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Assessment of TNF Alpha in Type 2 Diabetic Patients with Lactobacillus Acidophilus

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ABSTRACT

Background: Lactobacillus acidophilus is a probiotic strain that is widely used in traditional foods such as dairy products and nutritional supplements. It has been shown that L. acidophilus interacts with immune system cells and increases intestinal integrity with a beneficial effect on glucose balance as it has been found to delay the onset of glucose intolerance, hyperglycemia, dyslipidemia and oxidative stress in diet-induced diabetic rats.

Objectives: To assess the level of TNF alpha in type 2 diabetic patients with lactobacillus acidophilus.

Patients and methods: This study is a case control study that was conducted on 100 type 2 diabetic patients diagnosed according to the American diabetes association diagnostic criteria. They were recruited from the Outpatient Clinic of Internal Medicine and Endocrinology Department Unit of Ain Shams University Hospitals during the period from February 2019 to November 2019. They were divided into 2 groups, group 1 that included 50 type 2 diabetic patients with atherosclerosis and group 2, which included 50 type 2 diabetic patients without atherosclerosis.

Results: There was a statistically significant positive correlation between PCR cut-off threshold and HbA1c (p-value = 0.026), 2hr pp (p-value = 0.013) and intimal media thickness (p-value = 0.031) and there was a statistically significant positive correlation between TNF alpha level and HbA1c (p-value = 0.015), LDL cholesterol (p-value = 0.024) and intimal media thickness (IMT) (p-value = 0.033). In addition, there was highly statistically significant positive relation found between TNF alpha level and PCR cut-off threshold (p-value < 0.001).

Conclusions: Lactobacillus acidophilus has been found to delay the onset of glucose intolerance, hyperglycemia, dyslipidemia and oxidative stress in patients with type 2 diabetes mellitus.

Keywords: Lactobacillus acidophilus, Type 2 diabetes mellitus, TNF alpha.

INTRODUCTION

Probiotic bacteria can be defined as "living microorganisms" that, when eaten in sufficient quantities, confer a health benefit to the host. Upon surviving the passage of the gastrointestinal tract, probiotics can persist as part of the gut microbiota for a certain period after ingestion. There is increasing evidence that the gut microbiota effects outside the gut are exerted by regulating the entire body's energy balance through mechanisms associated with the breakdown and absorption of gut contents [(1)].

Additionally, the intestinal microbiota may be crucial to the development and homeostasis of the immune system as most of the body's immune cells adapt in the gut and the gut bacteria interact closely with the intestinal immune cells [(2)]. Therefore, processes such as energy harvesting and immunity can be affected by changes in the composition of the intestinal flora, which is caused by oral probiotic supplementation. Moreover, certain strains of microorganisms improve intestinal barrier function, and may reduce the transmission of microorganisms and their derivatives, for example, lipopolysaccharides (LPS), from the gut to the systemic circulation, by reducing the associated release for pro-inflammatory cytokines through Toll-like receptor (TLR) -4 signaling [(3)]. Chronic, low-grade inflammation with consistently high levels of inflammatory stimulant cytokines is a major pathogen component of insulin resistance and type 2 diabetes [(4)].

Lactobacillus acidophilus NCFM is a probiotic strain that is widely applied in traditional foods such as dairy products as well as nutritional supplements. L. acidophilus NCFM has been shown to interact with immune system cells and increase intestinal integrity [(5)]. The L. acidophilus NCFM strain was examined for its physiological, biochemical and fermentation properties in laboratory and animal studies, and its entire genome sequence was determined [(6)].

The salutary effect on glucose homeostasis is supported by a number of animal studies, in which certain strains of probiotic organisms such as Lactobacillus acidophilus have shown anti-diabetic properties. Thus, treatment with antibiotics may protect against diabetes-causing changes in rodents, possibly by altering the composition of the gut microbiota [(7)]. In addition, L. acidophilus has been found to delay the onset of glucose intolerance, hyperglycemia, dyslipidemia, and oxidative stress in rats with diet-induced diabetes [(8)]. Finally, probiotic supplementation improved the high-fat diet caused by insulin resistance and hepatic steatosis [(9)].

