



UNIVERSITI PUTRA MALAYSIA

EFFECTS OF A VITAMIN D ANALOG ON GLYCAEMIC CONTROL AND IMMUNE RESPONSE IN TYPE 1 DIABETIC CHILDREN AND ADOLESCENTS FROM IRAN

ASAL ATAIE-JAFARI

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BY

ASAL ATAIE-JAFARI

**Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
fulfillment of the Requirements for the Degree of Doctor of Philosophy**

Agust 2012

DEDICATION

I dedicate this thesis to my dearest parents, Afsaneh and Mohammad, who has supported me unconditionally in this journey, and to my beloved husband, Omid Chouhdari, for his immense support, patience, and encouragement.



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment
of the requirement of degree of Doctor of Philosophy

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AGUST 2012

Chairman: Prof. Asmah Binti Rahmat, PhD

Faculty: Medicine and Health Sciences

Type 1 diabetes mellitus (T1DM) results from autoimmune destruction of insulin producing pancreatic β -cells. At the time of T1DM diagnosis, although the majority of β -cells have been destroyed, some still produce insulin. A treatment that could protect β -cell function or decrease autoimmune destruction would provide important progress in T1DM therapy. Vitamin D has been known as an immunomodulator in this disease, which blockades β -cells destruction. In children with T1DM, vitamin D treatment produces moderate protective effects on residual β -cell function. However, limited doses can be used because of its hypercalcemic effect. This study investigated whether treatment with a vitamin D analog (1- α -hydroxyvitamin D₃) or alfacalcidol, can improve glycemic control and β -cell preservation, as well as improvements in immune responses. In this single-blind and placebo-controlled clinical trial, 60 patients (aged 8-18 years) with recent-onset T1DM were

randomized to alfacalcidol ($2 \times 0.25 \mu\text{g/day}$) or placebo, and followed up for 6 months. All patients received conventional insulin therapy, and insulin dosage was adjusted every two weeks. Dietary vitamin D intakes and sun exposure were assessed at baseline. Insulin requirement, as well as serum 25(OH)D, fasting C-peptide (FCP), hemoglobin A1C (HbA1C), interleukin-1 β (IL-1 β), interleukin-2 (IL-2), transforming growth factor β 1 (TGF- β 1), interferon-gamma (IFN- γ), and glutamic acid decarboxylase (GAD₆₅) autoantibody were assessed at baseline, and again 3 months and 6 months after the intervention. Repeated measures ANOVA was used to compare the alfacalcidol and placebo groups across time points during 6 months of intervention. Fifty-three patients completed the study. All patients were vitamin D deficient (77%) or insufficient (23%) at baseline. In a logistic regression model, it was shown that the risk of being vitamin D deficient was significantly decreased by sunlight exposure ≥ 15 minutes during the weekends versus < 15 minutes (odds ratio [OR]: 0.059 [95% confidence interval [CI]: 0.01–0.75]; $P=0.03$). Serum 25(OH)D dropped 19.7% and 39.2% in alfacalcidol and placebo group respectively, however the difference between groups was not significant [$F_{(1,51)}=1.69$; $P=0.195$]. HbA1C also decreased in both groups throughout the study as expected. Serum levels of FCP increased in both groups after 3 months of study, and then dropped from 0.49 ± 0.18 to 0.33 ± 0.15 ng/ml during month 3 to month 6 ($P=0.003$; unpaired t-test) in the placebo group, however it remained unchanged in the alfacalcidol group ($P > 0.05$). Insulin requirement per body weight did not change significantly in the placebo group; however, it decreased in the alfacalcidol group during the first two months of study, and then increased slightly by the end of 6 months follow-up. Changes of insulin requirement per body weight was significant

