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Randomized control trials

# A randomized placebo-controlled trial of alphacalcidol on the preservation of beta cell function in children with recent onset type 1 diabetes☆

Asal Ataie-Jafari [a,b], Seng-Cheong Loke [c,*], Asmah B. Rahmat [b], Bagher Larijani [a], Farzaneh Abbasi [a], Melvin K.S. Leow [d,e], Zaitun Yassin [b]

[a] Endocrinology and Metabolism Research Center (EMRC), Tehran University of Medical Sciences, Teheran, Iran
[b] Faculty of Medicine and Health Sciences (FMHS), Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia
[c] Institute of Gerontology (IG), Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia
[d] Singapore Institute for Clinical Sciences (SICS), Singapore
[e] Tan Tock Seng Hospital (TTSH), Singapore

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SUMMARY

*Background & aims:* This participant-blinded parallel-group randomized placebo-controlled study demonstrated that alfacalcidol (vitamin D analogue) preserves beta cell function in newly diagnosed type 1 diabetes (T1DM) in children.
*Methods:* Subjects from outpatient clinic were randomized to intervention and control groups. Inclusion: (1) age 8–15, (2) T1DM, (3) duration <8 weeks, (4) no chronic diseases, (5) stable diet. Exclusion: (1) vitamin D, calcium supplements or fortified foods, (2) hypercalcemia. Intervention group received alfacalcidol 0.25 μg twice daily, while control group received placebo. Insulin given physician-titrated to blood glucose. Safety monitored by serum calcium and phosphate. Beta cell function assessed at 0, 3, 6 months using fasting C-peptide (FCP) and daily insulin dosage per body weight (DID). Primary outcome measured using multivariate repeated measures GLM-ANOVA, with FCP and DID as primary measures and age, gender, sunlight exposure, 25-hydroxy vitamin D, and HbA1c as covariates.
*Results:* Of 61 subjects, 7 dropped out. GLM-ANOVA showed that groups were different ($p = 0.019$, Eta-squared = 0.087), with no significant covariates. FCP was higher and DID lower in the intervention group, with males having stronger responses to alfacalcidol ($p = 0.001$). No adverse effects were observed.
*Conclusions:* The study confirmed that alfacalcidol can safely preserve beta cell function in newly diagnosed T1DM in children, with a stronger effect in males.
*Clinical Trial Reg. No:* IRCT201205159753N1


## 1. Introduction

Type 1 diabetes in children imposes a significant health and economic burden to both patients and society. A recent study suggests that the lifetime costs of diabetes in type 1 diabetes patients are disproportionately higher than for type 2 diabetes, and that elimination of the disease would result in savings of an estimated $10.6 billion for each new cohort annually in the United States alone.[1]

The global variation in the incidence of type 1 diabetes among children is very large, both between and within ethnic groups, even in locations in close geographical proximity.[2] This suggests that both genetic and environmental factors play a role in the etiology of this disease.

In type 1 diabetes 1A subtype, there is autoimmune destruction of the insulin-producing beta cells in the islets of Langerhans. There is usually some residual beta cell function in newly diagnosed type 1 diabetes, and this proportion is higher in older children.[3] The genetic component of this condition is very strong and is known to be linked to the human leukocyte antigen (HLA) complex, while very little is known about the environmental factors that trigger its

*Abbreviations:* T1DM, type 1 diabetes; HLA, human leukocyte antigen; VDR, vitamin D receptor; Alfacalcidol, 1-alpha-hydroxycholecalciferol; HbA1c, glycated hemoglobin; SMBG, self-monitored blood glucose; FCP, fasting C-peptide; DID, daily insulin dosage per body weight; GLM-ANOVA, multivariate general linear modeling repeated measures ANOVA.

☆ The study data was presented as a poster at the International Diabetes Federation (IDF) meeting in Dubai, 2011.

* Corresponding author. Tel.: +60 3 8947 2755; fax: +60 3 8947 2744.
*E-mail addresses:* asal_ataie2003@yahoo.com (A. Ataie-Jafari), lokesengcheong@gmail.com, lokesengcheong@yahoo.com.sg (S.-C. Loke), asmah@medic.upm.edu.my (A.B. Rahmat), larijani@tums.ac.ir (B. Larijani), f_abbasi@sina.tums.ac (F. Abbasi), melvin_leow@sics.a-star.edu.sg (M.K.S. Leow), zaitun@medic.upm.edu.my (Z. Yassin).

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clinical onset.[4] As a significant proportion of monozygotic twins are discordant for type 1 diabetes, the evidence for non-genetic environmental factors influencing disease susceptibility is strong and deserves further investigation to unravel potentially effective interventional strategies.

The vitamin D hormone system has been implicated in the pathogenesis of several autoimmune diseases, including type 1 diabetes. Vitamin D appears to modulate the immune system, by regulating tolerance in both cellular and humoral immunity to self-antigens, protecting pancreatic islets against cytokine-induced apoptosis via down-regulation of the *Fas* receptor.[5] Vitamin D deficiency contributes to loss of self-tolerance and is associated with a higher incidence of autoimmune disease, while replacement leads to improvement of immune-mediated symptoms.[6] Polymorphism in the vitamin D receptor (VDR) has been shown to predict onset of type 1 diabetes, while sunlight exposure and consequent vitamin D production influences expression of the various VDR alleles.[7,8] In addition to its immunoregulatory functions, vitamin D may also play a direct role in the control of insulin gene expression by pancreatic beta cells.[9]

