Clinical Nutrition 32 (2013) 911–917

Contents lists available at SciVerse ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

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

ARTICLE INFO

Article history:
Received 26 July 2012
Accepted 18 January 2013

Keywords:
C-Peptide
Diabetes mellitus
Type 1
Hydroxycholecalciferols
Insulin-secreting cells
Iran
Randomized controlled trial

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).

0261-5614
http://dx.doi.org/10.1016/j.clnu.2013.01.012

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

removed, and to the nearest 0.1 kg (Seca 711 column scale, Seca, Birmingham, United Kingdom). The dosage was recorded only if it was stable for three consecutive days. If the dosage was recently changed, then the next stable dose was recorded instead.

Subjects were trained to self-monitor blood glucose (SMBG), which was done four times daily (pre-meal and bedtime). These readings were recorded together with any hypoglycemic symptoms, accompanied by a food and physical activity diary. At physician discretion, SMBG was performed up to eight times daily when glycemic excursions were frequent. The glucose target range was set as 80–120 mg/dl before meals and less than 180 mg/dl two hours after meals. A physician made weekly or biweekly contact with subjects or their parents by telephone to adjust the insulin dosage according to SMBG readings. They received short (regular) and intermediate-acting (NPH) insulin as two to three injections daily, depending on the glycemic pattern from SMBG. The SMBG record book together with the food and physical activity diary were inspected when subjects came for blood testing.

2.2. Measured biochemical indices

Serum calcium, phosphate, albumin, and HbA1c were determined by spectrophotometry using commercial kits (Pars Azmoon, Teheran, Iran) with a Hitachi 902 autoanalyzer (Roche Diagnostics GmbH, Mannheim, Germany). Serum 25-hydroxy vitamin D was measured using an enzymeimmunoassay (25-hydroxy vitamin D EIA kit; Immunodiagnostics Systems, Boldon, UK) with a measurement sensitivity of 5 nmol/l, and an intra- and inter-assay coefficient of variation of 5.9% and 5.1%, respectively. Serum C-peptide was measured after fasting overnight for at least 8 hours by immunoassay (C-Peptide AccuBind ELISA; Monobind, Lake Forest, California) with an inter- and intra-assay precision of 7.2% and 6.5%, respectively.

2.3. Statistical analysis

Baseline characteristics for the treatment groups, along with subjects who dropped out, were compared using an unpaired *T*-test or Chi-square test as appropriate (Table 1). Residual islet beta cell function was assessed using fasting serum C-peptide (FCP) and daily insulin dosage (DID) per body weight to maintain euglycemia.

The comparison between the treatment groups over the study period was quantified using Multivariate General Linear Modeling Repeated Measures ANOVA (GLM-ANOVA). Normality was checked for FCP and DID using the Kolmogorov–Smirnov test, and equality of variances using Levene's test. The assumptions of equality of covariances and sphericity were tested using Box's *M* and Mauchly's *W* tests, respectively. Besides FCP and DID as the within-subject factors, other covariates were age, gender, sunlight exposure score, 25-hydroxy vitamin D, and HbA1c at baseline. Multivariate comparisons were done using the Pillai–Bartlett Trace statistic as this is more robust to violation of model assumptions.[25] The Huynh–Feldt correction was applied when the sphericity assumption was violated. The GLM-ANOVA was evaluated using a 10% level of significance.

Power analysis was done using G*Power 3.1.3 for repeated measures ANOVA aiming to detect a partial Eta-squared of 0.059 (medium effect size), significance level of 0.05, 95% power, and two groups, giving a total sample size for both groups of 44 for three time periods (0, 3, 6 months).[26,27] Assuming a 25% dropout rate, a minimum of 59 subjects was required for the study.

All computations were performed using SPSS for Windows version 19.0 (SPSS Inc, Chicago, Illinois, USA) and Microsoft Excel 2007 (Microsoft Corp., Redmond, Washington, USA). Statistical tests were two-tailed and conducted at 5% level of significance unless otherwise stated.

