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Vitamin D Levels And Affecting Factors In Healthy Children Between 2 – 12 Years Of Age

2–12 Yaş Arası Sağlıklı Çocuklarda D Vitamini Düzeyleri Ve Etkileyen Faktörler

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ABSTRACT

Objective: Although Turkey is sunny in the northern hemisphere's temperate climate zone, vitamin D deficiency is a common public health problem. Within the scope of this research, we aimed to elucidate vitamin D levels and the affecting factors in healthy children between the ages of two and twelve.
Method: This prospective survey was conducted with 600 children aged 2–12 who applied to the pediatrics outpatient clinic. The clinical examination was performed, and body mass indexes were calculated. In the prepared survey, the month of participation in the study, birth weight, gender, type of vitamin D use, and duration of vitamin D use in the first year after birth were questioned.
Results: The lowest vitamin D level was in the nine-twelve age group, and the highest was in the two-four age group. Vitamin D deficiency was more common in girls than in boys, with a statistical significance (X2 = 14.236; p=0.003). When the cut-off values and vitamin D levels of the cases are examined according to age, 73.5% of our cases in the two-four age group with a cut-off value of 23.40 ng/ml, 83% of cases in the five-eight age group with a cut-off value of 24.80 ng/ml and 88.5% of the cases in the nine-twelve age group with a cut-off value of 25.50 ng/ml had vitamin D insufficiency or deficiency. When the average cut-off value of all age groups was evaluated as 24.49ng/ml, it was determined that 82% had vitamin D deficiency or insufficiency.
Conclusion: It has been observed that vitamin D insufficiency and deficiency occur severely during childhood, and its prevalence increases with age. For this reason, adopting a lifestyle that prevents vitamin D deficiency in children and evaluating them in the interim periods may be beneficial in preventing possible complications.

Keywords: Vitamin D Deficiency, Childhood, Adolescents, Rickets, Calcium.

ÖZET

Amaç: Türkiye, kuzey yarımkürenin ılıman iklim bölgesinde güneşli olmasına rağmen D vitamini eksikliği yaygın bir halk sağlığı sorunudur. Bu araştırma kapsamında 2–12 yaş arası sağlıklı çocuklarda D vitamini düzeylerinin ve etkileyen faktörlerin aydınlatılması amaçlandı.
Yöntem: Bu prospektif araştırmada, çocuk hastalıkları polikliniğine başvuran 2–12 yaş arası 600 sağlıklı çocuk ile gerçekleştirildi. Çocukların klinik muayeneleri yapıldı ve vücut kitle indeksleri hesaplandı. Hazırlanan ankette çalışmaya katılım ayı, doğum ağırlığı, cinsiyet, D vitamini kullanım şekli ve doğumdan sonraki ilk yıl D vitamini kullanım süresi sorgulandı.
Bulgular: En düşük D vitamini düzeyi dokuz-on iki yaş grubunda, en yüksek D vitamini düzeyi ise iki-dört yaş grubundaydı. D vitamini eksikliği kızlarda erkeklere göre istatistiksel olarak anlamlı derecede daha yaygındı (X2 = 14.236; p=0.003). Vakaların yaşa göre eşik değerleri ve D vitamini düzeyleri incelendiğinde, D vitamini eksikliği tespit edilen olguların %73.5'i iki-dört yaş grubunda, eşik değeri 23.40 ng/ml, %83'ü beş-sekiz yaş grubundaki eşik değeri 24.80 ng/ml, dokuz-on iki yaş grubundaki olguların %88.5'inde eşik değeri 25.50 ng/ml olarak bulundu. Tüm yaş gruplarının ortalama eşik değeri 24,49ng/ml olarak değerlendirildiğinde %82'sinde D vitamini eksikliği veya yetersizliği olduğu belirlendi.
Sonuç: D vitamini yetersizliği ve eksikliğinin çocukluk çağında ciddi oranda ortaya çıktığı, yaşla birlikte görülme sıklığının arttığı görülmüştür. Bu nedenle çocuklarda D vitamini eksikliğini önleyecek bir yaşam tarzının benimsenmesi ve ara dönemlerde değerlendirilmesi olası komplikasyonların önlenmesinde faydalı olabilir.

