

Vitamin D level and early cow's milk protein exposure in Type 1 Diabetes Mellitus

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Vitamin D level and early cow's milk protein exposure in type 1 diabetes mellitus

OBJECTIVE To analyze the association of vitamin D levels and cow's milk exposure in children with type 1 diabetes mellitus (T1DM). **METHOD** This is a case control study of children with T1DM aged 4–18 years and healthy control subjects. The children were grouped by level of vitamin D according to the 2011 guidelines of the Task Force: deficiency (≤ 20 ng/mL), insufficiency (>20 – 30 ng/mL), and sufficiency (>30 ng/mL). The children's history of cow's milk exposure was obtained. Statistical analysis was performed using the independent t-test, Mann-Whitney test, and logistic regression, with significance set at $p < 0.05$. **RESULTS** The study sample included 31 children with T1DM and 24 healthy control subjects. Vitamin D deficiency was detected in 4/31 children with T1DM, and none of the control subjects ($p < 0.001$). Vitamin D deficiency and insufficiency were detected in 77.41% of the children with T1DM and 25% of the control subjects ($p < 0.001$), and vitamin D deficiency and insufficiency were predictors of T1DM (odds ratio [OR]=3.09; $p < 0.001$). The proportion of children exposed to cow's milk in the first 3 months was 51.16% in the T1DM group and 50% in the control subjects ($p = 1.000$). Logistic regression analysis showed that the vitamin D level was significantly correlated with T1DM ($p = 0.001$). **CONCLUSIONS** Low vitamin D levels were shown to be correlated with T1DM, whereas early exposure to cow's milk was not. Vitamin D supplementation is essential since it has an immunomodulatory effect. It is recommended to be given to T1DM children.

Type 1 diabetes mellitus (T1DM) is mediated mainly by an autoimmune process. Based on the data of the International Diabetes Federation (IDF), in 2017, the number of children with T1DM worldwide was 1,106,500, with 132,600 newly diagnosed cases each year globally.¹ The mortality rate for T1DM is 3–18 times higher than that of healthy children of the same age.²

Risk factors for T1DM include genetics, microbial infections and environmental factors, such as vitamin D and early cow's milk exposure.^{1,3} Several studies reported that vitamin D deficiency exacerbated T1DM progression.⁴ Early exposure to cow's milk protein, i.e., before 3 months of age, is suspected to be associated with T1DM.⁵ It may lead to formation of antibodies that bind insulin and pancreatic β -cells.

Furthermore, the autoimmune process induces beta cell apoptosis in T1DM.^{6,7} Studies on the associations between vitamin D, early cow's milk exposure, and T1DM provide conflicting results.^{8,9} This study aimed to analyze vitamin D levels and cow's milk exposure in children with T1DM.

MATERIAL AND METHOD

This case-control study was conducted at the Dr Soetomo General Hospital, Surabaya, Indonesia, from March to May 2019, and was approved by the Ethics Committee of Health Research, Dr Soetomo General Hospital (Ethics no 1020/KEPK/III/2019). The participants in this study were children aged < 18 years with T1DM (diagnosis based on the International Society for Pediatric and Adolescent Diabetes protocol) and healthy control subjects of

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Επίπεδα βιταμίνης D και πρώιμη έκθεση στο αγελαδινό γάλα σε σακχαρώδη διαβήτη τύπου 1

Περίληψη στο τέλος του άρθρου

Key words

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the same age. Informed consent was obtained from the parents of all the participating children. Children who had received any multivitamins and those with a renal disorder or clinical signs suggestive of vitamin D deficiency (frontal bossing, pigeon chest, rachitic rosary, Harrison's sulcus, wrist widening, and double mal-leoli) were excluded from this study. Information about the early exposure to cow's milk before or at 3 months was obtained by the mother's recall method of the breast milk and cow's milk exposure.

Samples of venous blood for measurement of vitamin D were collected from patients and control subjects in heparinized amber-colored glass vials to prevent photodegradation. Plasma was extracted after centrifugation and then stored at -30 °C for subsequent analyses. The level of 25-hydroxyvitamin D [25(OH)D] in the serum was determined using a combination method of competitive enzyme immunoassay with enzyme-linked fluorescence assay (ELFA) from VIDAS. Subjects were categorized according to the serum vitamin D level as those with deficiency (≤ 20 ng/mL), insufficiency (>20 – 30 ng/mL), and sufficiency (>30 ng/mL), according to the 2011 guidelines of the Task Force of the Endocrine Society.