Table 1
Baseline characteristics of study subjects, and HbA1c at 3 and 6 months follow-up.

Variable	Intervention group ($n = 29$)	Control group ($n = 25$)	*P*-value
Age (years)	10.2 ± 2.5	11.1 ± 1.6	0.118
Gender			
Girls	22	17	0.520
Boys	7	8	
Time since diagnosis (days)	44 ± 14	38 ± 18	0.238
Serum 25(OH)D (ng/ml)	13.9 ± 6.0	12.5 ± 6.4	0.432
FCP (ng/ml)	0.40 ± 0.19	0.40 ± 0.21	0.997
Insulin dose (U/kg/day)	0.63 ± 0.19	0.60 ± 0.12	0.621
Sunlight exposure score	10.1 ± 1.9	9.6 ± 1.1	0.271
HbA1c (%) baseline	8.2 ± 1.0	8.2 ± 1.0	0.996
HbA1c (%) 3 months	7.1 ± 0.8	7.5 ± 1.0	0.088
HbA1c (%) 6 months	6.8 ± 0.9	7.1 ± 0.8	0.275

Comparisons between groups used an unpaired *T*-test for scalar variables or the Chi-square test for categorical variables. Data are means ± 1 s.d.

3. Results

A total of 61 subjects were recruited in autumn of 2010 (September to December), of which 7 dropped out for personal reasons mainly due to the travel distance to the study center. There was no significant difference in age or gender between those who dropped out and the other subjects ($p = 0.205$ and $p = 0.415$, respectively). Of the remaining 54 subjects, 29 belonged to the intervention group and 25 to the control group (Fig. 1). During the study, 5.1% of dispensed capsules were returned (4.6% alfacalcidol, 5.6% placebo). The mean age of the patients was 10.1 years (s.d. 2.1 years) and the mean duration since diagnosis was 43 days (s.d. 15 days). The treatment groups were similar in age, duration of disease, and other characteristics at baseline. There was a non-significant trend for HbA1c to be lower in the intervention group at months 3 and 6 (Table 1). Serum calcium and phosphate remained within normal limits for all subjects throughout the study.

All FCP and DID measurements had a normal distribution (Kolmogorov–Smirnov 0.256–0.911). Error variances were equal for FCP (Levene's test 0.107–0.548) but not for DID (Levene's test 0.001–0.129). The assumption of equality of covariances was satisfied (Box's *M* 0.154). The assumption of sphericity was satisfied for FCP (Mauchly's *W* 0.495) but not for DID (Mauchly's $W < 0.001$). For GLM-ANOVA, multivariate comparisons show that the treatment groups have a different pattern of change in FCP and DID over the study period ($p = 0.019$ partial Eta-squared = 0.087 overall, $p = 0.076$ partial Eta-squared = 0.078 FCP, $p = 0.056$ partial Eta-squared = 0.093 DID). None of the covariates tested showed a significant effect in the GLM-ANOVA analysis.

The within-subject contrasts show that the differences between the treatment groups were most marked between months 3–6 for FCP ($p = 0.049$) and between months 0–3 for DID ($p = 0.052$). This was confirmed on the charts of FCP and DID which showed that the changes in DID preceded that of FCP. In addition, it could be seen that the gap between the treatment groups for DID began to narrow towards the end of the study period where the difference became non-significant ($p = 0.285$) (Fig. 2).

The between-subject effects show that for DID, there were significant differences between the treatment groups ($p = 0.008$), and also between genders ($p = 0.001$), with a strong interaction component ($p = 0.011$). The interaction component was examined by looking at the DID chart which shows that males had a stronger response to alfacalcidol with a lower trough DID than females.

