Research Article
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Effects of vitamin D level and oxidant-antioxidant balance on postoperative morbidity in diabetic and non-diabetic patients undergoing off-pump bypass surgery

Abstract

Objective: Atherosclerosis is still a major problem that cannot be resolved despite significant developments in cardiology and is the leading cause of death over worldwide. Endothelial dysfunction is triggered by multifactorial causes of oxidative stress in the cellular dimension and is associated with atherosclerosis. Despite the associated mechanisms are still unclear vitamin D deficiency is considered as one of the causing factors.

Patient-method: In our study; randomized 100 consecutive patients with advanced atherosclerotic heart disease were classified as Group A (<20 ng /dl), Group B (21-29 ng /dl) and Group C (≥30 ng /dl) according to the vitamin D levels. They were underwent off-pump coronary artery bypass grafting. Clinical data of all cases, oxidant/antioxidant balance and thiol / disulfide levels were collected also.

Results: Preoperative CRP and procalcitonin levels were significantly high in Group A (p=0.028 and p=0.031) according to the other vitamin D levels. There was no statistically significant difference (p=0.46) between all groups in terms of total antioxidant status (mmol /L). In the vitamin D deficient group (<20 ng /ml), oxidant /antioxidant index (OSI) was significantly high (p=0.0378). There was no significant difference between total and native thiol levels and disulfide / thiol contents. While the duration of intubation periods were similar (p=0.056), intensive care and hospital stay times were significantly higher in the Group A with diabetics, respectively; p=0.0034, p=0.00021. There was no significant difference between the other groups. This suggests that the inflammatory process, and oxidative stress one of the basic mechanisms of atherosclerosis, may be more severe in the vitamin D deficient group, especially in diabetics.Our results confirm vitamin D, antioxidant and anti-inflammatory activity.

Keywords: atherosclerosis, coronary artery disease, vitamin D, off-pump coronary bypass surgery, CRP, procalcitonin, oxidant/antioxidant index, thiol, disulphide

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Introduction

Atherosclerosis is still an important problem that cannot be solved despite important developments in the cardiovascular field and is the most important cause of death worldwide. Recent studies have showed that atherosclerosis develops on the ground of endothelial dysfunction, which is triggered by oxidative stress caused by multifactors in the cellular dimension. Although the underlying mechanisms are still unclear, endothelial dysfunction is associated with dyspermiability, resulting in the both formation of endothelium-derived atherosclerotic platelets, which are the building blocks of atherosclerosis, and cytokines transition which triggers inflammatory process. Vitamin D deficieny is one of the biological factors that triggers endothelial dysfunction.[1]

Nowadays diabetes is a substantial social health problem by some of %50 increase over the worldwide ın the last decade. Recent studies suggest that the combination of diabetes and the vitamin D deficiency is a cause of atherosclerosis. In the Framingham study, although the rate of cardiovascular morbidity was significantly higher in the 5-year follow-up period in healthy adults with extremely low D vitamin levels (less than 15 ng /ml), some studies, such as NHANES III, did not confirm this association.[2]

In this clinical trial, we investigated this association in terms of clinical presentation of 100 consecutive patients randomized to a similar demographic and risk category, who underwent off-pump coronary bypass surgery.Vitamin D is a hormone in the steroid structure that is activated by the addition of a hydroxyl group twice in the body, first hepatic and last renal activation, and this active end product is involved in many metabolic pathways. Values of 20 ng / dl or less were reported as deficiency levels while circulating hepatic origin 25-OH-D vitamins were considered to be sufficient to have an average level of 30 ng /dl or more. Values above 50 ng /dl. are considered to be the toxic limit for side effects, although there is no definite final upper limit.

