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Interferon-γ, Interleukin 1-β and Tumor Necrosis Factor-α levels and Their Association with Lipid and Glycaemic Profiles in Diabetic Mice

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Authors' contributions

This work is an outcome of collaboration among all the authors. Author AJM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MAA and KHB managed the analyses of the study and revised the literature search. All authors read and approved the final manuscript.

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ABSTRACT

The cytokines IFN-γ, interleukin IL-1β, and TNF-α each up regulate the expression of major histocompatibility complex (MHC) and are therefore considered as inflammatory markers. The present study aimed to measure the serum levels of IFN-γ, interleukin IL-1β, and TNF-α in mice after induction of diabetes with streptozotocin and to correlate their levels to lipid and glycaemic profiles. The study included 40 Swiss Albino mice (20 males and 20 females), half of each male and female groups were made diabetic by intraperitoneal injection of streptozotocin. After 48 hours, the serum levels of INF-γ, IL1-β as well as TNF-α were measured with ELISA and their levels were correlated to lipid and glycaemic profiles. The levels of the cytokines, IFN-γ, IL1-β and TNF-α were measured and correlated to lipid profile, blood glucose and insulin. The serum levels of the three studied cytokines, IFN-γ, IL 1-β and TNF-α were statistically significantly higher among diabetic mice compared to the control group. Diabetic male mice (M-STZ mice) group showed significantly higher lipid profile compared to the control group (M-control). Cholesterol level was significantly higher among female control (F-control) compared to the M-control group. Cholesterol level was

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significantly higher among diabetic female mice (F-STZ mice) compared to the F-Control group. Regarding IFN-γ, IL 1-β and TNF-α levels, there were significant linear correlations with the glycemic profile (Glucose, insulin) reflected as positive correlation with blood glucose level and negative correlation with insulin level.

Keywords: Interferon; interleukin; necrosis; diabetic mice.

1. INTRODUCTION

The defining feature of diabetes mellitus is the presence of hyperglycaemia [1] which results from the body's insulin insufficiency or resistance. It is associated with risk of microvascular damage. The related elevated morbidity is attributed to particular complications associated with diabetes, an increased risk of macrovascular disease and a decreased quality of life [2]. The two known types of diabetes are type 1(TIDM), which is the result of deficiency of insulin as a consequence of pancreatic beta cell destruction; and type 2 (T2DM), which is largely due to insulin resistance. Obesity is an important risk factor for type 2 diabetes and it is on the rise [3].

T1DM is associated with an imbalance in pro-inflammatory and anti-inflammatory cytokines which is more evident as the disease progresses [4-5]. Increased serum levels of pro-inflammatory cytokines, such as IL-1β, tumor necrosis factor α (TNF-α), IL-6, and IL-7 can encourage the therapeutic approach of blocking these signaling pathways. IL-1 is an inflammatory cytokine involved in the differentiation of T helper 1 (T_H1) and T_H17 T cells that has profound pro-apoptotic effects on β cells [6-7]. In non-obese diabetic mice, blockage of the IL-1 receptor slowed the progression of T1DM, but could not prevent occurrence of the disease [8]. In T1DM patients, increased expression of IL-1β by monocyte is seen at time of diagnosis but returns to a normal level within 1 month [9]. This suggests anti-IL1 treatment may only be effective at early time in disease progression. TNF-α is a cytokine involved in controlling the maturation of dendritic cells and has been linked with the activation of islet-specific T cells in pancreatic lymph nodes [10]. Patients with T1DM have significant increased levels of plasma TNF-α compared to healthy subjects [11]. In experimental work on non-obese diabetic mice, the systemic injection of TNF-α produced an earlier onset of disease by 5 weeks and a higher incidence rate in comparison to control mice, [12] pointing to the therapeutic value in modulating this target.

Dyslipidaemia occurs commonly with T2DM than T1DM, and is associated with high levels of triglycerides and low-density lipoprotein (LDL) cholesterol particles, in combination with low levels of high-density lipoprotein (HDL) cholesterol [13]. Because of its atherogenic properties, diabetic dyslipidaemia is particularly important in the development of macrovascular complications [14]. High levels of plasma triglycerides are also associated with progression of diabetic neuropathy [15]. During the last decade, Diabetes mellitus has become one of the most common endocrine disorders. It has drawn more and more attention because of its serious and long-term complications on various body organs causing microvascular disease in the eyes, kidneys, and peripheral nervous system and macrovascular disease in major vessels including the coronary arteries, the carotids, and the peripheral arteries [16].