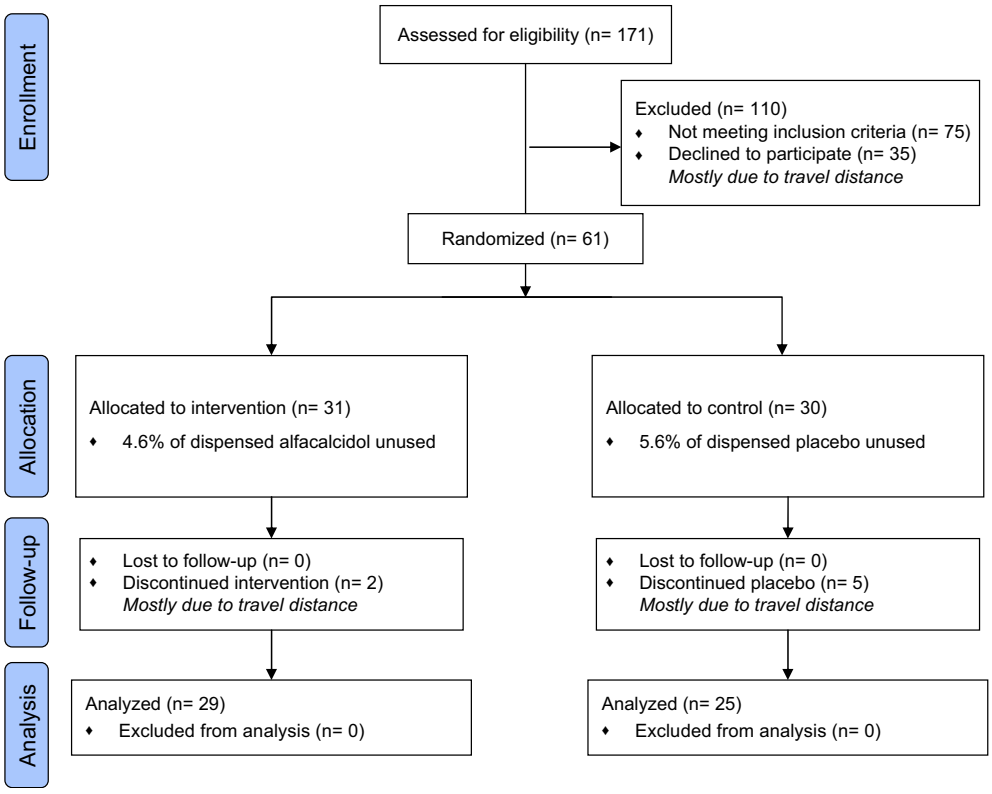

Fig. 1. Recruitment flow diagram according to the 2010 CONSORT statement.[34]

While the between-subject contrasts for FCP were not significant, the FCP chart shows that males improved their FCP levels at the end of the study period while females did not. The overlap between the treatment groups likely obscured the relationship and rendered the contrasts non-significant for FCP (Fig. 3).

4. Discussion

From the results of this study, it does appear that supplementation with alfacalcidol preserved islet beta cell function in newly diagnosed type 1 diabetes in children and adolescents as evidenced