Therefore, vitamin D is an important molecule that is still under investigation, as it causes 2 major consequences, such as atherosclerosis and cancer that threaten humanity, in excess or less. D vitamins are absorbed in small-intestine and participate in chilomicrons. Genetic-based studies have demonstrated common enzymatic catalysis between 25 hydroxylase enzymes with hepatic origin and cholesterol biosynthesis.[3–5]

Klotho glycoprotein, which plays a role in the atherosclerotic process, has been reported to supress the vitamin D which is renal activation point. Therefore, it may be considered that there is a correlation between atherosclerotic process and vitamin D level.[4,5]

On the other hand, the most important mechanism of formation of atherosclerosis is inflammation and its reflection on vascular

endothelium. The balance between oxidant and antioxidant levels in our bodies and their impairment in inflammatory processes initiates and / or promotes the atherosclerotic cascade process. Even though oxidant-antioxidant balance is established in the body as much as herne, it may require periodical external reinforcements in advanced interlaminar processes where this balance is impaired. Healing changes in the feeding regime and deficiencies related to nutritional deficiency need to be supported and replenished by world health policies.[6]

The purpose of our present study is; to investigate the correlation between atherosclerosis, the most frequent cause of death, and vitamin D deficiency, which is also significant in the whole world, and to investigate if there is any significant finding statistically that may indicate atherosclerosis severity. For this reason, a total of 100 diabetic and non-diabetic patients in the same cardiac risk group (intermediate risk group, Euroscore II) underwent coronary bypass surgery in our clinic were studied and correlations were investigated.

Our purpose in presenting our work; is to investigate the correlation between atherosclerosis, the most frequent cause of death, and vitamin D level, which is also significant in the world. If there is this correlation, we want to investigate whether this is an indicator that can determine the severity of atherosclerosis. For this reason, a total of 100 diabetic and non-diabetic patients in the same cardiac risk group underwent coronary bypass surgery in our clinic were studied and correlations were investigated. Our study differs from other studies in that it is the first study to compare the levels of vitamin D and oxidant-antioxidant balance and thiol-disulfide levels in terms of the diabetic factor of groups with the same cardiac risk level randomized and angiographically co-atherosclerosis.

Primer end-point, morbidity and intensive care, hospital stay lengths, and secondary end-point was defined as major atherosclerotic complication such as death due to cardiac death or stroke.

Patient and method

In our original and prospective-clinical trial; demographic characteristics (age, sex, BSA) and risk stratification (Euroscore II - moderate, mean: 4) of 100 patients with advanced atherosclerotic heart disease, angiographic findings and coronary artery bypass grafting in the beating heart without cardiopulmonary bypass , 1±0.4), and after approval of the ethics committee, all cases were divided into 2 groups according to whether they were diabetic or not: Group I (Diabetic group) and Group II (Nondiabetics group) (Table 1).

Table 1 Patient's characteristics

	NonDM (n=50)	T1DM (n=25)	T2DM (n=25)	Statistical analysis	P value	s/ns
Age (years)	58,1±4,5	59,3±4,3	58,5±4,2	F (2,55)=2,3	0,18	ns
Gender-Female/ Total[b]	21 /50	25-Nov	25-Oct	X^2=0,34	0,54	ns
Body mass index (BMI)[a]	1,65±0,5	1,67±0,7	1,66±0,5	X^2=0,137	0,61	ns
Smoking/Total[b]	18/50	25-Jun	25-Oct	X^2=3,952	0,12	ns
HbA1c(%)[a]	5,1±0,2	8,02±1,72*	7,4±0,5*@	X^2=86,824	0,001	s
Euroscore	4,20±0,5	4,22±0,6	4,32±0,6	X^2=1,824	0,402	ns
Ejection fractiona	57,5±812,95	56,16±4,70	55,96±3,82	X^2=0,886	0,642	ns
Hospital time(days)[a]	3,92±0,87	9,61±1,5*	5,51±1,3*	X^2=65,2	<0,001	s
ICU time (time/day)[b]	1,0±0,5	1,8±0,5*	2,2±0,5@	X^2= 75,00	<0,001	s
Entube time (hour)[a]	2,0±0,5	8,6±1,5*	2,56±0,5@	F =28.042	<0,001	s

Data are expressed as mean ± standard deviation, *p<0,001: Significantly different from the NonDM group, @p<0,001: Significantly different from the T1DM group, ns=non-significant mean comparison, s= significant mean comparison, a= Pearson Chi-Square, b= Kruskall-Wallis

All 2 groups were divided into 3 subgroups according to the blood vitamin D level preoperatively: Subgroup A (<20 ng /dl), subgroup B (21-29 ng /ml) and subgroup C (≥30 ng / dl). Definition according to vitamin D level[7,8] if the 25 (OH) D level is lower than 20 ng /mL, between 21 and 29 ng /mL, vitamin D deficiency,if it is higher than 30 ng /mL, adequate level (preferred range 40-60 ng / mL) and if it is higher than 150 ng / mL, D is considered to be an intoxication (Table 2).