Anahtar Kelimeler: D Vitamini Eksikliği, Çocukluk Çağı, Adölesan, Raşitizm, Kalsiyum.

INTRODUCTION

Vitamin D is a steroid vitamin that dissolves in fat tissue. Its most important effects are to keep calcium (Ca+2) and phosphorus (P+3) levels, along with parathyroid hormone (PTH), within the normal physiological range and thus ensure optimum bone mineralization. The main clinical finding of vitamin D deficiency is rickets in children and osteomalacia and osteoporosis in adults. Rickets occurs due to

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inadequate vitamin D intake, malabsorption, insufficient exposure to sunlight, and increased need during rapid growth. The most common type is nutritional rickets due to vitamin D and calcium deficiency (1). Rickets is a preventable disease, and in addition to many deformities in the skeletal structure, severe life-threatening findings such as tetany, convulsion, laryngospasm, muscle weakness, and dilated cardiomyopathy can be observed (2).

Vitamin D deficiency is common in our country. The prevalence of nutritional rickets, a problem in developing and developed countries, is reported to be 1.6–19% in Turkey (3). It is essential to provide vitamin D supplementation to babies from birth. Free vitamin D preparations are distributed to Family Health Centers within the scope of the 'Prevention of Vitamin D Deficiency and Protection of Bone Health Project' by the Ministry of Health, and 400 units (3 drops) of vitamin D support are provided to newborns starting from the first week for at least 12 months. In addition, 600 units of vitamin D per day are recommended for the period after age one (4). In national studies investigating vitamin D deficiency and insufficiency in children and adolescents, deficiency is between 10–80.3%, and insufficiency is 20–67.2% (5). In international studies, the prevalence of vitamin D insufficiency was reported to vary between 30–80% (6). However, due to differences in the geographic regions, lack of standardization in cut-off values, differences in patient groups and the seasons, and the origin of the research, whether it was hospital or community-based, outrageous differences have been observed in the outcomes of published studies (7).

Although Turkey is sunny in the northern hemisphere's temperate climate zone, vitamin D deficiency (VDE) is a common public health problem. Unfortunately, the support program provided with vitamin D drops free of charge from family health centers in the first year of life loses effectiveness after the second year. As is the case worldwide, changes in children's outdoor habits, use of sunscreens, clothing styles that reduce exposure to sufficient sunlight, insufficient physical activity, and limited consumption of seafood and dairy products are among the critical reasons for VDE. Hence, the vitamin D content in breast milk is insufficient (8).

In previous literature, the prevalence of vitamin D deficiency was reported as 8–21% in children and adolescents in Turkey. A study conducted in Erzurum in 2009 in the adolescent age group found that the rate of vitamin D deficiency was 17.7% and vitamin D insufficiency was 72% (9). This fact indicated our city's common vitamin D deficiency or insufficiency condition. Within the scope of this research, we aimed to elucidate vitamin D levels and the affecting factors in healthy children between the ages of two and twelve. In the second step, families could be informed about daily Ca+2 intake, the importance of nutrition, sunbathing, physical activity, and the long-term adverse effects of vitamin D deficiency or insufficiency.

METHOD

This prospective survey was conducted with 600 children aged 2–12 years who applied to the general pediatrics outpatient clinic of Erzurum Atatürk University. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Ethics committee approval has been granted from our institution, and informed consent has been obtained from all participants.

The weight and height of the subjects were measured using a sensitive scale, with their thick clothes removed and barefoot. Body mass indexes (BMI) were calculated. Five ccs of blood were taken from the participants into a Becton-Dickenson gel and vacuum biochemistry tube with a disposable sterile syringe No. 21 G.

In the prepared survey, the month of participation in the study, birth weight, gender, type of vitamin D use (regular/irregular), and duration of vitamin D use in the first year after birth were questioned. The duration and amount were determined if vitamin D and multivitamins were taken after one year of age. The three-day calcium amount was calculated from all food and drinks consumed during the day. Parental age, education level, economic income, time spent outdoors in winter and summer, sunbathing per day, physical activity outside, and time spent in front of TV and computer were recorded. The frequency of urinary tract infections (UTI) experienced by the cases per year and whether they had joint

disorders (bone pain, muscle pain, deformity in the legs, tremors in the hands, fractures, seizures) were questioned. The onset of complaints, age, duration, and frequency in which joint, if any, were recorded.