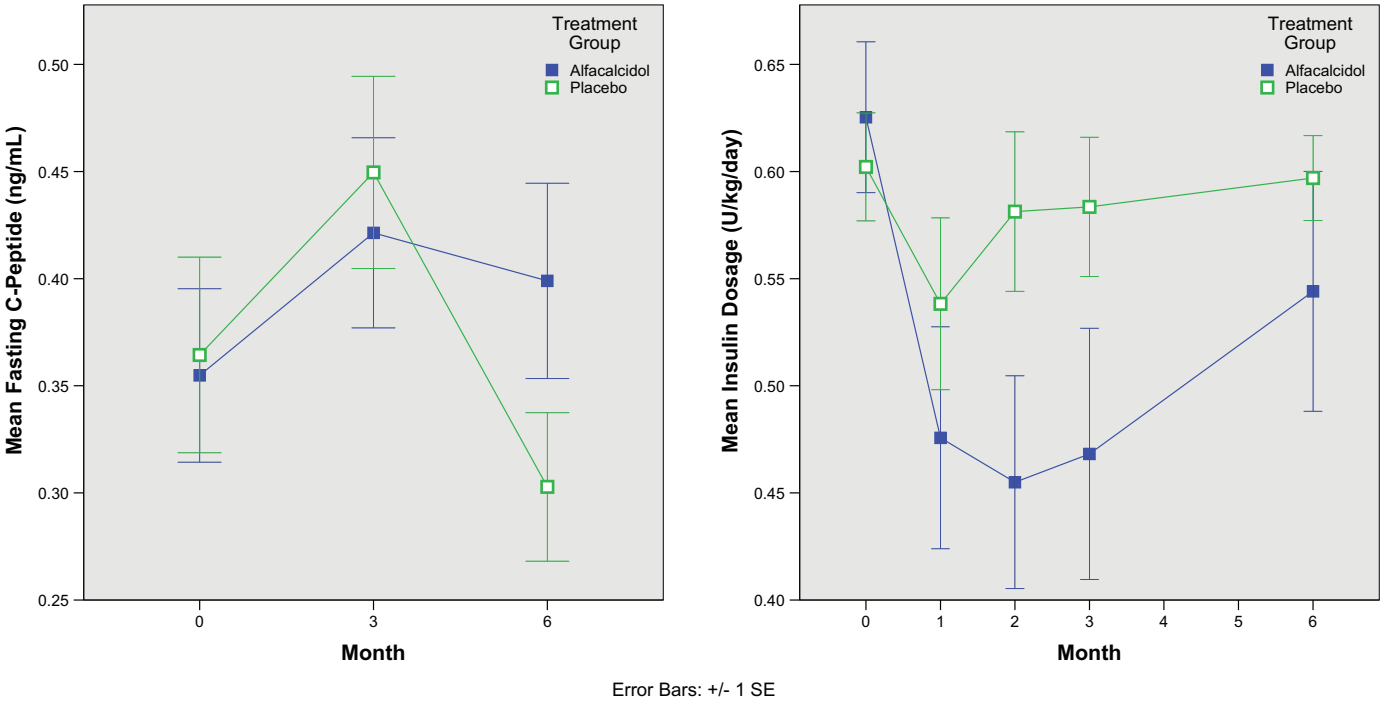

Fig. 2. Change in the mean fasting C-peptide and insulin dosage per body weight over the study period according to treatment group.