Table 2 Demographic, clinical and resuls sum data of all cases were shown in the table

	Group A (Vit D<20)	Group B (Vit D;20-30)	Group C (Vit D>30)
Vit D (ng/ml)	12±4.2	24±2.1	47±19
Euroscore	4.7±0.78	4.5±0.87	4.6±0.72
Age	60±6.5	59±9.3	61±3.7
Euroscore II	1±0.12	1±0.12	1.1±0.18
CABG	3.1±1.5	2.9±1.4	2.8±0.83
HbA1c (%)	6.7±1.8	6±1.4	5.7±1.1
EF	56±7.7	56±5.7	59±4.2
Pre-op creatinine (mg/dl)	0.91±0.26	0.87±0.31	1.0±1.2
ALT (U/L)	17±5.1	20±5.2	18±5.3
PCT (ng/ml)	1.5±0.93	0.56±0.45 x***	0.58±0.47 y***
CRP (mg/l)	4.4±3.6	1.5±1.4 x***	1±0.47 y***
Lactate (mmol/l)	1.3±0.52	0.95±0.21 x**	0.93±0.21 y**
Intubation	3±2.4	2.5±1.3	1.9±0.76
Icu	2.2±0.81	1.8±0.59	1.3±0.57 y***
Hospitalization	6.3±1.3	5.9±1.2	4.8±0.83 y*** z**
Osi index	1.1±0.53	0.8±0.34	0.76±0.22 y*
Total tiol	319±82	330±106	349±246
Native tiol	292.4±69.73	296.8±95.84	322.4±250
Disulfit	13±22	17±12	13±13

The overall clinical status of all patients enrolled in this study were similar; no cases were reoperations and there was no serious end-organ damage (kidney, liver, lung etc), no had active infection and multi-vasculopathy (carotid or peripheral pathology) preoperatively.

Oxidant-antioxidant balance and thiol/disulfide contents which is calculated spectrophotometrically with vitamin D (25-OH-D vitamin) determined by LC / MS / MS method. The angiographic findings of our cases and the number of atherosclerotic critical vessels were

equal in the number of distal anatomoses and all cases underwent the same surgical off-pump coronary bypass without arterial grafting and cardiopulmonary bypass. The cases were randomized with cardiac risk stratification (EuroScore II) and similar dermographic features. The inclusion criteria were- patients with moderate risk, age range 40-60 years, elective off-pump coronary artery bypass operation, multivessel disease

The exclusion criteria were- with advanced risk group and cases with immediate surgery, patients with advanced co-morbidity and advanced age are excluded from the study. Patients with fresh AMI trauma with the presence of active infection or new infections have been excluded from the study. In addition, patients who used vitamin D and / or antioxidant drugs in the last 6 months before surgery were not taken into study.

Ethics committee

The prospective clinical trial presented is approved by the decision of the Ethics Committee of Koç University, Faculty of Medicine, dated **25.01.2017** and numbered **2016.270.IRB2.127.** The submitted study does not contain any conflict of interest and sponsor assistance.

Biochemical analysis

Blood samples were drawn from a forearm vein of the participants at the end of an overnight fasting period. Venous blood samples were collected into dry tubes and centrifuged at 3500 × g at 4 °C for 10 min. Serum was collected and stored at − 80 °C until further analysis. Total antioxidant capacity of plasma was measured using an automated colorimetric measurement method developed by Erel.[9]

Total Antioxidant Status (TAS) levels were measured by spectrophotometrically method using commercial kit (Rel Assay, Turkey). Antioxidants in the sample reduce dark blue green colored ABTS radical to colorless reduced ABTS form. The change of absorbance at 660 nm is related with total antioxidant level of the sample. The assay is calibrated with a stable antioxidant standard solution which is traditionally named as Trolox Equivalent that is vitamin E analog. Total antioxidant activities were expressed in mmol Trolox Equiv/L of samples. Total oxidant capacity of plasma was measured using an automated colorimetric measurement method developed by Erel.[10]