Children whose physical examination revealed signs of infection such as organomegaly, hepatomegaly, cough, fever, and vomiting, who had signs of rickets such as scoliosis, rachitic rosary, Harrisson's groove, and who had malnutrition were not included in this study. Additionally, those with chronic diseases (lung, liver, kidney, gastrointestinal system, and endocrine system) and those who use medications that affect serum vitamin D levels (anticonvulsants, cortisone, heparin) were excluded.

Statistical Analysis

Patient data collected within the scope of the study were analyzed with the IBM Statistical Package for the Social Sciences (SPSS) for Windows 26.0 (IBM Corp., Armonk, NY) package program. Frequency and percentage for categorical data and mean and standard deviation for continuous data were given as descriptive values. For comparisons between groups, the “Independent Sample T–test” was used for two groups, and the “Pearson Chi-Square Test” was used to compare categorical variables. The results were considered statistically significant when the p-value was less than 0.05.

RESULTS

This prospective survey was conducted with 600 children aged 2–12. The age distribution of the participants was n=200 for 2–4 years, n=200 for 5–8 years, and n=200 for 9–12 years (Table 1).

Table 1. Baseline Demographic Characteristics Of The Study Population

		n	%			n	%
Place of Residence	**Rural**	174	29.0	**Mother’s Educational Level**	**Illiterate**	36	6.0
	Urban	426	71.0		**Literate**	204	34.0
Sex	**Female**	279	46.5		**Primary and Secondary**	284	47.3
	Men	321	53.5		**University**	76	12.7
Vit D Taking Period first time in the 1st year	**No**	78	13.0	**Mother’s Employment Status**	**Housewife**	497	82.8
	Regularly	360	60.0		**Working**	103	17.2
	Irregularly	162	27.0	**Father’s Educational Level**	**Illiterate**	12	2.0
Vit D Taking Period after the 1st year	**No**	372	62.0		**Literate**	170	28.3
	Regularly	58	9.7		**Primary and Secondary**	288	48.0
	Irregularly	170	28.3		**University**	130	21.7
Does he/she take multivitamins?	**No**	440	73.3	**Monthly Income**	**750 TL**	85	14.2
	Regularly	26	4.3		**750-1500 TL**	250	41.7
	Irregularly	134	22.3		**More than 1500 TL**	265	44.2
Nursing Time	**6 Months**	117	19.5	**Time Spent at Home or Outdoors**	**Less than 15 minutes**	58	9.7
	6-12 Months	158	26.3		**15-60 minutes**	200	33.3
	More than 12 Months	325	54.2		**More than 60 minutes**	342	57.0
Drinking cow’s milk	**1 glass**	313	52.2	**Time Spent Outdoors in the Winter**	**Less than 15 minutes**	265	44.2
	More than 1 glass	89	14.8		**15-60 minutes**	229	38.2
	None	198	33.0		**More than 60 minutes**	106	17.7
Eating Yogurt	**1 bowl**	437	72.8	**Sun Exposure Time During Day**	**Less than 15 minutes**	157	26.2
	More than 1 bowl	59	9.8		**15-60 minutes**	217	36.2
	None	104	17.3		**More than 60 minutes**	226	37.7
Maternal Age	**Younger than 18 years**	2	0.3	**Usage of Sunscreen**	**Yes**	47	7.9
	18-30 years	200	33.3		**No**	551	92.1
	Older than 30 years	398	66.3	**Time Spent Outdoors**	**Less than 20 minutes**	134	22.3
					20-60 minutes	241	40.2
					More than 60 minutes	225	37.5

The vitamin D levels of the participants revealed statistically significant differences between the age groups (X2=25.735; p=0.000). Among those aged between two and four, 6% had severe vitamin D deficiency, 52% had vitamin D deficiency, 30.5% had vitamin D insufficiency, and 11.5% had normal vitamin D levels. It was observed that 1.5% of the five-eight-year-old group had severe vitamin D deficiency, 62.5% had vitamin D deficiency, 29% had vitamin D insufficiency, and 6.5% had vitamin

D within the normal range. In the third group, aged nine to twelve, these values were 10.5%, 63%, 21.5%, and 5.0%, respectively. When the subjects participating in the study were evaluated in terms of vitamin D levels according to their age groups, it was determined that the lowest vitamin D level was in the nine-twelve age group, and the highest vitamin D level was in the two-four age group.