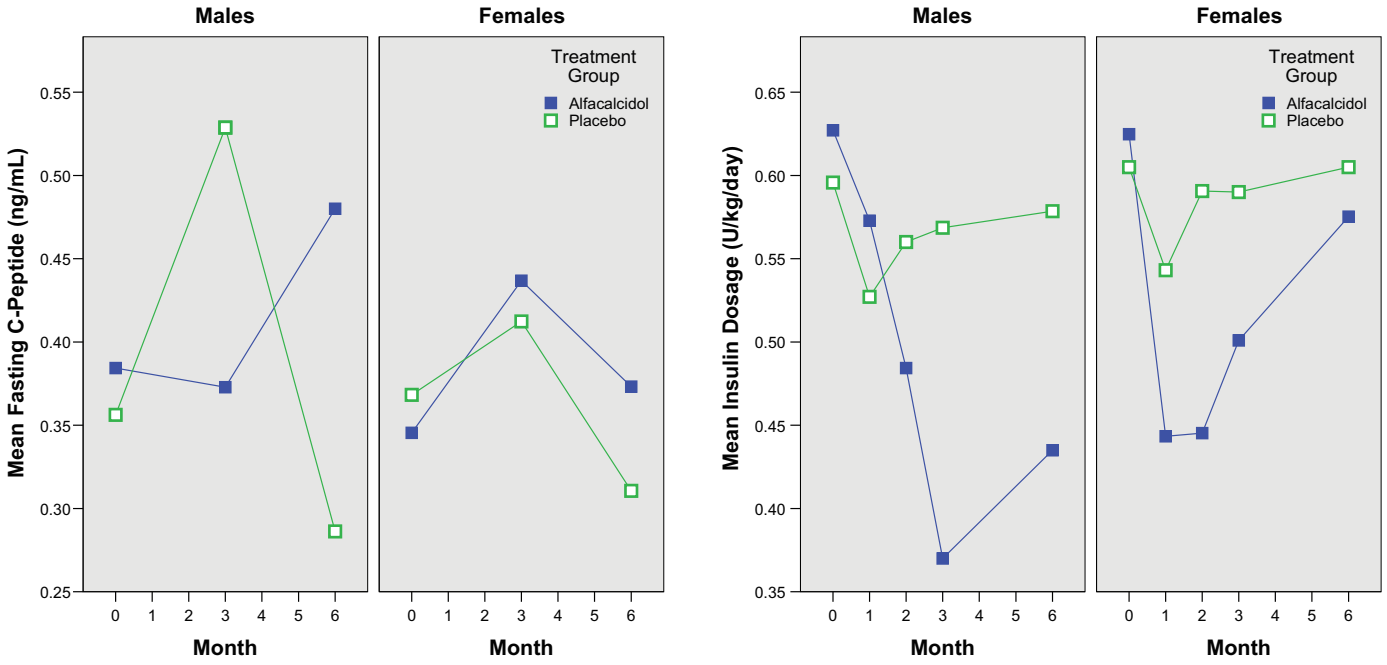

Fig. 3. Change in the mean fasting C-peptide and insulin dosage per body weight over the study period according to treatment group and gender.

by the higher FCP and lower DID for the intervention group (Fig. 2). The effect was however relatively modest with partial Eta-squared ranging between 0.078 and 0.093, which was only slightly above the medium effect size (partial Eta-squared 0.059) that this study was designed to detect.[27]

It was interesting to note that changes in DID occurred before there were any detectable differences in FCP. Logically, endogenous insulin production (FCP) should fall before the exogenous insulin requirement (DID) increases. In this case, the likely explanation is that FCP does not detect early beta cell loss as effectively as post-prandial C-peptide. When there is a modest reduction of beta cell reserve, the pancreas is still able to maintain basal insulin secretion, which is what FCP measures. The post-prandial insulin peak would however be blunted, thus requiring exogenous insulin to maintain euglycemia. A similar pattern has been observed in the evolution of type 2 diabetes with loss of post-prandial glycemic control preceding changes in inter-prandial and later fasting glycemia.[28]

Another interesting observation was that males had a stronger response to alfacalcidol than females. Baseline sun exposure scores and 25-hydroxy vitamin D levels were higher in males than females ($p = 0.001$ and $p = 0.012$, respectively), likely because males tend to spend more time outdoors during the hot Iranian summer while females are more modestly clothed, the effects of which were further enhanced by the timing of the study in autumn (Fig. 4). When DID and sun exposure scores were analyzed separately, GLM-ANOVA analysis showed highly significant and large between-subject effects ($p = 0.007$ partial Eta-squared $= 0.150$), further highlighting the association between sun exposure and DID.[27] One possible explanation for the differing response between genders is that sunlight exposure may moderate autoimmunity, thus rendering males more responsive to alfacalcidol treatment.[29]

4.1. Clinical relevance

The results from this study are generalizable to early type 1 diabetes in children older than 8 years worldwide, as the HLA alleles associated with type 1 diabetes prevalent in the Iranian population are similar to those in other populations, suggesting a comparable pattern of autoimmunity.[11,30] The economic implications of reducing the incidence of type 1 diabetes are huge, given that the lifetime costs of type 1 diabetes are much higher than for type 2 diabetes.[1]

The use of alfacalcidol instead of calcitriol allows higher dosages to be used with lower risk of hypercalcemia, and this increases its potential as a therapeutic agent. The previous studies using calcitriol were limited by the low dosages used to avoid adverse effects,

