ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Effect of Raw Camel Milk on ProinflammatoryAdipocytokines Levels in Children with Type 1 Diabetes Mellitus.

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ABSTRACT

Adipocytokines have been implicated in the pathogenesis and metabolic complications of diabetes mellitus. Camel milk (CM) is gaining increasing recognition, due to its beneficial effects, in patients with diabetes. The aim of the current study is to investigate the effects of CM on adipocytokines levels in children with type 1 diabetes mellitus (T1D). Thirty children with type 1 diabetes were selected from the Diabetic Endocrine Metabolic Pediatric Unit, children hospital, Cairo University. Measurements of HbA1c, lipid profile, superoxide dismutase (SOD), Interleukin-1 β (IL-1β), IL6, IL18 and lipocalin-2 levels were done at beginning and one month after consumption of CM daily. Thirty Healthy children with matched age and sex serve as control group.The baseline levels of lipid profile and HbA1c showed a significant improvement after CM consumption. Serum levels of IL-1β, IL-6, IL-18, lipocalin 2 in type 1diabetes post-treatment group were significantly decreased compared with pre-treatment group ($P \leq 0.001$).Camel milk has a unique composition that is rich in insulin. It exerts immunomodulatory effects. Regular consumption of CM could provide a natural way to improve glycemic control with a significant reduction in lipids in diabetic patients.

Keywords: Camel milk, Diabetes, Cytokines, Children, Lipocalin 2

Abbreviations:

LCN2; Lipocalin 2
CM; Camel Milk
T1D; Type1 diabetes
IDDM; Insulin dependent diabetes mellitus
TAC; Total antioxidant capacity
SOD; Superoxide dismutase

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ISSN: 0975-8585

INTRODUCTION

Diabetes mellitus is defined as a group of metabolic diseases characterized by hyperglycemia, which when uncontrolled can lead to long-term complications, including micro and macrovascular complications [1]. Continuous subcutaneous insulin infusion and daily insulin injections, established therapies for type 1 diabetes (T1D), are thought to prevent hyperglycemia and glucose level fluctuations [2]. Camel milk has been evaluated in patients with T1D[3]. Some mechanisms for lowering glucose concentration by camel milk consumption have been proposed, one of which is the higher level of insulin in camel milk in comparison to milk from other animals. Furthermore, insulin in camel milk is encapsulated in nanoparticles, which are capable of transporting this hormone intact into the blood[4].

Type 1 diabetes is thought to involve chronic inflammation, which is manifested by the production of different inflammatory mediators. Several proinflammatory cytokines were shown to be elevated in serum of diabetic patients with a new onset of diabetes or a longstanding disease [5].In type 1 or insulin-dependent diabetes mellitus (IDDM), the pancreatic islet beta-cells become the target of an autoimmune response which destroys their insulin-producing capacity [6]. Human enterovirus infections have long been suspected as environmental triggers of IDDM [7]. Cytokines have been implicated in the development of the characteristic islet mononuclear cell infiltrate (insulitis) and may also mediate cell damage in IDDM [8]. Multiple studies have suggested the potential involvement of the Interleukin-1(IL-1) pathway in islet destruction. IL-1 pathway is involved in the proinflammatory response leading to virus-induced T1D[9, 10]. IL-1, alone or combined with other proinflammatory cytokines such as IFN-β, can cause β-cell destruction in islets via pathways involving mitogen-activated protein kinase and nuclear factor -κB[11-13]. IL-6 is a pleiotropic cytokine with a key impact on both immunoregulation and non-immune events in most cell types and tissues outside the immune system, including but not limited to fibroblasts, endothelial cells, monocytes/macrophages, T cell lines, mast cells, and a variety of tumor cell lines [14]. IL-18 is produced mainly by monocytes/macrophages in response to stimuli of viral/bacterial origin, its production being therefore one of the effects of innate immunity initiated by host-pathogen interaction [15]. In addition, IL-18 is among the cytokines responsible for immune-mediated pathologies and is probably one of the factors that contribute to the pathogenesis of autoimmune diseases.

Lipocalin 2(LCN2) or neutrophil gelatinase-associated lipocalin belongs to the superfamily of lipocalins, and it was originally identified as a 25-kDa protein produced from human neutrophils. LCN2 is abundantly produced from adipocytes [16].The expression and secretion of LCN2 increases sharply after conversion of preadipocytes to mature adipocytes. Its expression can be induced by different inflammatory stimuli, including lipopolysaccharide and IL-1β [17, 18].

Our aimis to investigatepotential therapeutic benefits of camel milk, onglycemic control, through cytokinesimmunmodulatory effects, inchildren with type 1 diabetes.

MATERIALS AND METHODS

Subjects

The study group consisted of 30children (mean age; 8.8±2.85 years) diagnosed with type 1 diabetes that were randomly recruited from the Diabetic Endocrine Metabolic Pediatric Unit,Aboel-Reesh children hospital ,Cairo University. Patients with any complications like hypoglycemia, ketoacidosis, and cardiovascular event, renal or acute infection were not included in the study. Patients were not allowed to take insulin therapies or any other concomitant medicine throughout the study period.