across groups over 6 months of intervention [$F_{(1,51)} = 4.2$; $P = 0.017$]). Serum levels of IL-1 β was unchanged in the placebo group, but decreased in the alfacalcidol group throughout the study. Changes of serum IL-1 β was significant across groups over 6 months of intervention [$F_{(1,51)} = 4.88$, $P = 0.010$]. Serum concentrations of IL-2 did not change significantly in the alfacalcidol group during 6 months of the study (Friedman test, $P = 0.169$), but the trend of changes was highly significant in the placebo group (Friedman test, $P < 0.001$). The two-way repeated measures ANOVA also showed significant differences in serum TGF- β 1, IFN- γ , and GAD₆₅ autoantibody changes between the alfacalcidol and the placebo group during 6 months of study ($P = 0.045$, $P = 0.006$, $P = 0.006$; respectively, for interaction between time and group). Serum TGF- β 1 dropped from 115.5 ± 35.5 to 89.3 ± 35 pg/ml in the placebo group over 6 months of intervention ($P = 0.012$), but it remained unchanged in the alfacalcidol-treated patients. Conversely, serum IFN- γ was unchanged in the alfacalcidol group during 6 months of trial, but increased significantly in the placebo group. Serum levels of GAD₆₅ autoantibody decreased in both groups, with a higher rate in the alfacalcidol-treated patients. Six months treatment of newly diagnosed T1DM children with 1- α -(OH)D₃ seems to preserve β -cells function, and result in lower insulin requirement probably through induction of a shift from Th-1 to Th-2 cytokines produced by the autoimmune T cells.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah.

**KESAN ANALOG VITAMIN D KE ATAS PENGAWALAN GULA DARAH
DAN TINDAK BALAS SISTEM IMUNISASI TERHADAP KANAK-KANAK
DAN REMAJA DIABETES JENIS 1 DARI IRAN**

Oleh

ASAL ATAIE-JAFARI

AGUST 2012

Pengerusi : Prof. Asmah Binti Rahmat, PhD

Fakulti : Perubatan dan Sains Kesihatan

Diabetes mellitus jenis 1 (T1DM) terjadi disebabkan oleh kemusnahan sistem immunisasi automatik sel beta pankreas yang menghasilkan insulin. Semasa diagnosis, walaupun kebanyakan sel beta telah musnah, sesetengah daripadanya masih menghasilkan insulin. Rawatan yang dapat melindungi fungsi sel beta atau mengurangkan kemusnahan sistem immunisasi automatik akan memberikan resolusi penting dalam rawatan T1DM. Vitamin D telah dikenalpasti sebagai pengawal immunisasi bagi penyakit ini, yang mana ianya dapat menghalang kemusnahan sel beta. Bagi kanak-kanak yang menghidap T1DM, rawatan menggunakan vitamin D dapat menghasilkan kesan perlindungan yang sederhana pada fungsi sel beta yang masih ada. Kajian ini dijalankan untuk mengenalpasti sama ada rawatan menggunakan analog vitamin D ($1\text{-}\alpha\text{-hidroksivitamin D}_3$) atau al fakalsidol, boleh meningkatkan kawalan gula dalam darah serta tindak balas sistem immunisasi. Dalam

kajian klinikal ini, 60 pesakit (berumur 8-18 tahun) yang baru sahaja didiagnosa mempunyai T1DM telah dibahagikan secara rawak kepada 2 kumpulan: alfakalsidol ($2 \times 0.25 \mu\text{g/hari}$) atau plasebo, dan diikuti dengan 6 bulan pemeriksaan susulan. Kesemua pesakit akan menerima rawatan insulin terapi konvensional, dan dos insulin diubah setiap 2 minggu. Pengambilan diet vitamin D dan kadar pendedahan kepada cahaya matahari diukur pada permulaan kajian. Keperluan insulin serta serum 25(OH)D, C-peptida semasa puasa (FCP), hemoglobin A1C (HbA1C), interleukin-1 β (IL-1 β), interleukin-2 (IL-2), faktor yang merubah pertumbuhan β 1 (TGF- β 1), interferon-gamma (IFN- γ), dan asid glutamik dekarboksilase (GAD₆₅) antibodi automatik telah diukur pada peringkat awal, bulan ke-3 dan bulan ke-6 rawatan intervensi. *Repeated measures ANOVA* telah digunakan untuk membandingkan alfakalsidol dan kumpulan plasebo pada tempoh masa tertentu sepanjang 6 bulan rawatan intervensi ini. Lima puluh tiga pesakit telah berupaya melengkapkan kajian ini. Kesemua pesakit telah dikenalpasti sebagai kurang vitamin D (77%) atau mempunyai vitamin D yang tidak mencukupi (23%) pada awal kajian. Model regresi logistik menunjukkan bahawa risiko bagi status kurang vitamin D adalah berkurang secara signifikan dengan pendedahan kepada cahaya matahari selama atau lebih dari 15 minit semasa hari minggu jika dibandingkan dengan pendedahan kepada cahaya matahari kurang dari 15 minit (nisbah kemungkinan [OR]: 0.059 [95% kadar keyakinan [CI]: 0.01–0.75]; $P= 0.03$). Serum 25(OH)D berkurang sebanyak 19.7% bagi kumpulan alfakalsidol dan 39.2% bagi kumpulan plasebo. Walaubagaimanapun, tiada perbezaan secara signifikan ditemui [$F_{(1,51)}= 1.69$; $P= 0.195$]. Nilai HbA1C juga berkurangan bagi kedua-dua kumpulan sepanjang rawatan intervensi, dan seterusnya nilai tersebut menurun dari 0.49 ± 0.18