Total Oxidant Status (TOS) levels were measured by spectrophotometric method using commercial kit (Rel Assay, Turkey). Oxidants present in the sample oxidize the ferrous ion chelator complex to ferric ion. The oxidation reaction is prolonged by enhancer molecules, which are abundantly present in the reaction medium. The ferric ion makes a color complex with chromogen in an acidic medium. The color intensity which can be measured spectrophotometrically is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results were expressed in terms of micromolar hydrogen peroxide equivalent per litre (μm H_20_2 Equiv/L). The ratio of TOS to TAS was accepted as oxidative stress index (OSI). For the calculation; unit of TAS was converted to mmol/L, and the OSI value was calculated according to the following formula: OSI (arbitrary unit): TOS (μmH_20_2Equiv/L) / TAS (mmol Trolox Equiv/L).[11]

Clinical data and samples were prepared and stored at Koç University and measurements were made at the Department of Biochemistry at Medipol University. Clinical data and statistical analysis of the results and preparation of the article were made at the Department of Cardiovascular Surgery and Biochemistry of Koç University, consulted with the Professors of the Department of Biochemistry at Medipol University and the results were discussed and evaluated.[9,12]

Thiol/disulphide methods

Thiol disulphide homeostasis tests were performed with Biotek Synergy HTX multi-mode reader. The absorbance is measured at 415 nm. Reducible disulphide bonds were first reduced to form free functional thiol groups. Unused reductant sodium borohydride was consumed and removed with formaldehyde, and all thiol groups including reduced and native thiol groups were determined after the reaction with 5,5′-di- thiobis-(2-nitrobenzoic) acid (DTNB). Half of the difference between the total thiols and the native thiols gave the disulphide amount. After the native and total thiols were determined, disulphide amounts, disulphide/total thiol percent ratios, and disulphide/native thiol percent ratios were calculated. [13]

Results

The levels of vitamin D in the studied cases were as follows:

a. Subgroup (<20 ng /dl): n = 48 (48%) "Vitamin D deficient group"

b. Subgroup B (21-29 ng /ml): n = 28 (28%) "average vitamin D level"

c. Subgroup C (≥30 ng /dl): n = 24 (24%) "Vitamin D is a sufficient group"

As a result, randomized, near 100% of consecutive 100 coronary artery bypass grafts (48%) were missing vitamin D levels. Only vitamin D levels were satisfactory at ¼ of the cases (24%).Vitamin D levels were significantly lower in all diabetic cases. p=0.041 (Table 2).

There was no significant difference between Type 1 and 2 diabetics when examined from this direction. p=0.36

HbA1c levels of diabetic patients were found as 8.33±1.21. (In the non-diabetic group: 5.14±0.37). We investigated the correlations between oxidant / antioxidant balance (TAS / TOS), thiol / disulfide levels and cardiac morbidity and early postoperative period (intubation time and stay of length at intensive care unit and hospital) according to the level of Vitamin D in both diabetics or not (Table 3).

Table 3 Table based on OSI tas/tos index, Serum oxidant-antioxidant levels

Parameters		NonDM (n=50)	T1DM (n=25)	T2DM (n=25)	X2 (2) test	P value	s/ns
C-reactive protein (mg/L)	preOP	1,0±0,5	3,2±0,7	1,9±0,9	6,88	0,021	s
	postOP	2,62±1,45	3,71±2,80	2,35±1,41			
	Δpost-pre	1,26±1,44	0,19±3,82	0,81±2,27	6,346	0,042	ns
TAC/TOC (OSI index)	preOP	0,11±0,1	0,02±0,1	0,03±0,	9,06	<0,001[b]	s
	postOP	0,08±0,1	0,02±0,1	0,03±0,1			
	Δpost-pre	0,02±0,1	0,03±0,1	0,01±0,17	4,624	0,099	ns
				23,58±3,56	54,23	<0,001[b]	s

Citation: Ateş Ş, Alkan B T, İncir S, et al. Effects of vitamin D level and oxidant-antioxidant balance on postoperative morbidity in diabetic and non-diabetic patients undergoing off-pump bypass surgery. *Adv Tissue Eng Regen Med Open Access*. 2022;8(1):11–16. DOI: 10.15406/atroa.2022.08.00129

Table Continued...