1.1 Aim of the work

This study aimed to investigate serum levels of interferon-gamma (IFN-γ), Interleukin 1-β (IL-1β) and tumour necrosis factor-alpha (TNF-α) as inflammatory markers in mice, after injection of streptozotocin to induce diabetes in mice, aiming to prove the possible correlation between their levels to lipids and glycaemic profiles.

2. METHODS

A total number of 40 Swiss Albino mice, (20 males and 20 females) in the weight and age range of 30-35 g and 4-6 months respectively, were included in the study and housed in large cages with five mice per cage. The animals were kept under standard laboratory conditions (temperature 25°±2°C with dark/light cycle 12/12 h). Commercial pelleted food and tap water were given for each cage per day throughout the experimental period. The mice were divided into four groups each consisted of 10 mice: male control (M-control), female (F-control) control, male diabetic (M-STZ), female diabetic (F-STZ).

We selected mouse groups of different gender to see if gender has any effect on the level of the parameters included in the study. The study was

conducted in the duration from March 2018 to October 2019. Streptozotocin (Sigma- Aldrich ,Missouri, USA) was used to induce diabetes in mice through the intraperitoneal injection at dose of 60 mg/Kg body weight as reported by Han et al. 2017 [16]. STZ was prepared freshly by dissolving it in citrate buffer (50 mM, pH 4.5). The body weight of each mouse was taken using a digital scale before the beginning of the experiment.

At the end of the experiment, all mice from each group were set aside fasting for 12 hours and the blood was collected by a retro orbital plexus method in plane tube. Sera obtained after centrifugation at 7000 rpm were stored at -80°C until used for the estimation of serum biochemical assays. Serum glucose, triglycerides, cholesterol were determined calorimetrically. Fasting, serum insulin, IL-1 beta, IFN-gamma and TNF-alpha were determined by using ELISA (RayBiotech company, Norcross, United States). Diabetes was confirmed by the level of fasting blood glucose.

2.1 Statistical Analysis

All data were expressed as means ± standard deviation of the mean (SD). Statistical analysis was done using statistical package for social sciences (SPSS) computer software (version 25), IBM software, USA.

One-way analysis of variance (ANOVA) test was used to elucidate significance among group means, followed by Tukey's post-hoc test to compare mean values pair-wise. Differences were considered significant at $p < 0.05$. Total p value for ANOVA was calculated and written, while p-values of post hoc analysis were expressed as small letters (a,b,c,d).

Spearman's correlation analysis was done to evaluate linear relationship between studied cytokines and other parameters in each group separately and in all groups totally. Correlation graphs were drawn only for significant correlation which is considered significant at $P < 0.05$

Weak when r = >0 – 0.35, Moderate when r = >0.35 – 0.65; and Strong when r = > 0.65.

3. RESULTS

In the present study, diabetes was induced in mice with intra-peritoneal injection of streptozotocin and the level of cytokines (IFN-γ, IL- 1β, and TNF-α) was determined using ELISA. The diabetes induced group of mice consisted of males and females; they were compared to healthy mice. Blood samples were collected from cases after 48 hours following streptozotocin injection to induce diabetes, which was confirmed by a level of fasting blood glucose of more than 200 mg/dl and also from controls. The body weight of all mice ranged from (30-35 gm) with no statistically significant difference between the four groups (Table 1). The serum levels of the cytokines (IFN-γ, IL 1-β and was TNF-α) were statistically significantly higher among diabetic mice, male and female, compared to their control groups (Table 2).

In lipid profile assessment, M-STZ mice group showed significantly higher lipid profile as compared with M-control mice group. Cholesterol level was significantly higher among F-control as compared with M-control group. Cholesterol level was significantly higher among F-STZ mice as compared with F-Control group. LDL was significantly highest among M-STZ compared to other groups; however no statistically significant differences were detected between the other three groups. HDL was significantly highest among M-STZ compared to (M- control and F-Control) groups, however no statistically significant differences were detected between the other three groups. Triglycerides (TG) was significantly higher among M-STZ compared to M-Control group, and no statistically significant difference was detected between the remaining groups; the overall p-value was non-significant (Table 3).

