diabetic patients (28). One study showed serum vitamin D level were lower in subjects with diabetic retinopathy than subjects without diabetic retinopathy (29). This result is in concordance with our finding.

| Variables/Groups | Well Control | Poor Control | P-Value | Retinopathy | No-Retinopathy | P-Value | Neurapathy | No-Neurapathy | P-Value | No-Nephropathy | Nephropathy | P-Value |
|------------------|--------------|--------------|---------|-------------|----------------|---------|------------|--------------|---------|----------------|-------------|---------|
| PAB (HK arbitrary unit) | 57.1±25.10 | 90.6±63.21 | < 0.001 | 62.7±35.10 | 85.7±34.68 | < 0.01 | 69.4±37.89 | 98.7±33.28 | < 0.01 |
| Homocysteine (μmol/l) | 24.0±10.44 | 27.9±10.42 | < 0.01 | 24.5±10.47 | 27.4±10.44 | < 0.05 | 25.3±10.44 | 25.7±10.44 | < 0.05 |
| Oxidized LDL cholesterol (U/l) | 40.8±18.24 | 50.6±14.67 | < 0.05 | 48.6±15.86 | 42.8±18.98 | 0.134 | 44.7±15.96 | 49.7±18.98 | 0.407 |
| Vitamin D (nmol/l) | 58.0 (56.79-59.73) | 19.5 (14.25-26.84) | < 0.001 | 26.4 (19.42-57.83) | 22.8 (15.06-33.34) | 0.098 | 24.5 (18.36-57.23) | 29.3 (24.54-54.38) | 0.098 |

PAB: prooxidant-antioxidant balance; Oxidized LDL cholesterol: oxidized low density lipoprotein cholesterol. P-value determined by Independent-samples T test. *P-value determined by Mann-Whitney U test.
In current study, average PAB and oxidized LDL cholesterol levels were higher in poor control subjects compared to those well glycemic control diabetic patients. A high PAB value means more oxidants are present than antioxidants (18). Also, positive retinopathy and nephropathy diabetic patients had higher serum PAB levels compared to without retinopathy and nephropathy subjects. It seems that change in PAB levels is thought to be the cause of chronic diabetic complications, consistent with the literature. Kumawat et al. have reported that alteration in anti-oxidant status can help to predict the risk of diabetic retinopathy (30). Our results is consistent with a previous study shown that glycemic control is very important in order to avoid an increased susceptibility of LDLs to oxidation in diabetic patients (31). It is well documented that oxidized LDL cholesterol can activate the pathways associated with innate immunity and trigger pro-inflammatory events (32). Alkalin et al. showed that oxidized LDL cholesterol has been increased in diabetic patients and this may contribute to the increased atherogenesis in diabetes (33). Therefore, the outcome of our study shows the supportive role of well glycemic control decreasing the level of oxidant status parameters in type 2 diabetic patients.

Timar et al., have reported that high dose vitamin D supplementation (50000IU) once weekly during 9 weeks can increase the serum PAB levels in both groups of adolescents girls (aged 12-18) years with BMI <25 and ≥25 (kg/m²). This result may be because of the high dose, or long-term of vitamin D in this study (34). Therefore, whether mega dose is better than low dose is controversial among scholars yet.

Study limitations

Present study was a cross-sectional analysis which had some limitations. Firstly, it lacks baseline data on seasonal changes, sun exposure and thus vitamin D level. Secondly, we don't have enough information about drug use, vitamin D injection and nutritional status of volunteers.

Conclusions

This study indicates that there was a significant association between glycemic control with prooxidant-antioxidant balance and vitamin D serum levels.
Conflicts of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

References

1. Palanisamy Pasupathi JF, Palanisamy Chinnaswamy. Oxidant-antioxidant status, high sensitive C-reactive protein and homocysteine levels in type 2 diabetic patients with and without microalbuminuria. International Journal of Biological & Medical Research 2010; 1: 4–8.

2. International Diabetes Federation. IDF Diabetes Atlas. Global Burden. Epidemiology and Morbidity. Diabetes and Impaired Glucose Tolerance 2009: http://www.diabetesatlas.org/content/diabetes-and-glucose-tolerance Accessed 29 March 2010.

3. Choi SW, Benzie IF, Ma SW, Strain JJ, Hannigan BM. Acute hyperglycemia and oxidative stress: direct cause and effect? Free. Radic Biol Med 2008; 44: 1217-31.

