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Changes in the Levels of Cytokines in Both Diabetic/Non-Diabetic Type I Children Living in a Moderate Altitude Area in Saudi Arabia

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Abstract

Allam, Gamal, Adnan A. Alsulaimani, Hamed Alghamdi, Hameed Alswat, Burhan M. Edrees, Iftikhar Ahmad, and Amre Nasr. Changes in the levels of cytokines in both diabetic/non-diabetic type I children living in a moderate altitude area in Saudi Arabia. *High Alt Med Biol* 15:380–387, 2014.—The aim of the present study was to investigate the possible effects of living in moderate altitude area on pro/anti-inflammatory cytokines profile (IFN-γ, TNF-α, IL-6, IL-1β, IL-10, and IL-4) among type I diabetic (T1D) and non- T1D children compared with those living at sea level area. A prospective clinical study was carried out at pediatric outpatient endocrine clinics in Taif City, which is a moderate altitude area in Saudi Arabia, that stands about 1800–2000 meters above sea-level; and in Mecca City, which is a sea level area, that lies in the middle west of Saudi Arabia. Hemoglobin A1c (HbA1c) percentage was estimated and cytokine measurements were performed in sera by flow cytometry using Cytometric Bead Array (CBA) technology. In this study we included 600 children who were consecutively enrolled (sex and age were matched). The HbA1c was statistically significantly higher in children living in moderate altitude compared to those living at sea level (overall $p < 0.001$). Furthermore, T1D patients had higher values of serum cytokine levels (IFN-γ, TNF-α, IL-6, IL-1β, IL-4, and IL-10) in comparison to non-T1D control group (overall $p < 0.001$). In conclusion, the data of the present study clearly showed that in both T1D and non-T1D children, moderate altitude-natives expressed high HbA1c and both pro- and anti-inflammatory cytokines. Type I diabetic children living in moderate altitude or at sea level showed elevated levels of IFN-γ, TNF-α, IL-6, IL-1β, IL-4, and IL-10 than control subjects. Glycemic control in non-diabetic children was affected by living in moderate altitude, however, HbA1c significantly increased in diabetic children living in moderate altitude.

Introduction

High altitude is accompanied by hypoxia, which is known to elicit alterations in both the autonomic nervous system and endocrine function. Alterations in these systems can have an immediate as well as a longer lasting impact on immune function (van Gool et al., 1990; Soszynski et al., 1996; Mazzeo et al., 2001). High altitude exposure influences much immune function (Mazzeo et al., 1994; 1995; 2000), including mobilization of T-cells and NK cells, and regulation of cytokine production and release (Klokker et al., 1993; 1995; Pedersen and Steensberg, 2002). Regardless of the mechanism, it appears clear that acute exposure to altitude/hypoxia results in alterations of specific components of the immune system (Meehan et al., 1988; Klokker et al., 1993; 1995; Mazzeo et al., 1994; 2001; Pedersen and Steensberg 2002, Facco et al., 2005). Recently, more attention has been directed toward examining the influence of hypoxia on inflammatory cytokines. Upregulation of circulating interleukin (IL)-6, IL-1β, TNF-α, and C-reactive protein (CRP) have been

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observed during short-term high altitude exposure (Hartmann et al., 2000; Hagobian et al., 2006). A role for these inflammatory cytokines in the development of high-altitude pulmonary edema (HAPE) has been suggested (Kubo et al., 1998; Hartmann et al., 2000). It is now evident that the environmental stress of altitude/hypoxia exposure alone is sufficient to cause an elevation in circulating IL-6, even under resting conditions (Mazzeo, 2005).