When the vitamin D levels were examined according to gender, it was determined that cases of severe vitamin D deficiency and vitamin D deficiency were more common in girls, while cases of vitamin D deficiency and normal vitamin D levels were more common in boys and the differences were statistically significant (X2 = 14.236; p=0.003).

The participants' vitamin D levels and multivitamin usage rates were statistically significant (X2=16.994; p=0.009). A majority (86.1%) of those with severe vitamin D deficiency did not use multivitamins, and 13.9% used them irregularly. Additionally, 76.6% of those with vitamin D deficiency did not use it, 4.8% used it regularly, and 18.6% used it irregularly. It was observed that the rate of multivitamin use was higher in subjects with normal vitamin D levels. Of these cases, 58.7% were regularly using, 2.2% were using irregularly, and 39.1% were not using (Table 2).

Table 2. Vitamin D Levels And Status According To Age Groups

Vitamin D Levels	2-4 Years Old		5-8 Years Old		9-12 Years Old		P-value
	n	%	n	%	n	%	
<10ng/ml: Severe Vitamin D Deficiency	12	6	3	1.5	21	10.5	X^2=25.735 p=0.000
10-20ng/ml: Vitamin D Deficiency	104	52	125	62.5	126	63	
20-30ng/ml: Vitamin D Insufficiency	61	30.5	59	29.5	43	21.5	
>30ng/ml: Normal Vitamin D Level	23	11.5	13	6.5	10	5	

No statistically significant difference was detected between the laboratory values of Ca+2, P+3, and PTH in serum plasma levels. On the contrary, in osteocalcin correlation analysis, a negative significant relationship was found with vitamin D at 16.5%. The increased vitamin D levels were correlated with decreased osteocalcin levels. The osteocalcin value was highest in cases with severe vitamin D deficiency (99.0±48.4 ng/ml; F=6.064). While this was 89ng/ml in those with vitamin D deficiency, it was 79.6ng/ml in those with vitamin D insufficiency. In cases with normal vitamin D levels, serum osteocalcin was 75.8ng/ml (Table 3).

Table 3. Basic Laboratory Parameters Obtained From Blood Samples Of Participants

		2-4 years		5-8 years		9- 12 years		p-value
		n	%	n	%	n	%	
Ca	**Low** **Normal** **High**	2 196 2	1 98 1	0 200 0	0 100 0	0 200 0	0 100 0	X^2=8.054 p=0.090
PTH	**Low** **Normal** **High**	4 193 3	2 96.5 1.5	1 196 3	0.5 98 1.5	1 193 6	0.5 96.5 3	X^2=4.531 p=0.339
Osteocalcin	**Low** **Normal** **High**	1 16 183	0.5 8 91.5	2 12 186	1 6 93	0 4 196	0 2 98	X^2=9.492 p=0.050
P	**Low** **Normal** **High**	0 67 133	0 33.5 66.5	1 83 116	0.5 41.5 58	0 100 100	0 50 50	X^2=9.218 p=0.010

When the cut-off values and vitamin D levels of the cases are examined according to age, 73.5% of our cases in the two-four age group with a cut-off value of 23.40 ng/ml, 83% of cases in the five-eight age group with a cut-off value of 24.80 ng/ml and 88.5% of the cases in the nine-twelve age group with a cut-off value of 25.50 ng/ml had vitamin D insufficiency or deficiency. When the average cut-off value

of all age groups was evaluated as 24.49ng/ml, it was determined that 82% had vitamin D deficiency or insufficiency.

A significant correlation was found between symptoms such as bone pain, muscle pain, joint pain, numbness in hands, tremors in hands, convulsions, and Vitamin D levels. It was determined that 69.4% of the cases with severe Vitamin D deficiency had bone pain, 50% had muscle pain, and 47.2% had joint pain (Table 4).