Analysis of glycosylated hemoglobin (HbA1c), lipid profile, superoxide dismutase, lipocalin2, IL-1β, IL-6 and IL-18 were measured at beginning and one month after consumption of 250 ml of raw CM daily. The control group consisted of 30 age and sex-matched individuals recruited from outpatient clinic. All subjects gave informed consent and the study followed the principles of the Declaration of Helsinki and was approved by the local Ethics Committee.

Determination of serum IL-1 IL-6, IL-18, Lipocalin2and Superoxide dismutaseLevels

IL-1β and IL-6, measured by using commercialkit, Human IL-1β ELISA Kitand IL-6Human IL-6 ELISA kit,were from(Anogen-YES Biotech Laboratories Ltd, Ontario, Canada). Assay range2-400 pg/mL and 7-2000 pg/mL respectively.IL-18 was measured using commercial ELISA kit (CUSABIO Company,China). Il-18 level is expressed in pg/mL, and the detection range is 31.25– 2000 pg/mL.Determination of Serum Lipocalin 2 Level byLipocalin-2/NGAL Human ELISA kit(BioVendor Research and Diagnostic Products, Czech Republic). Its limit of detection is 0.02 ng/ml.Human Superoxide dismutase was measured using commercial ELISA kit(Kamiya biomedical company, Seattle, USA).

Camel milk analysis

Camel milk was collected aseptically from twelve healthy domestic camels. The collection of milk was usually conducted by experienced attendants. Milk was allowed to flow directly into stainless steel containers and then transferred todrinking glass bottles. Camel samples were transported to the laboratory as soon as practical (within 4 h) and stored at4^{o}C. TAC was measured using ABTS AntioxidantAssay Kit (Zen-Bio Inc., Research Triangle Park, NC).

CM insulin was measured by Human Insulin ELISAkit (RayBiotech, Norcross, USA) .The minimal detectable dose of human insulin was determined to be 4µIU/ml.

The results of CM analysis are shown in table 1.

Table 1: Insulin level and total antioxidant capacity in milk for 12 camels

No.	Camel no	Code number	Insulin uIU/ml	Total anti oxidant µm/ml
1-	Camel milk	1/1	37.8	415.2
2-	Camel milk	2/1	40.1	297.6
3-	Camel milk	3/1	38.6	386.6
4-	Camel milk	1/2	43.2	319.6
5-	Camel milk	2/2	46.2	442.3
6-	Camel milk	3/2	50.1	367.9
7-	Camel milk	1/3	47.6	384.2
8-	Camel milk	2/3	53.4	374.9
9-	Camel milk	3/3	56.1	512.3
10-	Camel milk	1/4	36.5	497.2
11-	Camel milk	2/4	39.1	367.4
12-	Camel milk	3/4	38.2	395.1

Statistical Analysis

SPSS for Windows, version 15.0 software was used for statistical analysis. Data are represented as mean ± SD for quantitative data, and as frequency and percentage for qualitative data. The t-test was used to compare between 2 independent means. The paired t-test was used to compare between 2 dependent means. A p value of less than or equal to 0.05 was considered statistically significant.

RESULTS

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The mean age observed in diabetic patients group and control group was 8.8±2.85and 9.83±2.81 years, respectively, (difference found was statistically non significant). Male to female ratio in diabetic patients was 17:13.

The present study was performed to study the effect of camel milk consumption on oxidative stress as well as proinflammatoryadipocytokineslevels on type 1 diabetic patients by measuring the serum levels of superoxide dismutase, IL-1β, IL-6, IL-18and lipocalin2.

Apart from SOD,all measured parameters showed significant changes after camel milk consumptions. Serum levels of IL-1β, IL-6, IL-18, lipocalin 2 in type 1diabetic patients'post-treatment group were significantly decreased compared with pre-treatment group. Serum levels SOD in post treatment diabetic group were elevated compared with pre treatmentgroup. However, it does not reach statistically significant level. .Significant differences were revealed in all measured parameters compared with normal controls (Table 2, P ≤ 0.001).

Table2: Effect of camel milk on the level of SOD and adipocytokines in type 1 diabetic patients:

Variables	Controls(N=30) Mean ± SD	Diabetic patients(N=30)	
		Before Treatment Mean ± SD	After Treatment Mean ± SD
SOD (U/ml)	222.537±14.899	167.490±9.344[a]	224.290±179.82
Lipocalin 2(Pg/ml)	25.663±3.852	65.933±8.082[a]	36.153±4.713[b]
Interleukin -1β(Pg/ml)	3.403±0.880	7.763±1.119[a]	4.930±1.172[b]
Interleukin -6(Pg/ml)	4.980±1.071	10.007±1.115[a]	6.383±1.288[b]
Interleukin-18(Pg/ml)	15.463±2.870	22.347±2.672[a]	15.927±2.060[b]

SOD,superoxide dismutase. Correlation is significant at ≤0.05.
a significant difference from normal group.
b Significant difference from diabetic patients before therapy.