The data were analyzed using the Statistical Package for the Social Sciences (SPSS) (SPSS, Chicago, IL, USA) version 16.0. Comparisons between patients with T1DM and control subjects were evaluated by the independent t-test and the Mann-Whitney test. The odds ratio (OR) was calculated using the χ^2 test with the equation, $y=9.453+3.232$ (cow's milk) – 0.247 (vitamin D), based on a statistical journal from Peng et al.¹⁰ For all statistical analyses, $p < 0.05$ was considered statistically significant with a 95% confidence interval (95% CI).

RESULTS

This study enrolled 31 children with T1DM (18 boys and 13 girls) and 24 healthy control subjects (10 boys and 14 girls). The baseline characteristics of the study subjects are shown in table 1.

The median duration of consuming cow's milk was 12.0 (1.0–60.0) months in patients with T1DM and 6.0 (0–24.0) months in the control subjects ($p=0.128$). The median age at introduction of cow's milk was 3.0 (0–48.0) months in the patients with T1DM and 2.0 (0–24.0) months in the control subjects ($p=0.162$). The median vitamin D levels were significantly lower in the patients with T1DM than in the control subjects: 26.11 (13.95–52.11) ng/mL vs 34.39 (26.73–47.57) ng/mL; $p < 0.001$.

It was found that 12.9% of the children with T1DM had vitamin D deficiency, while none of the healthy control subjects had vitamin D deficiency (tab. 2). The majority of subjects (54.54%), both in the T1DM group (77.41%) and the control group (25.0%), were either vitamin D deficient or insufficient. The risk of having T1DM was three times higher in subjects with vitamin D deficiency or insufficiency

Table 1. Baseline characteristics of study children with type 1 diabetes mellitus (T1DM) and control subjects.

Characteristics	T1DM (n=31)	Control subjects (n=24)	p
Sex			
Male (n)	18	10	
Female (n)	13	14	
Age (years; mean \pm SD)	11.22 \pm 4.15	7.55 \pm 3.18	0.309*
Weight [kg; median (min–max)]	30.0 (13.0–65.0)	26.0 (11.0–57.0)	0.465**
Height (cm; mean \pm SD)	132.54 \pm 19.49	122.08 \pm 18.69	0.509*
BMI (mean \pm SD)	17.35 \pm 4.32	18.54 \pm 5.23	0.160*
Duration of T1DM [years; median (min–max)]	1.0 (0–11.0)	–	
Race			
Javanese	29	22	
Madurese	1	1	
Chinese	1	–	

*Unpaired t-test; **Mann-Whitney test

SD: Standard deviation, BMI: Body mass index

Table 2. Vitamin D status and early exposure to cow's milk in children with (T1DM) and control subjects.

	T1DM (n=31)	Control subjects (n=24)	p	OR (95% CI)
Vitamin D status				
Deficiency	4	0	<0.001	
Insufficiency	20	6		
Sufficiency	7	18		
Deficiency insufficiency	24	6	<0.001	3.09 (1.51–6.35)
Age at cow's milk introduction				
≤ 3 months	16	12	1.000	1.06 (0.36–3.09)
> 3 months	15	12		

T1DM: Type 1 diabetes mellitus, OR: Odds ratio, 95% CI: 95% confidence interval

(≤ 30 ng/mL), compared with those with levels of > 30 ng/mL (OR=3.09; 95% CI=1.51–6.35; $p < 0.001$). No difference was detected in the risk of developing T1DM in infants who first received cow's milk at ≤ 3 or > 3 months of age (OR=1.06; 95% CI=0.36–3.09; $p=1.000$; tab. 2). Logistic regression analysis showed vitamin D to be significantly risk factor for T1DM ($p=0.001$).

DISCUSSION

This study found that most of the subjects with T1DM (77.41%) had either vitamin D deficiency or insufficiency, with a median vitamin D level of 26 ng/mL. In contrast, most of the control subjects (75%) had vitamin D sufficiency, with a median vitamin D level of 34 ng/mL. Several previous case-control studies have also reported that children with T1DM had lower serum vitamin D levels than the control group.^{17–14} Our study has shown that the risk of T1DM was significantly increased in the vitamin D deficient and insufficient groups compared to the sufficient group [OR=3.09 for 25(OH)D ≤30 nmol/L; p<0.001]. According to the study of Franchi and colleagues, low serum vitamin D levels increased the odds of developing T1DM [OR=5.56 for 25(OH)D ≤50 nmol/L].¹² Some reports, however, have stated that serum vitamin D levels were lower in subjects with DM than in the control subjects.^{15,16} The study of Miettinen and colleagues reported that the mean serum 25(OH)D concentration in children with T1DM was lower than in control subjects.¹⁷