Type I diabetes mellitus (T1D) is an autoimmune disease most frequently affecting children, characterized by infiltrating mononuclear cells in the islets of Langerhans (insulinitis) and selective destruction of the insulin-producing β-cells (Bach 1994; Lehuen et al., 2010). Cellular components of this infiltration include monocytes, macrophages, and $CD4^+$ and $CD8^+$ T-cells, which all participate in the autoimmune attack of the pancreatic islets (Bach, 1994; Morran et al., 2008; Shao et al., 2012). Several lines of evidence in both animals and humans indicate that Th1 populations, which secrete cytokines including IFN-γ and IL-2, are key mediators of β-cell auto-reactivity (reviewed in Azar et al., 1999). Conversely, induction of Th2 cells, which are associated with the cytokines such as IL-4, IL-5, IL-10, and IL-13, results in a dominant protective effect against autoimmunity in T1D (Suarez-Pinzon and Rabinovitch, 2001). Therefore, the balance between Th1 and Th2 cells is crucial in the pathogenesis of T1D. A bias toward the pro-inflammatory Th1 cytokines promotes insulinitis and T1D (Suarez-Pinzon and Rabinovitch, 2001; Amirshahrokhi et al., 2008). Released cytokines, IL-1β, TNF-α, and IFN-γ, exert cytotoxic effects specifically on beta-cells in the islets of Langerhans, in part via the induction of free radical production (Mandrup-Poulsen et al., 1990; Mandrup-Poulsen, 1996; Yamagata et al., 1996). Th2 anti-inflammatory cytokines, IL-4, IL-5, and IL-10, suppress insulinitis, beta-cell damage, and T1D in multiple-low-dose streptozotocin (MLDS)-induced diabetic mice and in non-obese diabetic mice (Cameron et al., 1997; Amirshahrokhi et al., 2008). Furthermore, immune system deviations other than Th1/Th2 imbalance have been detected in peripheral blood of T1D patients, such as circulating islet antigen specific autoreactive $CD8^+$ and $CD4^+$ T cells, found in both patients and some at-risk individuals, and occasionally also in nondiabetic individuals (reviewed by Huntington and Tarlinton, 2004). Functional impairment of T cell regulation has also been reported in T1D patients (Brusko et al., 2005; Lindley et al., 2005), and this impairment has later been ascribed to an inability of effector cells to respond properly to regulation, rather than a defect intrinsic to regulatory cells (Brusko et al., 2005; Lawson et al., 2008). Consequently, it appears that there are multiple cellular and cytokine immunoregulatory defects in human T1D.

Previous altitude studies have shown that glucose homeostasis is influenced by hypoxia, both acutely and after more prolonged exposure. In response to acute hypoxia (hours), fasting plasma glucose concentration is unchanged (Cooper et al., 1986; Brooks et al., 1991), but is increased after 3 days of hypoxia (Sawhney et al., 1991; Larsen et al., 1997). After short-term exposure to altitude, men and women appear to be less sensitive to insulin than at sea level (Braun et al., 2001; Jakobsson and Jorfeldt, 2006). With increasing altitude, diabetic mountaineers report a reduction in metabolic control (Pavan et al., 2003; Leal, 2005) as demonstrated by elevated HbA1c, insulin requirements, and capillary blood glucose (Moore et al., 2001; Pavan et al., 2004). After further altitude acclimatization, fasting glucose concentration is the same (Sawhney et al., 1991; Young et al., 1992) or below sea level value (Brooks et al., 1991). The insulin concentration increases acutely (Sawhney et al., 1991; Young et al., 1992) and remains elevated for up to 1 week (Sawhney et al., 1991) and returns to sea level value after 15–21 days (Brooks et al., 1991; Sawhney et al., 1991). On the other hand, hypoxia induced by chronic altitude exposure in nondiabetic subjects, stimulates glucose production with decreased hepatic insulin sensitivity, but increases peripheral insulin sensitivity (Sauerwein and Schols, 2002).

The aim of the present study was to investigate the possible effects of living in moderate altitude area on pro/anti-inflammatory cytokines profile (IFN-γ, TNF-α, IL-6, IL-1β, IL-10, and IL-4) among T1D and non-T1D children compared with those living at sea level.

Materials and Methods

Study area

This study was conducted in a moderate altitude area in the Kingdom of Saudi Arabia (KSA), Taif City which stands about 1800–2000 meters above sea-level on the eastern slopes of Al-Sarawat Mountains; and at Mecca City, a sea level area, which lies in the middle west of KSA.

Study design and subjects

A cross-sectional study was carried out at pediatric outpatient endocrine clinics from June 2012 to May 2013 in Prince Mansour Military Hospital (PMMH) and King Abdul Aziz Specialist Hospital (KASH) in Taif (moderate altitude), and Al-Noor Specialist Hospital (ASH) in Mecca City (sea level). This study included 300 children with T1D residing in Taif and Mecca, and 300 nondiabetic controls from both regions.