Parameters	NonDM (n=50)	T1DM (n=25)	T2DM (n=25)	X2 (2) test	P value	s/ns
			22,70±3,18			
			-0,88±2,90	4,177	0,124	ns

Data are expressed as mean ± standard deviation. □p<0.05: Significantly different from the Control group *p<0.05. Significantly different from the NonDM group, ^Δ^p<0.05: Significantly different from the T1DM group, ns=non-significant mean comparison, s= significant mean comparison, a= Pearson Chi-Square, b= Kruskall-Wallis

According to the results obtained, it is thought that potential atherosclerotic process becomes more complicated by replacing deficiency of vitamin D present in elective cases of coronary artery bypass grafts, and it is thought that atherosclerotic new processes in the grafts can be prevented, thus contributing to community health.

The effects of the outcomes on intensive care and hospital stay were investigated and the correlations and predictive values, if any, during the clinical follow-up period of late postoperative 6 months-1 year were investigated.

The duration of intubation, intensive care and hospital stay were significantly higher in the group with diabetic plus vitamin D deficiency (<20 ng / ml). Respectively; p=0.0012, p=0.0034, p=0.00021. There was no significant difference between the other groups. The lethal complications of atherosclerosis, the sequela of termination, and mortality have not developed in any case of study. None of the cases were re-admitted to the cardiac-related patient during the first month of operation (Table 4).

Table 4 Early postoperative parameters (entubation time, ICU and hospital duration) after off-pump coronary bypass surgery according to D vitamin levels (N: 40-60 mg/ml)

D vitamin levels	Non-diabetics	Type I - Diabetics	Type II - Diabetics
Intubation time (h/hs)			
D <20	7,33±1,16	10,44±2,81	10±2,36
D =21-29	3,06±3,4	5,8±3,03	10,43±5,35
D ≥30	1,95±1,6	3±2,1	4±3
ICU time (day/s)			
D <20	2±0,1	2,06±0,6	2,09±0,5
D =21-29	1,31±0,7	1,6±0,8	3±1
D ≥30	1,05±0,1	1±0,9	1±0,1
Hospitalisation (day/s)			
D <20	9±1,0	11,15±2,34	10,64±2,5
D =21-29	5,56±1,46	6,61±1,52	4,67±0,58
D ≥30	4,25±0,44	4±0,4	4,18±0,58

There was no significant difference (p=0.46) between vitamin D deficient group (<20 ng /ml) and vitamin D deficient group (>30 ng / ml) in terms of total antioxidant status (mmol / L) in terms of antioxidant / oxidant index There was no statistically significant difference between the two groups (p<0.05). D vit levels of 20 mg / mL or less will produce oxidative stress for the body, and the events triggered by them become evident at the end of the chain developing atherosclerosis.

There was no significant difference between total and native thiol levels between vitamin D deficient and adequate group, but there was a significant difference between disulfide and disulfide / thiol contents; p=0.048 and p=0.0076, respectively. Progressive lowering of disulfide and disulfide / thiol groups in the vitamin D deficient group confirms that the inflammatory process, one of the basic mechanisms of atherosclerosis, is more severe in the absence of vitamin D deficiency. It is also suggested that the possible graft patency and prognosis are more risky in this group. In this regard, we believe that by exploring larger series in different risk groups, we can have more detailed information.

Discussion

Vitamin D deficiency in our body triggers the inflammatory process on the one hand and increases oxidative stress on the other. Increasingly worldwide, diabetic patients are developing atherosclerosis with an increase of nearly 50% over the last 10 years. How are these atherosclerotic structures of diabetic patients with lacking of vitamin D? In our clinical trial we searched for this question.[1,2] In an observational study published in 2011, 100-case sera investigators who underwent coronary angiography reported significantly lower levels of brachial artery flow in patients with a D vitamin concentration below 20 ng / dL.[2–4]

Of the 100 patients we randomized to receive consecutively, only 1/4 of the patients had adequate vitamin D levels. The worst group in terms of clinical follow-up was group D with vitamin deficiency and HbA1c level of 8% or more (n = 48, 42 diabetic patients, of which HbA1c level was 7.5% and above, mean: 7.7 ± 0.4).[5] In the patients who will be followed in this direction after the coronary bypass operation, the aim is to keep the blood glucose level at 180 mg / dl and below, thus the HbA1c level should be kept at 7 and below.[7,8]