Tumor necrosis factor alpha (TNF-α) is a cytokine that is released predominantly from macrophages, but is also released from a variety of other immune cells. As a pyrogen, tumor necrosis factor alpha

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(TNF-α) is important in the acute phase of inflammation and infection, with signals being transmitted through NF-B. It also acts as a pro-apoptotic signal by the TNF receptor decease domain [(10)].

Previous studies have shown that lactic acid bacteria (LAB) are helpful in treating various mucosal disorders including inflammatory damage even in the absence of an infection. They also evaluated the effects of probiotic strains on immune responses, and showed that probiotics-mediated protection against pathogen-induced infections, in part, from maintaining the equilibrium of production of pro-inflammatory and anti-inflammatory cytokines in immune cells. The role of L. acidophilus, a well-known probiotic, has been confirmed to regulate inflammation in humans [(11)].

Aim of the work was to assess the level of TNF alpha in type 2 diabetic patients with lactobacillus acidophilus.

PATIENTS AND METHODS

This study is a case control study that was conducted on 100 type 2 diabetic patients diagnosed according to the American diabetes association diagnostic criteria. They were recruited from the Outpatient Clinic of Internal Medicine and Endocrinology Department Unit of Ain Shams University Hospitals during the period from February 2019 to November 2019. They were divided into 2 groups, group 1 that included 50 type 2 diabetic patients with atherosclerosis and group 2, which included 50 type 2 diabetic patients without atherosclerosis.

All patients were subjected to full medical history taking, (emphasizing on age, sex, duration of diabetes, complications, treatment type and duration) and thorough clinical examination including anthropometric measurements of weight, height, body mass index (BMI) and fundus examination.

Laboratory investigations included serum creatinine (0.5-1.2 mg/dl) , ALT (7-56 U/L), AST (10-40 U/L) , CRP (< 3 mg/L), fasting blood glucose (100 mg/dl), two hrs post prandial blood glucose (<140 mg/dl), HbA1c (< 5.7%), cholesterol (200 mg/dl), triglycerides (< 150 mg/dl), LDL cholesterol (<100 mg/dl), HDL cholesterol (> 60 mg/dl), fasting serum insulin (< 25 micro IU/L) and albumin / creatinine ratio. All measured by ELISA. In addition, HOMA IR (0.5–1.4) as an estimate for insulin resistance calculated according to the formula: fasting insulin x fasting glucose (nmol/L)/22.5, and identification of stool lactobacillus acidophilus by PCR semi-quantitative technique.

Radiological investigations included carotid artery intima media thickness (IMT) (0. 59-0. 95 mm) using carotid arterial duplex.

Ethical and patients' approval:

Ethical approval was obtained from the Medical Research Ethics Committee, Ain Shams University, Faculty of Medicine. Informed consents were taken from all patients.

Exclusions criteria:

History of any renal disease, liver disease, recent myocardial infarction, recent cerebrovascular stroke or recent infection, patients who have chronic pulmonary disease such as chronic obstructive pulmonary disease, bronchial asthma, interstitial pulmonary fibrosis or sarcoidosis and history of drugs that alter normal bacterial flora as antibiotics bile acid sequestrates and inflammatory or autoimmune diseases.

Methods:

4 ml of venous blood was collected by venipuncture in the morning after 8 hrs fasting. 2 ml of the sample were taken on EDTA containing tube for measurement of HbA1c stored at 4 ºC to be examined within one week. The other 2 ml were taken in fluoride containing tube for measurement of fasting blood glucose. 2 ml of blood were collected after intake of 75 gm for two hours post-prandial blood glucose level. 2 ml were collected on another day after 12 hrs fasting for lipid profile. The remainder of samples were separated by centrifugation to measure CRP, liver function tests and creatinine. They were frozen at -20 °C until assayed.