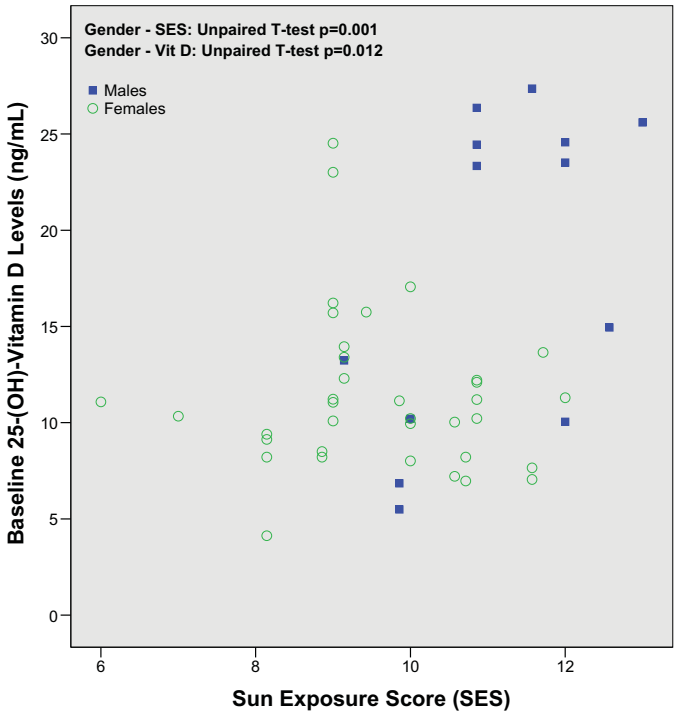

Fig. 4. Baseline sun exposure scores and 25-hydroxy vitamin D levels according to gender.

and this likely contributed to the small effect sizes even though the trends were broadly similar to the results from this study.[16,17,31]

It is interesting to note that one of the studies which demonstrated preservation of beta cell function utilized cholecalciferol, which has a long terminal half-life of weeks to months and has some protection against toxicity due to feedback inhibition of renal 1-alpha hydroxylase.[19,32] There is also the possibility that the short half-life of calcitriol leads to fluctuating serum levels which may inadequately suppress the autoimmune process.

4.2. Study weaknesses

There are a number of areas in which the study could have been improved. The use of FCP was less appropriate than post-prandial C-peptide, which was used in other studies investigating early type 1 diabetes.[33] There was also no data on VDR gene polymorphisms which may affect individual responses to alfacalcidol, although most of the previous studies similarly lack this information. The study design only allowed detection of a medium effect size, and as the actual effects were modest, we used a 10% level of significance in the GLM-ANOVA analysis so that we could pick up any trends that were present. Finally, the study period was too short to detect whether beta cell function could be preserved in the longer term, although the trends observed in this study suggest that this may not be the case as in the earlier studies using calcitriol (Fig. 2).[16,17]

4.3. Research implications

While the effects in this study were modest, the lack of significant changes in calcium and phosphate suggest that there is room to increase the dosage of alfacalcidol in subsequent studies. Improvements in methodology such as a larger sample size, a longer follow-up period, and the use of post-prandial C-peptide would certainly help to study early changes as well as demonstrate long-term efficacy. It appears that autoimmunity in type 1 diabetes is harder to suppress in younger patients, and stratification by age and inclusion of an older post-adolescent cohort would allow this factor to be properly explored.[18,33] Finally, the link between gender, autoimmunity, and sunlight exposure needs to be examined in greater detail with autoantibody testing, dendritic cell function, T-cell phenotype, as well as stratification of males by sunlight exposure, as this may provide useful insights into the mechanism of action of alfacalcidol in early type 1 diabetes.

4.4. Conclusions

The study confirmed that supplementation with alfacalcidol can safely preserve islet beta cell function in newly diagnosed type 1 diabetes in children and adolescents, with the effect being stronger in males than females. However, the effects are modest at the dosage used, and long-term outcomes are still unknown.

Funding

The study was run using internal funding from the Endocrinology and Metabolism Research Center (EMRC), Tehran University of Medical Sciences, and was registered with the Iranian Registry of Clinical Trials, a Primary Registry in the WHO Registry Network.

Conflict of interest

The authors declare no relevant conflicts of interest. A.A.J. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Acknowledgments

A.A.J. was the primary investigator and involved in data research. S.C.L. was involved in study design, wrote the manuscript, and analyzed data. A.B.R. conceptualized the project and was involved in study design. B.J. was the site coordinator for research in Iran. F.A. was involved in data research. M.K.S.L. reviewed/edited the manuscript. Z.Y. was involved in study design.