In sun-rich Iran where this study was conducted, the incidence of type 1 diabetes in children is relatively low, with a male to female ratio of 0.7.[10] The greater incidence in females is possibly related to the cultural practice of full body clothing in public, and hence reduced sunlight exposure compared to males. It has been previously shown that HLA-DR and -DQ alleles together with VDR gene polymorphisms are strongly associated with type 1 diabetes in Iran.[11] Another study in nearby Qatar showed that type 1 diabetes occurs more frequently with low serum vitamin D levels, poor dietary vitamin D intake, and limited sunlight exposure.[12] It has been demonstrated that temperature, latitude, and hence sunlight exposure account for about 40% of the variance in type 1 diabetes risk.[13]

Alfacalcidol (1-alpha-hydroxycholecalciferol) is an analogue of vitamin D that is converted to calcitriol by hepatic metabolism, but with a much longer duration of action (15–20 days for alfacalcidol, 3–5 days for calcitriol).[14] Although both compounds have a similar risk of hypercalcemia, the prolonged duration of action and reduced renal load give a favorable risk-benefit profile for alfacalcidol compared with calcitriol.[15]

A previous clinical study using calcitriol and nicotinamide in early type 1 diabetes was inconclusive and showed only a temporary reduction in insulin dosage, while having a non-significant effect on beta cell function.[16] Another study utilizing only calcitriol showed no significant effects on any of the observed parameters, although there was a temporary slowing of beta cell functional decline midway through the study period.[17] However, a third study on adult-onset latent autoimmune diabetes did demonstrate preservation of beta cell function using alfacalcidol, while another study showed that cholecalciferol slowed deterioration of residual beta cell function.[18,19] In spite of the strong laboratory and epidemiological data linking vitamin D insufficiency to type 1 diabetes risk, we can see that the clinical evidence is somewhat ambivalent.

As alfacalcidol has been used with success in adult-onset latent autoimmune diabetes, this study is intended to demonstrate that supplementation with alfacalcidol can preserve islet beta cell function in newly diagnosed type 1 diabetes in children and adolescents. This was done by comparing fasting C-peptide and insulin requirement over the study period between the intervention and control groups.

## 2. Materials and methods

The study was approved by the Ethics Committee of the Endocrinology and Metabolism Research Center, Tehran University of Medical Sciences (E-00100) in accordance with current guidelines on Good Clinical Practice and the Declaration of Helsinki, with informed consent obtained from parents of the children.

The study was designed as a single blinded parallel-group randomized controlled clinical trial of efficacy. Subjects were recruited from the pool of patients referred for newly diagnosed type 1 diabetes to the outpatient diabetes clinic in Shariati Hospital and the Children Medical Center of the Tehran University of Medical Sciences.

Inclusion criteria were: (1) age between 8 and 15 years old at the time of recruitment, (2) satisfied the criteria for diagnosis of type 1 diabetes, (3) duration of clinical disease less than 8 weeks, (4) without any medical co-morbidities or chronic diseases, and (5) been on a stable diabetic diet for the previous week. Patients were excluded if: (1) they had consumed cholecalciferol, calcium, multivitamin, or mineral supplements during the previous 3 months, (2) consumed vitamin D-fortified foods on a regular basis, or (3) had hypercalcemia defined as a serum calcium greater than 2.7 mmol/L (10.8 mg/dl).

The diagnosis of type 1 diabetes was made based on criteria from the American Diabetes Association, without the use of HbA1c (glycated hemoglobin) (pre-2010 criteria) as there is some controversy over its use for diagnosis of type 1 diabetes.[20] Differentiation from type 2 diabetes was made solely on clinical grounds as the utility of tests such as autoantibody markers and measures of islet beta cell function have not been established.[21]

Patients who met the criteria and consented for inclusion were assigned to an intervention and control group using computer-generated simple randomization with a 1:1 allocation ratio. Randomization and assigning of participants were supervised by the primary investigator. The control group received standard insulin treatment plus placebo (Zahravi Pharmaceutical Company, Tabriz, Iran) while the intervention group received standard insulin treatment plus alfacalcidol (One-Alfa; LEO Pharma, Ballerup, Denmark) in labeled packaging. This was done for a period of six months, with subjects and laboratory personnel blinded to the intervention, but not the care providers or investigators performing the analysis.

In the first two weeks of the study, subjects received one capsule of alfacalcidol (0.25 μg daily) or placebo with lunch, after which serum calcium was checked corrected for albumin using Payne's formula.[22] If serum calcium was within the normal range (8.5–10.5 mg/dl) for the intervention group, the dose was increased to one capsule of alfacalcidol each with lunch and dinner, otherwise only one capsule of alfacalcidol with one placebo capsule was given. The dosage for the control group was automatically increased to two placebo capsules.

Drug toxicity was monitored by measuring serum calcium and phosphate every two weeks for the first month, then monthly for the remainder of the study period. If hypercalcemia was detected, alfacalcidol was stopped immediately until serum calcium normalized, and then restarted at half the previous dose. Every two weeks, patients returned unused capsules to the clinic, and were given new capsules for the next two weeks. Adherence to treatment was monitored by counting the number of capsules returned. Sunlight exposure was quantified using a sunlight exposure questionnaire modified from an existing instrument that has been validated for use in children and adolescents, with a score range from 4 to 14.[23,24]

### 2.1. Standard insulin treatment

Blood samples were drawn at 0, 3, and 6 months to measure the biochemical indices listed below. The subject's insulin requirement was recorded at months 0, 1, 2, 3, and 6, together with body weight. This was done with the subjects standing, shoes and heavy clothing