All participants fulfilled the following criteria: no infectious diseases, no autoimmune disorders, no asthma, no eczema or allergies. In addition, nondiabetic controls were consecutively enrolled in study from outpatient dentistry clinics, had no type I or II diabetes, and no first-degree relatives with diabetes or autoimmune disorders. Numbers, sex, and age were matched between the diabetic/nondiabetic groups living in both Taif and Mecca City.

Sample collection

One hundred μL of blood were collected on filter paper (Schleicher & Schuell; n° 903TM) for cytokine detection, and 1 mL of venous blood sample was collected in EDTA BD Vacutainer® Plasma Tube, 143 USP Units of sodium heparin freeze dried. Plasmas were obtained by centrifugation and were kept at $-80°C$ until usage.

Quantification of hemoglobin A1c (HbA1c)

Hemoglobin A1c (HbA1c) percentage was estimated by using reagent kits purchased from Abbott Diagnostics (Abbott Park, IL, USA) according to manufacturer instructions.

Determination of cytokines in sera samples using cytometric bead array

Cytokine measurements were performed in sera by flow cytometry using Cytometric Bead Array (CBA) technology,

previously described (Cook et al., 2001). Human Inflammation CBA Kit (BD Biosciences, San Jose, CA) was used to quantitatively measure IFN-γ, TNF-α, IL-6, IL-1β, IL-10, and IL-4 levels. The sensitivity of Human Inflammation CBA is comparable to conventional ELISA (Chen et al., 1999). Samples were analyzed using a BD FACSC alibur flow cytometer (BD Biosciences), according to manufacturer's instructions.

Statistical analysis

The data were analyzed using SPSS software version 16 for Windows (SPSS, Inc., Chicago, IL, USA). Assessment of association of cytokine levels (dependent variable) with disease outcome was made according to different cytokine levels interaction. The linear regression analysis with different assumptions about the mode of living area was used to assess higher levels of cytokine-associated disease outcome. Overall, the 95% confidence interval (CI) for unstandardized coefficients (the values for predicting the dependent variable from the independent variable) (B) that do not cross 1.00 were statistically significant. Regression is the determination of the statistical relationship between two or more variables (Khothari, 2004). Regression equations for stature estimation were developed using the upper limb measurements. The simple linear regression models for estimation of stature were derived as *Y* (cytokines) = *a* (constant) + *b* (regression coefficient of the independent variable) *X* ((1) nondiabetic and (2) diabetic patients) ± SEE (standard error of the estimate). The multiple regression models were derived as *Y* (cytokines) = *a* (constant) + *b1* (regression coefficient of the first variable) *X1* (first variable) + *b2* (regression coefficient of the second variable) *X2* (second variable) + … *bn* (regression coefficient for the coefficient variables) *Xn* (coefficient variable) ± SEE (Khothari, 2004). Therefore we fitted a separate model for each cytokine. We modelled each cytokine as a continuous dependent variable, and altitude, sex, and age as independent variables. Each of these models was fitted separately for (1) nondiabetic and (2) diabetic patients.

Ethical considerations

The work described here was performed in accordance with the Declaration of Helsinki. Ethical approval was obtained from the Research Ethics Committee of the College of Medicine at Taif University, Armed Forces Hospitals, and King Abdul Aziz Specialist Hospital at Taif, Saudi Arabia. Informed consent was obtained from children and their responsible guardians before participation.

Results

In this study we consecutively enrolled 600 children, who were divided into four study groups that were matched for sex and age within each group (75 Female: 75 Male in each group); mean ± Standard deviation (SD) (10.01 ± 3.30). Group I: T1D ($n = 150$ patients). Group II: Control or non-T1D group ($n = 150$ individuals), both groups I and II were living at sea level (Mecca). Group III: T1D group ($n = 150$ patients). Group IV: Control or non-T1D ($n = 150$ individuals), both Groups III and IV were living in a moderate altitude area (Taif).

General characteristics of each analyzed group

T1D patients had higher values of HbA1c% and serum cytokine level pg/mL (IFN-γ, TNF-α, IL-6, IL-1β, IL-4, and IL-10) in comparison to the age and sex-matched non-T1D young individuals from the control group (Table 1).The HbA1c was statistically significantly higher in children living in moderate altitude compared to those living at sea level (overall $p < 0.001$) (Table 1).