References

1. Tao B, Pietropaolo M, Atkinson M, Schatz D, Taylor D. Estimating the cost of type 1 diabetes in the U.S.: a propensity score matching method. *PLoS One* 2010;**5**:e11501.
2. Karvonen M, Viik-Kajander M, Moltchanova E, Libman I, LaPorte R, et al. Incidence of childhood type 1 diabetes worldwide. Diabetes Mondiale (DiaMond) Project Group. *Diabetes Care* 2000;**23**:1516–26.
3. Picardi A, Visalli N, Lauria A, Suraci C, Buzzetti R, et al. Metabolic factors affecting residual beta cell function assessed by C-peptide secretion in patients with newly diagnosed type 1 diabetes. *Horm Metab Res* 2006;**38**:668–72.
4. Haller MJ, Atkinson MA, Schatz D. Type 1 diabetes mellitus: etiology, presentation, and management. *Pediatr Clin North Am* 2005;**52**:1553–78.
5. Riachy R, Vandewalle B, Moerman E, Belaich S, Lukowiak B, et al. 1,25-Dihydroxyvitamin D3 protects human pancreatic islets against cytokine-induced apoptosis via down-regulation of the Fas receptor. *Apoptosis* 2006;**11**:151–9.
6. Szodoray P, Nakken B, Gaal J, Jonsson R, Szegedi A, et al. The complex role of vitamin D in autoimmune diseases. *Scand J Immunol* 2008;**68**:261–9.
7. Motohashi Y, Yamada S, Yanagawa T, Maruyama T, Suzuki R, et al. Vitamin D receptor gene polymorphism affects onset pattern of type 1 diabetes. *J Clin Endocrinol Metab* 2003;**88**:3137–40.
8. Ponsonby AL, Pezic A, Ellis J, Morley R, Cameron F, et al. Variation in associations between allelic variants of the vitamin D receptor gene and onset of type 1 diabetes mellitus by ambient winter ultraviolet radiation levels: a meta-regression analysis. *Am J Epidemiol* 2008;**168**:358–65.
9. Bourlon PM, Billaudel B, Faure-Dussert A. Influence of vitamin D3 deficiency and 1,25 dihydroxyvitamin D3 on de novo insulin biosynthesis in the islets of the rat endocrine pancreas. *J Endocrinol* 1999;**160**:87–95.
10. Pishdad GR. Low incidence of type 1 diabetes in Iran. *Diabetes Care* 2005;**28**: 927–8.
11. Rabbani A, Abbasi F, Taghvaei M, Rabbani B, Moradi B, et al. HLA-DRB, -DQA, and DQB alleles and haplotypes in Iranian patients with diabetes mellitus type I. *Pediatr Diabetes* 2012.
12. Bener A, Alsaied A, Al-Ali M, Hassan AS, Basha B, et al. Impact of lifestyle and dietary habits on hypovitaminosis D in type 1 diabetes mellitus and healthy children from Qatar, a sun-rich country. *Ann Nutr Metab* 2008;**53**:215–22.
13. Moltchanova EV, Schreier N, Lammi N, Karvonen M. Seasonal variation of diagnosis of Type 1 diabetes mellitus in children worldwide. *Diabet Med* 2009;**26**:673–8.
14. Roche, LEO Pharmaceutical. Rocalctrol and one-Alfa drug information pamphlets.
15. Bjelakovic G, Gluud LL, Nikolova D, Whitfield K, Wetterslev J, et al. Vitamin D supplementation for prevention of mortality in adults. *Cochrane Database Syst Rev* 2011:CD007470.
16. Pitocco D, Crino A, Di Stasio E, Manfrini S, Guglielmi C, et al. The effects of calcitriol and nicotinamide on residual pancreatic beta-cell function in patients with recent-onset Type 1 diabetes (IMDIAB XI). *Diabet Med* 2006;**23**: 920–3.
17. Bizzarri C, Pitocco D, Napoli N, Di Stasio E, Maggi D, et al. No protective effect of calcitriol on beta-cell function in recent-onset type 1 diabetes: the IMDIAB XIII trial. *Diabetes Care* 2010;**33**:1962–3.
18. Li X, Liao L, Yan X, Huang G, Lin J, et al. Protective effects of 1-alpha-hydroxyvitamin D3 on residual beta-cell function in patients with adult-onset latent autoimmune diabetes (LADA). *Diabetes Metab Res Rev* 2009;**25**:411–6.
19. Gabbay MA, Sato MN, Finazzo C, Duarte AJ, Dib SA. Effect of cholecalciferol as adjunctive therapy with insulin on protective immunologic profile and decline of residual beta-cell function in new-onset type 1 diabetes mellitus. *Arch Pediatr Adolesc Med* 2012;**166**:601–7.
20. American Association of Clinical Endocrinologists. American Association of Clinical Endocrinologists/American College of Endocrinology statement on the use of hemoglobin A1c for the diagnosis of diabetes. *Endocr Pract* 2010;**16**: 155–6.
21. National Collaborating Centre for Chronic Conditions (UK). In: *Type 1 diabetes in adults: national clinical guideline for diagnosis and management in primary and secondary care. 2011/09/23*. London, UK: Royal College of Physicians; 2004.
22. Payne RB, Carver ME, Morgan DB. Interpretation of serum total calcium: effects of adjustment for albumin concentration on frequency of abnormal values and on detection of change in the individual. *J Clin Pathol* 1979;**32**:56–60.
23. Glanz K, Yaroch AL, Dancel M, Saraiya M, Crane LA, et al. Measures of sun exposure and sun protection practices for behavioral and epidemiologic research. *Arch Dermatol* 2008;**144**:217–22.