4. Koubaa N, NABI A, Smaoui M, Abid N, Chaaba R, Abid M, et al. Hyperhomocysteinemia and elevated ox-LDL in Tunisian type 2 diabetic patients: role of genetic and dietary factors. Clin Biochem 2007; 40: 1007-14.

5. Liguori A, Abete P, Hayden JM, Cacciatore F, Rengo F, Ambrosio G, et al. Effect of glycemic control and age on low-density lipoprotein susceptibility to oxidation in diabetes mellitus type 1. Eur Heart J 2001; 22:2075-84.

6. Outinen PA, Sood SK, Liaw PC, Sarge KD, Maeda N, Hirsh J, et al. Characterization of the stress-inducing effects of homocysteine. Biochem J 1998; 15: 213-21.

7. Olivares-Corichi IM, Medina-Santillan R, Fernandez del Valle-Laisequilla C, Alvarez P, Hicks-Gomez JJ. Increase of human plasma antioxidant capacity with a novel formula. Nutr Res 2002; 22:45-7.

8. Telci A, Cakatay U, Kayali R, Erdogan C, Orhan Y, Sivas A, et al. Oxidative protein damage in plasma of type 2 diabetic patients. Horm Metab Res 2000; 32: 40-3.

9. Hamed S, Brenner B, Absi Z, Aharon A, Daud D, Roguin A. Hyperglycemia and oxidized-LDL exert a deleterious effect on endothelial progenitor cell migration in type 2 diabetes mellitus. Thromb Res 2010; 126:166-74.

10. Hsu RM, Devaraj S, Jialal I. Autoantibodies to oxidized low-density lipoprotein in patients with type 2 diabetes mellitus. Clin Chim Acta 2002;317: 145–50.

11. Aguillo-Ortuno MT, Albaladejo MD, Parra S, Rodriguez-Manotas M, Fenollar M, Ruiz-Espejo F, et al. Plasmatic homocysteine concentration and its relationship with complications associated to diabetes mellitus. Clin Chim Acta 2002; 326: 105-12.

12. Smulders YM, Rakic M, Slats EH, Treskes M, Sijbrands EJ, Odekerken DA et al. Fasting and post-methionine homocysteine levels in NIDDM. Determinants and correlations with retinopathy, albuminuria, and cardiovascular disease. Diabetes Care 1999; 22:125–32.

13. Mattila C, Knekter P, Mannisto S, Rissanen H, Laaksonen MA, Montonen J, et al. Serum 25-hydroxyvitamin D concentration and subsequent risk of type 2 diabetes. Diabetes Care 2007; 30: 2569–70.

14. Polidoro L, Properzi G, Marampon F, Gravina GL, Festuccia C, Di Cesare E, Scarsella L, Ciccarelli C, Zani BM, Ferri C. Vitamin D protects human endothelial cells from H 2 O 2 oxidant injury through the Mek/Erk-Sirt1 axis activation. Journal of cardiovascular translational research, 2013, 6, 2: 221–231.

15. World Health Organization. Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia. http:// hoint/diabetes/publications/en/. Accessed 5 August 2008.

16. Maxwell CS, Wood RJ. Update on vitamin D and type 2 diabetes. Nutr Rev 2011; 69: 291–5.

17. Alamdari DH, Paletas K, Piegou T, Sarigianni M, Befani C, Koliasos G. A novel assay for the evaluation of the prooxidant-antioxidant balance, before and after antioxidant vitamin administration in type II diabetes patients. Clin Biochem 2007; 40: 248-54.

18. Arjmand MH, Ahmad Shah F, Saleh Moghadam M, Tara F, Jalili A, Mosavi Bazaz M, et al. Prooxidant-antioxidant balance in umbilical cord blood of infants with meconium stained of amniotic fluid. Biochem Res Int. 2013;2013:270545. doi: 10.1155/2013/270545. Epub 2013 Nov 28.

19. Holick MF. Vitamin D deficiency. The New England Journal of Medicine 2007; 357(3): 266-81. doi: 10.1056/NE JIma070553.

20. International Diabetes Federation. IDF Diabetes Atlas. 6th ed. Brussels, Belgium: International Diabetes Federation; 2013. Available from: http://www.idf.org/sites/default/files/EN_6E_Atlas_Full_0.pdf. Accessed 2 September, 2014.