Plasma cytokine and HbA1c levels in non-T1D patients living in moderate altitude compared to non-T1D patients living at sea level

The serum concentrations of six cytokines were statistically significantly higher in non-T1D children living in moderate altitude area compared with non-T1D children living at sea level: IFN-γ [adjusted B = 1.07, 95% CI (0.94–1.20) and $p < 0.001$], TNF-α [adjusted B = 1.64, 95% CI (1.50–1.90) and $p < 0.001$]; IL-6 [adjusted B = 1.80, 95% CI (1.58–2.01) and $p < 0.001$]; IL-10 [adjusted B = 1.22, 95% CI (1.09–.34) and $p < 0.001$]; IL-4 [adjusted B = 2.20, 95% CI (1.93–2.48) and $p < 0.001$]; and IL-1β [adjusted B = 2.02, 95% CI (1.85–2.20)and $p < 0.001$] (Table 2). Furthermore, the level of HbA1c was statistically significantly higher in non-T1D children living in moderate altitude compared to non-T1D children living at sea level [adjusted B = 2.26, 95% CI (1.93–2.60) and $p < 0.001$] (Table 2).

Table 1. Mean and Standard Deviation (SD) for Age/Years, HbA1c%, and Cytokine Levels pg/mL (IFN-γ, TNF-α, IL-6, IL-1β, IL-4, and IL-10) for the Various Study Groups

Region	*Study groups*		*Age/ years*	*HbA1 c%*	*IFN-γ pg/mL*	*TNF-α pg/mL*	*IL-1β pg/mL*	*IL-6 pg/mL*	*IL-4 pg/mL*	*IL-10 pg/mL*
Sea level	Control $n^* = 150$	Mean	9.76	3.99	3.17	4.23	1.43	4.23	2.16	1.42
		SD	2.66	0.72	0.18	0.26	0.44	0.26	0.32	0.44
	Diabetes type I $n^* = 150$	Mean	9.45	7.44	10.84	13.02	6.82	13.02	8.14	6.82
		SD	3.17	0.68	2.67	3.29	1.48	3.29	2.86	1.48
Moderate altitude	Control $n^* = 150$	Mean	10.63	4.43	4.11	5.98	3.48	5.97	3.38	3.48
		SD	3.54	1.15	0.51	0.83	0.52	0.83	0.38	0.52
	Diabetes type I $n^* = 150$	Mean	10.19	11.47	19.97	27.84	12.26	27.84	18.28	12.26
		SD	3.84	1.90	6.53	6.87	2.56	6.87	4.48	2.56
Overall *P* value$^{\Psi}$			< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

*n represents number of individuals; $^{\Psi}P$ values are derived from one-way ANOVA test.

Table 2. Linear Regression Analysis of Circulating Cytokine Levels pg/mL (IFN-γ, TNF-α, IL-6, IL-1β, IL-4, and IL-10) and HbA1c% in Control Groups Living in Moderate Altitude Area Compared to Control Group Living at Sea Level

	Unadjusted B[a] 95% CI	P *value*	*Adjusted B (95% CI)[b]*	*P value*
IFN-γ	0.94 (0.85–1.02)	<0.001	1.07 (0.94–1.20)	<0.001
TNF-α	1.55 (1.46–1.65)	<0.001	1.64 (1.50–1.90)	<0.001
IL-6	1.75 (1.61–1.89)	<0.001	1.80 (1.58–2.01)	<0.001
IL-10	1.21 (1.13–1.29)	<0.001	1.22 (1.09–1.34)	<0.001
IL-4	2.01 (1.83–2.19)	<0.001	2.20 (1.93–2.48)	<0.001
IL-1β	2.05 (1.94–2.16)	<0.001	2.02 (1.85–2.20)	<0.001
HbA1c%	2.44 (2.22–2.66)	<0.001	2.26 (1.93–2.60)	<0.001

[a]B represents unstandardized coefficients (the values for predicting the dependent variable from the independent variable) while CI represents confidence intervals; [b]B (95%CI) adjusted with sex and age.