24. Ataie-Jafari A, Rahmat A, Abbasi F, Loke SC, Qorbani M, et al. Vitamin D status and associated factors in recent-onset type 1 diabetic children in Iran. *J Diabetes Metab Disorders* 2012;**11**:12.
25. Olson CL. On choosing a test statistic in multivariate analysis of variance. *Psychol Bull* 1976;**83**:579.
26. Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. *Behav Res Methods* 2009;**41**:1149–60.
27. Cohen J. *Statistical power analysis for the behavioral sciences*. L. Erlbaum Associates; 1988.
28. Monnier L, Colette C, Dunseath GJ, Owens DR. The loss of postprandial glycemic control precedes stepwise deterioration of fasting with worsening diabetes. *Diabetes Care* 2007;**30**:263–9.
29. Ponsonby AL, McMichael A, van der Mei I. Ultraviolet radiation and autoimmune disease: insights from epidemiological research. *Toxicology* 2002;**181**–**182**:71–8.
30. Erlich H, Valdes AM, Noble J, Carlson JA, Varney M, et al. HLA DR-DQ haplotypes and genotypes and type 1 diabetes risk: analysis of the type 1 diabetes genetics consortium families. *Diabetes* 2008;**57**:1084–92.
31. Walter M, Kaupper T, Adler K, Foersch J, Bonifacio E, et al. No effect of the 1alpha,25-dihydroxyvitamin D3 on beta-cell residual function and insulin requirement in adults with new-onset type 1 diabetes. *Diabetes Care* 2010;**33**:1443–8.
32. Marcus R. Agents affecting calcification and bone turnover. In: Hardman J, Limbird L, editors. *Goodman and Gilman's: the pharmacological basis of therapeutics*. 9th ed. New York: McGraw-Hill; 1996. p. 1519–46.
33. Ergun-Longmire B, Marker J, Zeidler A, Rapaport R, Raskin P, et al. Oral insulin therapy to prevent progression of immune-mediated (type 1) diabetes. *Ann N Y Acad Sci* 2004;**1029**:260–77.
34. Schulz KF, Altman DG, Moher D. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. *PLoS Med* 2010;**7**. e1000251.