Stool sample was collected in a clean, dry and sterile container then stored in a stool tube and frozen at 4 ºC to be examined within week.

Statistical analysis:

The collected data were coded, processed and analyzed using the SPSS (Statistical Package for Social Sciences) version 22 for Windows® (IBM SPSS Inc, Chicago, IL, USA). Data were tested for normal distribution using the Shapiro Walk test. Qualitative data were represented as frequencies and relative percentages. Chi square test ($\chi 2$) to calculate difference between two or more groups of qualitative variables. Quantitative data were expressed as mean ± SD (Standard deviation). Independent samples t-test was used to compare between two independent groups of normally distributed variables (parametric data). P value < 0.05 was considered significant.

RESULTS

Table (1): Correlation between PCR cut-off threshold and the other the other studied parameters in all patients

Correlations		
	PCR cut-off threshold (**PCRCT)**	
	r	**P-value**
Age (yrs)	-0.035	0.818
Weight (Kg)	-0.162	0.282
Height (cm)	-0.053	0.725
BMI	-0.118	0.435
Systolic (mmHg)	-0.040	0.794
Diastolic (mmHg)	0.025	0.868
HbA1c (%)	0.328	0.026*
Cholesterol (mg/dl)	0.111	0.463
TG (mg/dl)	0.097	0.522
LDL (mg/dl)	0.103	0.498
HDL(mg/dl)	-0.035	0.815
FBS (mg/dl)	-0.035	0.816
@2hrsPP (mg/dl)	0.364	0.013*
F. insulin (microIU/L)	-0.039	0.796
HOMAIR	-0.086	0.571
IMT(mm)	0.318	0.031*
HbAlb\creat ratio	0.023	0.880

Table (1) showed that there was a statistically significant positive correlation between PCR cut-off threshold and glycated hemoglobin, 2 hr pp and intimal media thickness.

Table (2): Relation between PCR cut-off threshold and the other studied parameters in all patients

		PCRCT				**T-Test or ANOVA**	
		N	**Mean**	**±**	**SD**	**T or F**	**P-value**
Sex	**Male**	22	26.073	±	6.549	-1.438	0.158
	Female	24	28.728	±	5.978		
Smoking	**Non smoker**	24	28.078	±	6.772	0.689	0.494
	Smoker	22	26.782	±	5.895		
Hypertension	**Non HTN**	24	26.972	±	7.641	-0.540	0.592
	HTN	22	27.989	±	4.623		
Antidiabetics	**Not on medications**	3	32.010	±	2.504	0.845	0.437
	On insulin	19	27.365	±	5.918		
	On oral drugs	24	26.963	±	6.892		
Fundus	**Normal**	21	27.485	±	6.338	0.029	0.971
	NPDR	19	27.261	±	6.322		
	PDR	6	27.988	±	7.461		
PCR	**Negative PCR**	0	0.000	±	0.000	-	-
	Positive PCR	46	27.458	±	6.331		
Peripheral neuropathy	**Negative**	22	27.774	±	6.754	0.320	0.750
	Positive	24	27.169	±	6.049		

Table (2) showed that there was no statistically significant correlation found between PCR cut-off threshold and other studied parameters in all patients.

Table (3): Correlation between TNF alpha and the other studied parameters in all patients

Correlations		
	TNF alpha	
	r	P-value
Age (yrs)	-0.117	0.359
Weight (kg)	0.053	0.676
Height (cm)	0.126	0.319
BMI	-0.065	0.611
Systolic (mmHg)	0.087	0.495
Diastolic (mmHg)	0.019	0.880
HbA1c (%)	0.303	0.015*
Cholesterol (mg/dl)	-0.036	0.778
TG (mg/dl)	0.098	0.440
LDL (mg/dl)	0.282	0.024*
HDL (mg/dl)	-0.045	0.725
FBS (mg/dl)	0.058	0.649
@2hrsPP (mg /dl)	0.199	0.116
F. insulin (microIU/L)	0.110	0.387
HOMAIR	0.075	0.557
IMT(mm)	0.266	0.033*
HbAlb\creat ratio	-0.040	0.752

Table (3) showed that there was a statistically significant positive correlation between TNF alpha level and glycated hemoglobin, LDL cholesterol and intimal media thickness (IMT).