Plasma cytokines and HbA1c levels in T1D patients living in moderate altitude as compared to other study groups

Serum samples from the children living in moderate altitude and sea level area were analyzed for levels of IFN-γ, TNF-α, IL-6, IL-10, IL-4, and IL-1β. When comparing T1D children living in moderate altitude area and controls living at sea level regardless, higher level of IFN-γ [adjusted B = 10.89, 95% CI (9.54–12.23) and $p < 0.001$]; TNF-α [adjusted B = 12.10, 95% CI (10.78–13.43) and $p < 0.001$]; IL-6 (adjusted B = 14.68, 95% CI (13.40–15.98) and $p < 0.001$]; IL-10 (adjusted B = 10.04, 95% CI (9.15–10.94) and $p < 0.001$]; IL-4 (adjusted B = 16.03, 95% CI (14.99–17.07) and $p < 0.001$); and IL-1β (adjusted B = 5.42, 95% CI (4.92–5.92) and $p < 0.001$) were associated with T1D patients living in moderate altitude (Table 3).

The levels of HbA1c were statistically significantly higher in T1D children living in moderate altitude compared to T1D children living at sea level (adjusted B = 4.00, 95% CI (3.66–4.34) and $p < 0.001$) (Table 3).

Analysis of cytokines and HbA1c concentrations in relation to T1D living in moderate altitude area

In this study, serum cytokine concentration was analyzed in the study groups to quantify the independent relationship between the risk of T1D infection and moderate altitude. Linear regression analysis showed that higher levels of cytokines and HbA1c were associated with T1D children living in moderate altitude area than those with non-T1D patients living in the same study area, IFN-γ (adjusted B = 15.93; 95% CI 14.86–17.01 and $p < 0.001$); TNF-α (adjusted B = 17.04; 95% CI 15.81–18.26 and $p < 0.001$); IL-6 (adjusted B = 21.77; 95% CI 20.63–22.91 and $p < 0.001$); IL-10 (adjusted B = 14.87; 95% CI 14.13–15.61 and $p < 0.001$); IL-4 (adjusted B = 21.59; 95% CI 20.67–22.50 and $p < 0.001$); and IL-1β (adjusted B = 8.78; 95% CI 8.35–9.21 and $p < 0.001$) (Table 4).

Higher levels of HbA1c were significantly associated with T1D patients living in moderate altitude area compared to non-T1D group living in the same study area (adjusted B = 5.00; 95% CI 4.64–5.36 and $p < 0.001$) (Table 4).

Discussion

High altitude is defined as 2500 m above sea level where arterial oxygen saturation (Sao_2) measurably begins to fall (Krampl et al., 2000; Moore et al., 2011). Other studies have defined high altitude as 3000–5000 m and extreme altitude as more than 5000 m (Brubaker 2005; West 2004). This study was carried in Taif City which lies about 1800–2000 m above sea level. Therefore, we consider the study area as a moderate altitude area according to Pyne et al., (2000).

Little is known about how chronic altitude exposure affects immune function of both normal and diseased humans. The effect of living in moderate altitude on cytokine profile of nondiabetic and diabetic patient has not yet been studied. Therefore, the present study was carried out to investigate the effects of living in moderate altitude on pro- and anti-inflammatory cytokine profile of both T1D and non-T1D children. This was achieved by looking at plasma cytokine levels in T1D children and non-T1D controls living in moderate altitude and sea level area in Saudi Arabia. The study has clearly shown that both T1D and non-T1D children living in moderate altitude expressed a higher plasma level of pro-inflammatory (IL-β, IL-6, TNF-α, IFN-γ) and anti-inflammatory (IL-4, IL-10) cytokines than sea level natives. This result runs in parallel with the findings of Pyne et al., (2000), who reported an increase in mitogen-induced IL-1β, IL-4, and IFN-γ production from peripheral blood lymphocytes of both athletic swimmers and control groups after medium-level altitude exposure. As moderate and high altitudes are characterized by hypoxia, previous studies have demonstrated that hypoxia increases IL-1β and TNF-α production by human peripheral blood mononuclear cells (Ghezzi et al., 1991). Klausen et al. (1997) examined the influence of acute hypoxia in humans and found an increased