The intubation periods and the intensive care and hospital stay processes were significantly longer in this group. In some studies, it has been reported that the deficiency of vitamin D is effective from the insulin resistance to the obvious diabetes location.[13] Vitamin D is a steroid hormone that is a steroidal pro-hormone that transforms into skin by sunlight (UVB) and then takes the second hydroxyl group at the renal level as hepatic and then primary and becomes active form in the cellular critical biologic processes. Vitamin D levels are generally found around the world at 40% of individuals aged 70 years or older.[14,15]

An association was found between vitamin D deficiency and low LDL. Some publications suggesting vitamin D supplementation are those in which the statin group to reduce the level of LDL, which is a major component of atherosclerosis, constitutes approximately 1/10 of the drugs and muscle masses that can reach a size that requires drug withdrawal and rarely have side effects such as muscle weakness, have been reported to return valuable blood for this atherosclerosis after replacement therapy. This may be interpreted as an additional contribution of vitamin D to this important group of patients, as well as a potential inhibitor of oxidative stress.[16,17]

Clinical trials involving prospective long follow-up results are accompanied by cardiovascular-induced morbidity and lack of mortality D vitamins. D vitamins have been associated with inflammatory process, high calcium content and endothelial damage. Nevertheless, the number of publications that clearly demonstrate the positive effects of potential vitamin D supplementation on the coronary atherosclerosis process is poor and extensive randomized trials are inadequate.

Malnutrition, which varies from country to country at various levels and especially in the geriatric population, also contributes

to the lack of vitamin D and therefore the vascular endothelin dysfunction.[18] A review of the place of vitamin D in the protection from cardiovascular disease by Pilz et al. has resulted in a marked increase in cardiovascular risk at lower levels of vitamin D <20 ng / mL. In the same study, vitamin D has been shown to be beneficial in the atherosclerotic process, primarily through anti-inflammatory effects, moderator regulation in vascular calcification, effect on cholesterol uptake into macrophages, inhibition of foam cell formation and thrombogenicity reduction and vascular repair and remodeling effects. The results of our study also confirm this clinically in terms of more favorable clinical processes between vitamin D levels and post-operative intensive care procedures.[19]

In some series, a significant difference was found in the number of distal anastomoses in our study, although there was a significant difference in the group with low coronary angiographic high bypass bypass requiring multivessel disease and / or prevalence and low D vitamin content.[20] This may be due to the fact that the incidence is quite similar for diabetes and other co-morbidities and the risk score is on average 4 levels. Furthermore, a further low D group of vitamin D below 15 ng /mL was studied in the indicated group. For this, a similar study is planned for patients with advanced risk groups and lower levels of vitamin D, and the results are compared.[20,21]

Although there are studies that suggest that thrombocyte aggregation is facilitated at the cytological level in the absence of vitamin D deficiency, no significant change in thrombocyte count or thromboembolic event was observed in any of the 100 cases that were present.[22] It is known that atherosclerosis is triggered in the inflammatory zone and that markers such as IL-6 and CRP increase in the acute phase at advanced levels in their markers. These markers have been shown to be significantly increased in the acute coronary syndrome group with acute MI, particularly with vitamin D deficiency.[23]

There was no significant difference between CRP levels (p=0.52) in cases in which the study was not performed, and all of the cases taken for the study could be explained as elective cases. In a further study in this regard, more specific interleukin and adhesion molecules were planned to be examined.[22–24]

Conclusion

Of the 100 patients included in our study, only 1/4 'had adequate vitamin D levels (>30 ng /ml). Vitamin D adequacy was determined at the level of ¼ of the cases (<20 ng /ml). In diabetic atherosclerotic patients, the level of vitamin D was found to be significantly lower. This emphasizes the clinical importance of our work. According to the short practical result of our study in our clinical routine, we will now try to establish adequate blood levels in these cases in terms of vitamin D by starting the necessary replacement therapy by looking at vitamin D level in each patient before the coronary artery bypass grafting and the next treatment. Elective surgery will be planned by bringing vitamin D to an adequate level. We believe that patients with coronary artery bypass grafting may increase graft patency rate in future periods. We are aiming to carry out further studies in this regard.

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None

Conflicts of interest

The author declares no conflicts of interest.

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