Table 4. The Relationship Between Vitamin D Levels And Clinical Features

		Vitamin D (ng/ml)								
		Sev. Vit D Def. <10ng/ml		D Vit. 10-20ng	Insuff. /ml	Vit D Insuff. 20- 30ng/ml		Normal Vit D >30ng/ml		P-value
		n	%	n	%	n	%	n	%	
Bone Pain	**Yes**	25	69.4	99	27.9	20	12.3	5	10.9	X^2=58.744
	No	11	30.6	256	72.1	143	87.7	41	89.1	p=0.000
Muscle Pain	**Yes**	18	50.0	85	23.9	24	14.7	5	10.9	X^2=25.579
	No	18	50.0	270	76.1	139	85.3	41	89.1	p=0.000
Joint Pain	**Yes**	17	47.2	86	24.2	28	17.2	9	19.6	X^2=15.460
	No	19	52.8	269	75.8	135	82.8	37	80.4	p=0.001
Deformity in legs	**Yes**	6	16.7	31	8.7	9	5.5	1	2.2	X^2=7.536
	No	30	83.3	324	91.3	154	94.5	45	97.8	p=0.057
Numbness in hands	**Yes**	7	19.4	18	5.1	10	6.1	0	0	X^2=15.394
	No	29	80.6	337	94.9	153	93.9	46	100	p=0.002
Tremor and Spasm in Hands	**Yes**	5	13.9	10	2.8	3	1.8	0	0	X^2=16.885
	No	31	86.1	345	97.2	160	98.2	46	100	p=0.001
Convulsion	**Yes**	5	13.9	15	4.2	6	3.7	0	0	X^2=10.190
	No	31	86.1	340	95.8	157	96.3	46	100	p=0.017
Fracture	**Yes**	2	5.6	16	4.5	2	1.2	0	0	X^2=5.900
	No	34	94.4	339	95.5	161	98.8	46	100	p=0.117

DISCUSSION

Many studies have been conducted on vitamin D in recent years when its extra-bone effects were noticed, but there is no clear consensus on the definition of "deficiency." According to the American Academy of Pediatrics (APA) Guidelines, if 25 (OH) D3 levels are <5 ng/mL, it is called "severe deficiency". If it is <15 ng/mL, it is a "deficiency". If it is 15–20 ng/mL, it is "insufficiency;" if it is 20–100 ng/mL, it is called “sufficient;” if it is between 101–150 ng/mL, as “excess”, and >150 ng/mL as “toxic level” (10). In the 2015 report of the International Endocrine Society (IES), deficiency was defined as <30 nmol/mL (<12ng/mL), and insufficiency was defined as 30–50 nmol/mL (12–20 ng/mL). Adequacy was defined as >50 nmol/mL (>20ng/mL). The limit value of 30 nmol/mL = 12 ng/mL was considered the lowest value that "provides the prevention of nutritional rickets due to vitamin D deficiency" (1 ng/mL=2.5 nmol/L) (11). In a study published in 2017 from Korea, the cut-off value was defined as 18 ng/mL, and the peak value at which the PTH value did not increase in children (12). However, a study conducted with healthy children in the USA reported that a threshold value for 25 (OH) D cannot be determined in individuals who continue to grow. The cases should be evaluated together with PTH, and the diagnosis of deficiency should be made individually if there is an increase in PTH (13).

One of the essential findings of our study is that vitamin D deficiency increases with age, which creates a significant difference, especially in girls. These results, which are compatible with the literature, may have many reasons: During infancy and early childhood, physicians and families are more inclined to take vitamin supplements. Our study observed the lowest Vitamin D level in cases aged 9-12 years while the highest Vitamin D level was 2-4 years.

In the study of Yakarış et al., the rate of those with vitamin D deficiency was 9.0%, those with vitamin D deficiency was 22.9%, and those with vitamin D intoxication was 0.9%. Vitamin D deficiency and

insufficiency were statistically significantly lower in winter and spring than in summer and autumn, in girls compared to boys, and in the 2–12 age group compared to the 0–2 age group (14). Badem et al. conducted a study on 2672 adolescents; 84.9% of girls had vitamin D deficiency, and 12.1% had vitamin D insufficiency. These results were 59.5% and 31.4% for boys (15). Due to sociocultural reasons, this difference may be the closed clothing style and less time spent outdoors than men. Other research is needed to make more definitive interpretations of the cause.