Table 3. Linear Regression Analysis of Circulating Cytokine Levels pg/mL (IFN-γ, TNF-α, IL-6, IL-1β, IL-4, and IL-10) and HbA1c% in Diabetes Type I Patients Living in Moderate Altitude Area *Compared to Diabetes Type I Patients Living at Sea Level*

	Unadjusted B[a]95% CI	P *value*	*Adjusted B (95% CI)[b]*	P *value*
IFN-γ	9.13 (8.00–10.27)	<0.001	10.89 (9.54–12.23)	<0.001
TNF-α	11.89 (10.62–13.15)	<0.001	12.10 (10.78–13.43)	<0.001
IL-6	14.81 (13.59–16.04)	<0.001	14.68 (13.40–15.98)	<0.001
IL-10	10.13 (9.28–10.99)	<0.001	10.04 (9.15–10.94)	<0.001
IL-4	15.68 (14.67–16.69)	<0.001	16.03 (14.99–17.07)	<0.001
IL-1β	5.44 (4.96–5.91)	<0.001	5.42 (4.92–5.92)	<0.001
HbA1c%	4.03 (3.71–4.36)	<0.001	4.00 (3.66–4.34)	<0.001

[a]B represents unstandardized coefficients (the values for the regression equation for predicting the dependent variable from the independent variable) while CI represents confidence intervals. [b]B (95%CI) adjusted with sex and age.

Table 4. Linear Regression Analysis of Circulating Cytokine Levels pg/mL (IFN-γ, TNF-α, IL-6, IL-1β, IL-4, and IL-10) and HbA1c% in Diabetes Type I Patients Compared to Controls Living in Moderate Altitude Area

	Unadjusted B[a] *95% CI*	P *value*	*Adjusted B (95% CI)*[b]	P *value*
IFN-γ	15.93 (14.81–16.92)	<0.001	15.93 (14.86–17.01)	<0.001
TNF-α	16.86 (15.66–18.05)	<0.001	17.04 (15.81–18.26)	<0.001
IL-6	21.86 (20.75–22.98)	<0.001	21.77 (20.63–22.91)	<0.001
IL-10	14.90 (14.18–15.62)	<0.001	14.87 (14.13–15.61)	<0.001
IL-4	21.29 (20.39–22.20)	<0.001	21.59 (20.67–22.50)	<0.001
IL-1β	8.79 (8.34–9.21)	<0.001	8.78 (8.35–9.21)	<0.001
HbA1c%	5.04 (4.69–5.40)	<0.001	5.00 (4.64–5.36)	<0.001

[a]B represents unstandardized coefficients (the values for the regression equation for predicting the dependent variable from the independent variable) while CI represents confidence intervals; [b]B (95%CI) adjusted with sex and age.

level of serum IL-6. Furthermore, both acute and chronic high altitude (4300 m) exposure results in elevated resting IL-6 levels in humans. The elevation in IL-6 persists while subjects are becoming acclimatized to high-altitude exposure for several weeks (Mazzeo et al., 2001). Such increase in IL-6 level could be mediated via norepinephrine acting on the α-adrenergic receptors, which is a primary factor responsible for the sustained elevation in IL-6 levels over time at altitude. Blockage of α-adrenergic by prazosin resulted in completely abolished elevated level of IL-6 in chronic high altitude exposure (Mazzeo et al., 2001). The physiological significance of the IL-6 response to hypoxia remains unknown; however, a number of possibilities have been suggested, such as that IL-6 can promote angiogenesis (Motro et al., 1990) and may play a role via the induction of vascular endothelial growth factor (VEGF). Treatment of various cell lines with IL-6 results in a significant induction of VEGF mRNA that is comparable to the documented induction of VEGF mRNA by hypoxia (Cohen et al., 1996). Additionally, IL-6 can modulate production of erythropoietin as the addition of IL-6 to hypoxic human hepatoma cells resulted in a dose-dependent stimulation of hypoxia-induced erythropoietin production by as much as 81%. The associated increase in erythrocytes number and oxygen carrying capacity are well-documented markers of adaptation to high altitude (Faquin et al., 1992). However, the significance of increase in the other cytokines (IL-β, TNF-α, IFN-γ, IL-4, and IL-10) levels in moderate altitude natives is unclear and warrants further investigation.