Table (4): Relation between TNF alpha level and the other studied parameters in all patients

		TNF alpha				T-Test or ANOVA	
		N	Mean	±	SD	T or F	P-value
Sex	**Male**	33	30.636	±	11.680	-0.250	0.804
	Female	31	31.323	±	10.180		
Smoking	**Non smoker**	36	31.667	±	11.329	0.578	0.565
	Smoker	28	30.071	±	10.452		
Hypertension	**Non HTN**	34	29.853	±	12.354	-0.870	0.387
	HTN	30	32.233	±	9.012		
Antidiabetics	**Not on medications**	4	32.500	±	6.608	0.097	0.908
	On insulin	23	30.261	±	9.569		
	On oral drugs	37	31.243	±	12.148		
Fundus	**Normal**	29	29.276	±	12.047	0.773	0.466
	NPDR	28	32.857	±	10.029		
	PDR	7	30.429	±	9.289		
PCR	**Negative PCR**	18	40.444	±	12.650	5.161	<0.001*
	Positive PCR	46	27.261	±	7.473		
Peripheral neuropathy	**Negative**	35	32.257	±	11.708	1.040	0.303
	Positive	29	29.414	±	9.807		

Table (4) showed that there was highly statistically significant positive correlation found between TNF alpha level and PCR cut-off threshold.

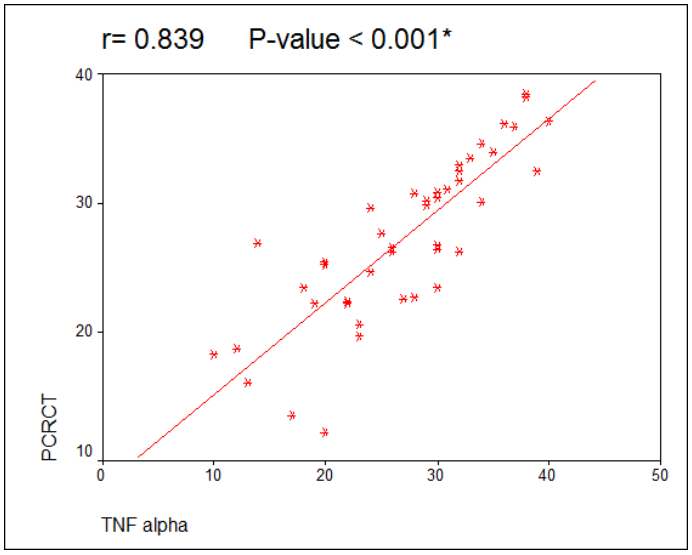

Figure (1): Correlation between PCR cut-off threshold and TNF alpha level in all cases

There was positive correlation between PCR cut-off threshold and TNF alpha level (Fig.1).

DISCUSSION

Probiotics contribute to the maintenance of a healthy host's digestive system by regulating intestinal growth and plant balance, and some have been shown to enhance the host's immune responses by regulating cytokine and chemokine production. The *L. acidophilus* strain is a well-characterized probiotic bacterium, which has been reported to improve the productive performance of animals and enhance immune responses [(12)].

Several cytokines are important in affecting bowel function. For example, IL-8 has been associated with changes induced by the pathogen to narrow connections, and TNF-α has a remarkable functional duplication that is strongly involved in both tissue regeneration and tissue destruction. Several in vitro studies demonstrated the ability of *L. acidophilus* to modulate the production of inflammatory-stimulating cytokines, including IL-8, TNF-α, and IL-6 in intestinal epithelial cells [(13)]. Here, we observed a significant decrease in the pro-inflammatory TNF alpha cytokines by *L. acidophilus*.