21. Asemi Z, Samimi M, Tabassi Z, Shakeri H, Esmaillzadeh A. Vitamin D supplementation affects serum high-sensitivity C-reactive protein, insulin resistance, and biomarkers of oxidative stress in pregnant women. Journal of Nutrition 2013;143(9):1432-8. doi: 10.3945/jn.113.177550. Epub 2013 Jul 24.

22. Daneshvar P, Hariri M, Ghiasvand R, Askari G, Darvishi L, Iraj B, et al. Dietary behaviors and nutritional assessment of young male isfahan wrestlers. Int J Prev Med 2013;4(Suppl 1):S48–52.

23. Bonakdaran Sh, Fakhraee F, Saberi Karimian M, Mirhafes SR, Roknie H, Mehebati M, et al. Association between Serum 25-hydroxyvitamin D concentrations and prevalence of metabolic syndrome. ADVMS. http://dx.doi.org/10.1016/j. advms.2016.01.002

24. Ryu OH, Lee S, Yu J, Choi MG, Yoo HJ, Mantero F. A prospective randomized controlled trial of the effects of vitamin D supplementation on long-term glycemic control in type 2 diabetes mellitus of Korea. Endocr J 2014;61:167–76.

25. Kampmann U, Mosekilde L, Juhl C, Moller N, Christensen
B, Rejnmark L, et al. Effects of 12 weeks high dose vitamin D3 treatment on insulin sensitivity, betacell function, and metabolic markers in patients with type 2 diabetes and vitamin D insufficiency—a double-blind, randomized, placebo-controlled trial. Metabolism 2014;63:1115-24.

26. Calvo-Romero JM, Ramiro-Lozano JM. Metabolic effects of supplementation with vitamin D in type 2 diabetic patients with vitamin D deficiency. Diabetes Metab Syndr. 2015 Oct 9. pii: S1871-4021(15)30034-5. doi: 10.1016/j.dsx.2015.09.008.

27. Ahmadieh H, Azar ST, Lakkis N, Arabi A. Hypovitaminosis D in patients with type 2 diabetes mellitus: a relation to disease control and complications. ISRN Endocrinol 2013; doi: 10.1155/2013/641098. eCollection 2013

28. Oh J, Weng S, Felton SK, Bhandare S, Rick A, Butler B, et al. 1,25(OH)2 vitamin d inhibits foam cell formation and suppresses macrophage cholesterol uptake in patients with type 2 diabetes mellitus. Circulation 2009; 25: 687-98.

29. Suzuki A, Kotake M, Ono Y, Kato T, Oda N, Hayakawa N, et al. Hypovitaminosis D in type 2 diabetes mellitus: association with microvascular complications and type of treatment. Endocr J 2006; 53: 503-10.

30. Kumawat M, Kharb S, Singh V, Singh N, Singh SK, Nada M. Plasma malondialdehyde (MDA) and anti-oxidant status in diabetic retinopathy. J Indian Med Assoc. 2014;112(1):29-32.

31. Oranje WA, Rondas-Colbers GJ, Swennen GN, Jansen H, Wolffenbuttel BH. Lack of effect on LDL oxidation and antioxidant status after improvement of metabolic control in type 2 diabetes. Diabetes Care 1999; 22: 2083-4.

32. Lopes-Virella MF, Hunt KJ, Baker NL, Lachin J, Nathan DM, Virella G. Levels of oxidized LDL and advanced glycation end products-modified LDL in circulating immune complexes are strongly associated with increased levels of carotid intima-media thickness and its progression in type 1 diabetes. Diabetes 2011; 60: 582-9.

33. Akalin A, Temiz G, Akcar N, Sensoy B. Short term effects of atorvastatin on endothelial functions and oxidized LDL levels in patients with type 2 diabetes. Endocr J 2008; 55: 861-6.

34. Timar A, Saberi-Karimian M, Ghazizadeh H, Reza Parizadeh SM, Sabbagh-zadeh R, Emadzadeh M, Eshaghi F, Tavallaie S, Ferns GA, Ghayour-Mobarhan M. Evaluation of the serum prooxidant-antioxidant balance before and after vitamin D supplementation in adolescent Iranian girls. Adv Med Sci. 2019 Mar;64(1):174-180.

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