Türe et al. conducted a study on 4153 children and adolescents regarding vitamin D cut-off values. In that study, they accepted levels below 20 ng/ml as a deficiency and levels below 30 ng/ml as a deficiency; 65.0% (n=2700) of the patients had vitamin D deficiency, and 23.1% (n=959) had vitamin D insufficiency (16). In previous research from Turkey, the rate of children with vitamin D deficiency was between 12.8% and 26.7%, and the rate of children with insufficiency was between 26.7% and 68.7% (17, 18). Considering the international literature, the rate of vitamin D deficiency in children and adolescents was between 12.8% and 26.7%, and insufficiency varied between 19–61%, while deficiency was between 7% and 68% (19). The main reasons for these studies' differences were geographical areas (latitude, longitude) and cut-off values. On the other hand, differences between countries were also caused by factors such as sociocultural differences, nutritional habits, and vitamin D prophylaxis programs. In our study, both vitamin D deficiency and vitamin D insufficiency were significantly higher in girls than in boys. This finding was compatible with the literature. Additionally, the vitamin D cut-off value was also similar to the previous literature.

In the guidelines and consensus reports prepared to prevent vitamin D deficiency and insufficiency, it is recommended that all babies be given 400 IU/day of vitamin D supplementation before the age of one, regardless of nutrition style (20, 21). In the program implemented in our country, all babies are given 400 IU/day of vitamin D supplementation starting from the 15th day until the age of one (4).

The balance between vitamin D, Ca+2, P, ALP, and PTH determines bone turnover and Ca–P balance. The inverse relationship between PTH and 25 (OH) D is known, but at what cut-off point and how many days PTH is triggered may vary geographically and individually. Ca2+ and PTH are reliable laboratory variables that help define this balance. However, P+3 and ALP are not as reliable and are highly affected by environmental conditions, especially hemolysis, a common condition in pediatric blood samples. “Adequate vitamin D level” is defined as “the threshold value that allows the PTH curve to plateau” (22). Our study found a significant difference between the age groups in the PTH and osteocalcin levels. In our cases, an increase in the serum ALP level with osteocalcin was determined. It was determined that the serum ALP level began to increase when the vitamin D level began to drop below 20 ng/ml. Serum osteocalcin level tends to increase depending on age. PTH level indicates the extent of osteonecrosis. However, the PTH level also represents bone development in cases at developmental age. In subclinical Vitamin D efficiency cases, PTH level can be within the normal range. Indeed, it would be appropriate to evaluate cases according to clinical examination findings. A negative correlation was found between the PTH and vitamin D levels. Our study determined significant differences between the PTH level, age, and the Vitamin D level. It was seen that the PTH level decreased as the Vitamin D level increased.

Vitamin D deficiency and lower calcium levels lead to numbness and cramps in extremities and overall weakness in the body. A correlation was found between the Vitamin D level in the children in the adolescent age group, cortical bone development, and muscle and bone joint complaints. It was determined that 13.9% of the cases in our study with tremors and spasms in the hands had severe Vitamin D deficiency. It was found that the cases with normal Vitamin D levels did not have numbness, spasms in their hands, convulsions, and fractures.

Although we are one of the countries in the world that rarely gets sun in all seasons throughout the year, we cannot effectively benefit from sunlight, which is our most important source of vitamin D, due to various cultural reasons such as our closed clothing habits, a diet poor in vitamin D, and spending too much time in closed areas. Spending more time outdoors in schools when sunny and weather conditions are suitable and giving importance to a diet rich in vitamin D and calcium in our nutritional sources are extremely important for healthy young generations. When we looked at our cases' vitamin D and cut-off values according to age groups, we observed that 73.5% of the two-four-year-olds, 83% of the five-

eight-year-olds, and 88.5% of the nine-twelve-year-olds had vitamin D insufficiency and/or deficiency. Considering the results of our literature review and our data, we recommend taking prophylactic vitamin D in chronic diseases and the childhood age group.

CONCLUSION

Regarding the outcomes of this research, it has been observed that vitamin D insufficiency and deficiency occur at a severe rate during childhood, and its prevalence increases with age. For this reason, adopting a lifestyle that prevents vitamin D deficiency in children and evaluating them in the interim periods may be beneficial in preventing possible complications.

DESCRIPTIONS

No financial support.

No conflict of interest.

Ethical Declaration: All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Ethics committee approval has been granted from our institution. Informed consent was obtained from all participants.

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