A case control study that was held to evaluate level of TNF alpha in diabetic patients with lactobacillus acidophilus. It was conducted on 100 type diabetic patients diagnosed according to American diabetes association diagnostic criteria. They were divided into 2 groups, group 1 that included 50 type 2 diabetic patients with atherosclerosis and group 2, which included 50 type 2 diabetic patients without atherosclerosis.

This study showed that there was a statistically significant positive correlation between PCR cut-off threshold and glycated hemoglobin, 2hr pp and intimal media thickness. There was also a statistically significant positive correlation between TNF alpha level and glycated hemoglobin, LDL cholesterol and intimal media thickness (IMT) as well as a highly statistically significant positive relation found between TNF alpha level and PCR cut-off threshold. This is in agreement with **Hsieh *et al.*** [(14)] who performed a randomized, double-blinded, placebo-controlled trial with a total of 68 T2DM patients to examine the beneficial effects of oral consumption of Lactobacillus strains to investigate the associated changes in intestinal flora using a quantitative PCR method to analyze 16 S rRNA in fecal specimens. Significant reductions in HbA1c and serum cholesterol were observed in participants in the live ADR-1 consumption group (n = 22) after 3 months of intake when compared to those in the placebo group (n = 22). Nevertheless, there was no obvious change in serum inflammatory cytokines or antioxidant proteins in participants after intake of lactobacillus strains, except for a reduction in IL-1β in the ADR-3 consumption group after 6 months of intake, which contradicts results of our study. This discrepancy can be explained by the difference in the Lactobacillus strains used in both studies, as we used in our study Lactobacillus acidophilus, which was already present in a patient infected with other strains. However, **Hsieh *et al.*** [(14)] depended in their study on oral consumption of specific strain, which is lactobacillus reuteri strains ADR-1 and ADR-3 for only 3 months, which may be not enough period to reduce other cytokines level.

Another case control study was conducted by **Halawa *et al.*** [(15)] on 30 diabetic patients and 10 control individuals revealed that stool Lactobacillus acidophilus PCR count was lower among type 2 diabetic patients, which may show relationship of lactobacillus with type 2 diabetes mellitus. However, further studies are needed to determine correlation or causation of this relationship, which agrees with our study. Similarly, a cohort study done by **Ivey *et al.*** [(16)] of elderly women, reported that yogurt intake was beneficially associated with CCA-IMT, which suggests that increased consumption of yogurt (rich in probiotics including lactobacillus) may prevent thickening of the common

carotid artery intima-media which may play a role in stroke and atherosclerosis prevention.

In contrast to our study, **Rangavajhyala *et al.* (17)** suggested that Lactobacillus acidophilus strain DDS-1 (LA1) has a suppressive effect on chemically-induced tumors in experimental animals by inducing the production of higher levels of IL-1 alpha and TNF-alpha. This result, which disagrees with our results, may be explained by that they utilized specific strain of lactobacillus acidophilus (strain DDS-1 (LA1)) but we assessed TNF alpha level in presence of all strains.

Whereas similar to our study, a study done by **Lihua Chen *et al.* (18)** suggested that administration of *L. acidophilus* ATCC 4356 can attenuate the development of atherosclerotic lesions in $ApoE^{-/-}$ mice. This occurred through reducing oxidative stress and inflammatory response by decreasing atherosclerotic lesion size in en face aorta and decreasing levels of serum malondialdehyde (MDA), oxidized low density lipoprotein (oxLDL) and tumor necrosis factor-alpha (TNF-α). Also, another study done by **Singh *et al.* (19)** on patients with chronic fatigue syndrome with high levels of TNF alpha showed that consumption of lactobacillus acidophilus produced significant decrease ($P < 0.05$) in TNF-α as compared with CFS group.

CONCLUSION

Lactobacillus acidophilus has been found to delay the onset of glucose intolerance, hyperglycemia, dyslipidemia and oxidative stress in patients with type 2 diabetes mellitus.

RECOMMENDATIONS

Further studies should be done to assess the impact of different strains of lactobacillus acidophilus on diabetes and oxidative stress, caused by TNF alpha & other types of cytokines.

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