Our study showed that early cow's milk exposure in cases and control subjects was not significantly different. Several studies, however, have reported that early exposure to cow's milk protein (i.e., conventional infant formula) is associated with the subsequent risk of β-cell autoimmunity and clinical disease.^{18–20} The study of Lamb and colleagues showed that increased childhood cow's milk protein intake is associated with the risk of islet autoimmunity (IA).²¹ Based on the study by Šipetic and colleagues (2005),²² the

early introduction of supplementary milk was associated with a higher risk of DM (OR=5.75, 95% CI=2.91–11.36), although other studies, as ours, failed to reveal that very early exposure to cow's milk affects the occurrence of T1DM.^{23,24} Rosenbauer and colleagues reported that the introduction of cow's milk/formulas at age 7 weeks to 4 months had no association with T1DM, with an OR (95% CI) of 0.85 (0.61–1.18) and p of 0.332.²³

A limitation of our study is that the number of patients with T1DM was low, because in Asia, the numbers of T1DM cases are lower than in Caucasians, although the study was conducted at a referral hospital in East Indonesia. Another limitation is that this study was single-center, so that it was also difficult to conduct a controlled trial study for this case. Nevertheless, our study provides some information on low vitamin D status and early exposure to cow's milk protein before the first 3 months as the risk factors of T1DM.

In conclusion, this study found that low vitamin D level was correlated with T1DM in children, whereas early exposure to cow's milk was not. Vitamin D supplementation is very important and is recommended for children with T1DM, because of its immunomodulatory effect.

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ΠΕΡΙΛΗΨΗ

Επίπεδα βιταμίνης D και πρώιμη έκθεση στο αγελαδινό γάλα σε σακχαρώδη διαβήτη τύπου 1

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ΣΚΟΠΟΣ Ανάλυση του επιπολασμού των επιπέδων βιταμίνης D και της έκθεσης στο αγελαδινό γάλα στον σακχαρώδη διαβήτη τύπου 1 (T1DM). **ΥΛΙΚΟ-ΜΕΘΟΔΟΣ** Πρόκειται για μια μελέτη ελέγχου περίπτωσης. Συμπεριλήφθηκαν παιδιά με T1DM, ηλικίας 4–18 ετών, και υγιείς μάρτυρες. Ταξινομήθηκαν ανάλογα με το επίπεδο της βιταμίνης D σύμφωνα με τις οδηγίες του Task Force για το 2011: Έλλειψη (≤20 ng/mL), ανεπάρκεια (>0–30 ng/mL) και επάρκεια (>30 ng/mL). Ελήφθη υπ' όψιν το ιστορικό λήψης αγελαδινού γάλακτος. Η στατιστική αξιολόγηση διενεργήθηκε με τις δοκιμασίες t-test, Mann-Whitney και λογιστική παλινδρόμηση και η στατιστική σημαντικότητα ήταν p<0,05. **ΑΠΟΤΕΛΕΣΜΑΤΑ** Αξιολογήθηκαν 31 περιπτώσεις T1DM και 24 υγιείς μάρτυρες. Τέσσερα από τα 31 παιδιά με T1DM είχαν έλλειψη βιταμίνης D (p<0,001). Έλλειψη και ανεπάρκεια βιταμίνης D εντοπίστηκαν σε ποσοστό 77,41% των περιπτώσεων T1DM και στο 25% των μαρτύρων (p<0,001). Ο σχετικός λόγος (OR) αποκάλυψε ότι η έλλειψη και η ανεπάρκεια βιτα-

μίνης D ήταν προγνωστικοί παράγοντες του T1DM (OR=3,09, $p<0,001$). Το ποσοστό των παιδιών που εκτέθηκαν στο αγελαδινό γάλα τους πρώτους 3 μήνες ήταν 51,16% στην ομάδα T1DM και 50% στους μάρτυρες ($p=1,000$). Ο έλεγχος λογιστικής παλινδρόμησης έδειξε ότι τα επίπεδα της βιταμίνης D συσχετίστηκαν σημαντικά με τον T1DM ($p=0,001$). **ΣΥΜΠΕΡΑΣΜΑΤΑ** Τα χαμηλά επίπεδα βιταμίνης D συσχετίζονται με τον T1DM, ενώ δεν συσχετίζεται η πρώτη έκθεση στο αγελαδινό γάλα.

Λέξεις ευρητηρίου: Αγελαδινό γάλα, Βιταμίνη D, Παιδιά, Παράγοντες κινδύνου, Σακχαρώδης διαβήτης τύπου 1

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