to 0.33 ± 0.15 ng/ml dari bulan ketiga hingga bulan keenam rawatan ($P= 0.003$) dalam kumpulan plasebo, namun nilai FCP tidak berubah bagi kumpulan alfavalsidol ($P> 0.05$). Keperluan insulin bagi berat jisim tubuh tidak berubah secara signifikan dalam kumpulan plasebo, namun nilai tersebut berkurangan dalam kumpulan alfavalsidol pada dua bulan pertama kajian, dan meningkat sedikit pada bulan keenam. Perubahan keperluan insulin bagi berat jisim tubuh adalah signifikan bagi kesemua kumpulan setelah 6 bulan rawatan intervensi [$F_{(1,51)}= 4.2$; $P= 0.017$]. Nilai serum IL-1 β pula tidak berubah bagi kumpulan plasebo, tetapi menurun bagi kumpulan alfavalsidol sepanjang kajian dijalankan. Perubahan nilai serum IL-1 β adalah signifikan bagi kesemua kumpulan sepanjang 6 bulan rawatan intervensi [$F_{(1,51)}= 4.88$, $P= 0.010$]. Selain itu, kepekatan tidak berubah secara signifikan bagi kumpulan alfavalsidol sepanjang 6 bulan rawatan intervensi (ujian Friedman, $P= 0.169$), tetapi nilai perubahan serum IL-2 mempunyai signifikan yang ketara bagi kumpulan plasebo (ujian Friedman, $P< 0.001$). Analisis ANOVA juga menunjukkan perbezaan perubahan yang signifikan dalam nilai serum TGF- β 1, IFN- γ , dan GAD₆₅ antibodi automatik antara kumpulan alfavalsidol dan kumpulan plasebo ($P= 0.045$ bagi TGF- β 1; $P= 0.006$ bagi IFN- γ ; $P= 0.006$ bagi GAD₆₅ bagi perkaitan antara masa serta kumpulan). Nilai serum TGF- β 1 berkurangan dari 115.5 ± 35.5 to 89.3 ± 35 pg/ml bagi kumpulan plasebo setelah 6 bulan rawatan intervensi, tetapi tidak berubah bagi kumpulan alfavalsidol. Sebaliknya, nilai serum IFN- γ tidak berubah bagi kumpulan alfavalsidol setelah 6 bulan kajian, tetapi meningkat secara signifikan dalam kumpulan plasebo. Nilai serum GAD₆₅ antibodi automatik berkurangan bagi kedua-dua kumpulan, dengan kadar pengurangan yang lebih tinggi dalam kumpulan alfavalsidol. Oleh itu, enam bulan rawatan intervensi bagi kanak-kanak yang baru

didiagnosa penyakit T1DM menggunakan $1\text{-}\alpha\text{-(OH)D}_3$ berupaya untuk mengekalkan fungsi beta-sel, dan mengurangi keperluan insulin luar yang mana ianya mungkin disebabkan oleh induksi peralihan dari Th-1 kepada Th-2 sitokines yang terhasil daripada sel immunisasi automatik T.



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I certify that a Thesis Examination Committee has met on 9 August 2012 to conduct the final examination of Asal Ataie-Jafari on her thesis entitled "Effects of a vitamin D analog on glycaemic control and immune response in type 1 diabetic children and adolescents from Iran" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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