Most of our current information on cytokines implicated in the pathogenesis of T1D comes from studies using non-obese diabetic mice and BB rat models of the human disease. Association between serum levels of pro- and anti-inflammatory cytokines, and the possible roles of these cytokines in the pathogenesis of the human disease are less well characterized. The data of the current investigation showed an elevated serum level of IL-β, IL-6, TNF-α, IFN-γ, IL-4, and IL-10 in T1D patients living at both sea level and moderate altitude. Studies of serum levels of different cytokines, as well as secretion of cytokines by peripheral blood mononuclear cells (PBMCs) from patients with T1D, have not yielded consistent results (Cavallo et al., 1991; Faquin et al., 1992). Previous studies demonstrated that cells in whole blood from patients with T1D produced significantly higher amounts of IFN-γ and TNF-α than cells from normal control subjects, whereas production of IL-4 and IL-10 was similar in diabetic and control subjects (Kallmann et al., 1997). Another study reported that secretion of IL-4 and IL-10 was inhibited and IFN-γ and TNF-α increased in activated PBMCs from diabetic subjects (Rapoport et al., 1998). A previous study by Berman et al. (1996) found decreased IL-4 secretion from stimulated PBMCs and T cells of diabetic subjects and normal IFN-γ expression. It is unclear from these studies whether changes in serum levels or production of cytokines by cells from patients with T1D preceded or resulted from DM. It was previously suggested that the circulating levels of IL-1α, TNF-α, IL-2, and IFN-γ were reported to be elevated at the time of diagnosis of T1D and in the prediabetic period (Hussain et al., 1996). Similarly, circulating levels of TNF-α and soluble IL-2 receptor were found to be elevated in nondiabetic first-degree relatives of patients with T1D. Furthermore, IL-1α and TNF-α production by mitogen-stimulated PBMCs was increased in both diabetic and nondiabetic family members (Hussain et al., 1998). In another study, the ratio of IFN-γ/IL-4 production by PBMCs was significantly increased in high-risk first-degree relatives of T1D children (Karlsson et al., 2000).

The data of the present study suggest that living in a moderate altitude significantly alters HbA1c levels of T1D and non-T1D subjects. Previous altitude studies have demonstrated that glycemic control is decreased in normal individuals in response to acute or chronic altitude exposure, whereas after further altitude acclimatization, a restoration to basal levels of both glycemia and insulin sensitivity was found indicating adaptation (Brooks et al., 1991; Sawhney et al., 1991; Young et al., 1992; Larsen et al., 1997). On the other hand, the current study has clearly shown that HbA1c levels significantly increased in diabetic subjects living in moderate altitude compared to diabetic individuals living at sea level. It has been demonstrated that diabetic mountaineers report a reduction in metabolic control (Pavan et al., 2003; Leal, 2005), as demonstrated by elevated HbA1c, insulin requirements, and capillary blood glucose (Moore et al., 2001; Pavan et al., 2004). Reduced insulin sensitivity is thought to be the major factor contributing to these effects (Brubaker 2005; Leal 2005). It was recently suggested that the influence of hypoxia is associated with high glucose (Kanafi et al., 2013). In addition, a study by Yang et al. (2011) found that when people used easy relaxation techniques, they dramatically decreased the levels of HbA1c significantly (Yang et al., 2011). In fact, there is an association between stress and diabetes, suggested in three different ways: (A) Stress hormones such as cortisol speed up breathing and heart rate and release stored glucose into the blood to make energy available to the muscles (Yang et al., 2011). (B) Stress

contributes to insulin resistance (Yang et al., 2011). (C) Chronic stress induces cortisol secretion that is known to increase appetite leading to weight gain (Yang et al., 2011). All these reasons could be useful to explain how moderate altitude can affect the levels of HbA1c.

Conclusion

The data of the present study clearly show that in both T1D and non-T1D children, moderate altitude-natives expressed high HbA1c and both pro-and anti-inflammatory cytokines. Type I diabetic children living in moderate altitude or at sea level showed elevated levels of IL-β, IL-6, TNF-α, IFN-γ, IL-4, and IL-10 than control subjects. Glycemic control in nondiabetic children was affected by living in moderate altitude; however, HbA1c significantly increased in diabetic children living in moderate altitude.

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The authors declare that they have no competing interests.

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