The baseline levels of lipid profile and HbA1c showed a significant improvement after CM consumption. The ingestion of CM by diabetic patients resultedin significant decreases in HbA1C, cholesterol, triglycerides and LDL and increased the HDL levels (Table 3, p < 0.001).

Table 3: Lipid profile and HGA1c in children with type 1 diabetes before and after Camel milk

	Mean ± SD	*P* value
Cholesterol Before After	 195.33±23.422 133.37±19.937	<0.001*
Triglycerides Before After	 163.60±21.146 120.73±19.705	<0.001*
HDL Before After	 39.20±1.627 46.93±2.935	0.001*
LDL Before After	 123.97±22.489 76.13 ±13.485	<0.001*
HbA1c (%) Before After	 7.067±0.872 4.870±0.686	<0.001*

*Correlation is significant at≤ 0.05. HbA1c, hemoglobin A1c.

DISCUSSION

Recently, camel milk was also reported to have potential therapeutic properties such as anticarcinogenic, antimicrobial, antioxidant, angiotensin I-converting enzyme-inhibitory activities,

hypocholesterolemic, hypoglycemic, and hypoallergenic effects attributed to the presence of bioactive compounds in milk [19-25]. Reduction of the uncontrolled cytokines production and activity in autoimmunity would allow for novel therapeutic targets to effectively block autoimmune activation and inhibit subsequent tissue damage.

The production of several cytokines could be dysregulated in type 1 diabetes. In particular, the activation of T helper type 1cells has been proposed to underlie the autoimmune pathogenesis of the disease [26].Different inflammatory cells producing variousproinflammatory cytokines could also be involved, and pancreatic β-cell toxicity accompanies the inflammatory response (insulitis) within the islets [27].

In our study, we demonstrated the differences in proinflammatory cytokine (IL-1β, IL-6 and IL-18) levels between diagnosed IDDM patients compared to age-matched healthy controls.

Blocking the IL-1 pathway protected from type 1 diabetes in animal models and treating non–diabetes-prone animals with IL-1 lead to transient insulinopenic diabetes [28]. Earlier reports have linked IL-1 cytokine family members, including IL-1β, IL-1R1, and IL-1R2, with human IDDM [29].

IL-6 promotes inflammation [30] and adaptive immune responses [31]; however, it also suppresses the functions of various cell subsets, including macrophages and synovial fibroblasts. IL-6 induces anti-inflammatory factors, such as IL-1 receptor antagonist and glucocorticoids, inhibits the production of proinflammatory cytokines, such as IL-1, tumor necrosis factor, and IL-12. Thus, reduced IL-6 responses in seropositive individuals could potentially be part of early mechanisms leading to loss of immune regulation and consequently T1D [32].

In addition, it has been previously reported that hyperglycaemia increases serum concentrations of IL-6 possibly through augmented production in monocytes[33]. Thus patients with diabetes have elevated blood levels of IL-6, which, together with TNF-α are known to increase inflammation and the development of vascular disease, possibly by increasing oxidative stress [34].The SOD level is increased in post CM intake group ,but it doesn't reach statistically significant level compared to pretreatment group. In children with T1D, IL-6 levels were found to be statistically significantly increased in the group with long T1D duration, as well as in those newly diagnosed, when compared with healthy controls, with the highest levels in the newly diagnosed patients [35].IL-18 belongs to the IL-1 superfamily of cytokines .In synergy with IL-12; IL-18 activates polarization of Th1 cells, augments activity of NK cells, and induces IFN-γ production. Furthermore, some authors linked IL-18 or its receptor polymorphism with T1D [36].

Adipose tissue secretes a large numberof adipokines and cytokines that work to regulate inflammation, insulin action, and glucosemetabolism locally and systemically [37]**.**Yan 2007 suggested that LCN2 may be added to the growing list of secreted molecules that adipocytes use to regulate glucose homeostasis [38].In this study, we identify increased levels of LCN 2in diabetic patients and it isdramaticallyimproved byCM intake. HbA1c is marker of glycemic control and improvement in its values clearly indicates better glycemic control[3] .The significant reduction is observed in HbA1c levels in camel-milk receiving group versus pretreatment (7.067±0.872 and 4.870±0.686%respectively) .Meanwhile, the lack of hypoglycemic events in this study is encouraging. In addition, significant improvement in the lipid profile, post treatment, could be directly related to the content of high L-carnitine, which decreases cholesterol absorption [39]. The cholesterol-lowering activity of camel milk has been also reported in rats [21].

The results of our study lead us to conclude that Camel milk has immunomodulatory function on patients with type 1 diabetes. Camel milk is safe and efficacious in improving glycemic control and lipid profile in T1D children.Although these results are promising, more extensive and longer study is recommended.

ACKNOWLEDGMENTS

This study has been supported by the Diabetic Endocrine Metabolic Pediatric Unit,Aboel-Reesh children hospital ,Cairo University.

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