Regarding glycaemic profile, fasting blood glucose level was significantly higher among diabetic mice of both groups compared to the control groups. Insulin blood level was significantly lower among diabetic mice compared to the control groups (Table 4). Regarding correlation of cytokines level with weight in diabetic and control group, there was non-significant linear correlation in all studied cytokines blood level with mice weight. However; in M-Control mice group there was a strong significant negative correlation between IFN-γ level and mouse weight (Fig. 1).

In the correlation between IFN-γ and glycaemic profile, in diabetic and control group, there was a significant linear correlation (high strong positive correlation with blood glucose level and negative correlation with insulin level) (Table 5). Correlating IL 1-β with glycaemic profile in

diabetic and control group, showed significant linear correlation (high strong positive correlation with blood glucose level and negative correlation with insulin level) (Table 6).

Table 1. Initial weight distribution among the diabetic and control group (N=40):

	Weight (gm.) Mean ±SD	p-value
M-Control	32.00 ±1.05	**0.577**
F-Control	32.82 ±1.40	
M-STZ	32.16 ±1.44	
F-STZ	32.44 ±1.59	

Each value represents a mean of 10 values ± SD.
Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Tukey's post hoc analysis

Table 2. The profile of IFN-γ, IL- 1β, and TNF-α in diabetic and control group (N= 40)

	IFN-γ (pg/ml) Mean ±SD	IL 1-β (pg/ml) Mean ±SD	TNF-α (pg/ml) Mean ±SD
M-Control	259.37 ±11.48c,d	30.51 ±4.12 c,d	10.80 ±8.24 c,d
F-Control	261.02 ±13.52 c,d	31.08 ±4.98 c,d	11.90 ±8.38 c,d
M-STZ	361.29 ±56.52 a,b	78.47 ±7.43 a,b	22.00 ±5.12 a,b
F-STZ	352.51 ±58.76 a,b	79.88 ±7.37 a,b	22.20 ±4.07 a,b
p-value	**<0.001***	**<0.001***	**<0.001***

**p-value ≤0.05 is considered statistically significant*
Each value represents a mean of 10 values ± SD.
Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Tukey's post hoc analysis.
a Significantly different from M-Control at p≤ 0.05, b Significantly different from F-Control at p≤ 0.05, c Significantly different from M-STZ at p≤ 0.05, d Significantly different from F-STZ at p≤ 0.05

Table 3. Lipid profile in diabetic and control group (N= 40):

	Ch (mmol/L) Mean ±SD	LDL (mmol/L) Mean ±SD	HDL (mmol/L) Mean ±SD	TG (mmol/L) Mean ±SD
M-Control	2.73 ±0.23 b,c,d	1.18 ±0.16 c	1.07 ±0.10 c	1.03 ±0.08 c
F-Control	3.85 ±1.08 a	1.62 ±0.50 c	1.38 ±0.25	1.14 ±0.29
M-STZ	4.48 ±0.42 a,d	2.19 ±1.00 a,b,d	1.88 ±1.15 a,b	1.36 ±0.58 a
F-STZ	3.72 ±1.01 a,b	1.50 ±0.41 c	1.5 ±0.41	1.21 ±0.28
p-value	**<0.001***	**0.007***	**0.044***	**0.235**

**p-value ≤0.05 is considered statistically significant; Each value represents a mean of 10 values ± SD. Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Tukey's post hoc analysis. a Significantly different from M-Control at p≤ 0.05, b Significantly different from F-Control at p≤ 0.05, c Significantly different from M-STZ at p≤ 0.05, d Significantly different from F-STZ at p≤ 0.05*

Table 4. Glycaemic profile (Glucose, insulin) in diabetic and control mice; (N= 40):

	Glucose (mg/dl.) Mean ±SD	Insulin (µu/L) Mean ±SD
M-Control	135.00 ±3.68c, d	14.23 ±1. 43c.d
F-Control	132.30 ±3.05c, d	15.34 ±1. 69c.d
M-STZ	259.50 ±63.70a, b	9.66 ±1.55a, b
F-STZ	301.20 ±77.15a, b	10.07 ±2.99a, b
p-value	**<0.001***	**<0.001***

**p-value ≤0.05 is considered statistically significant; Each value represents a mean of 10 values ± SD. Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Tukey's post hoc analysis. a Significantly different from M-Control at p≤ 0.05, b Significantly different from F-Control at p≤ 0.05, c Significantly different from M-STZ at p≤ 0.05, d Significantly different from F-STZ at p≤ 0.05*

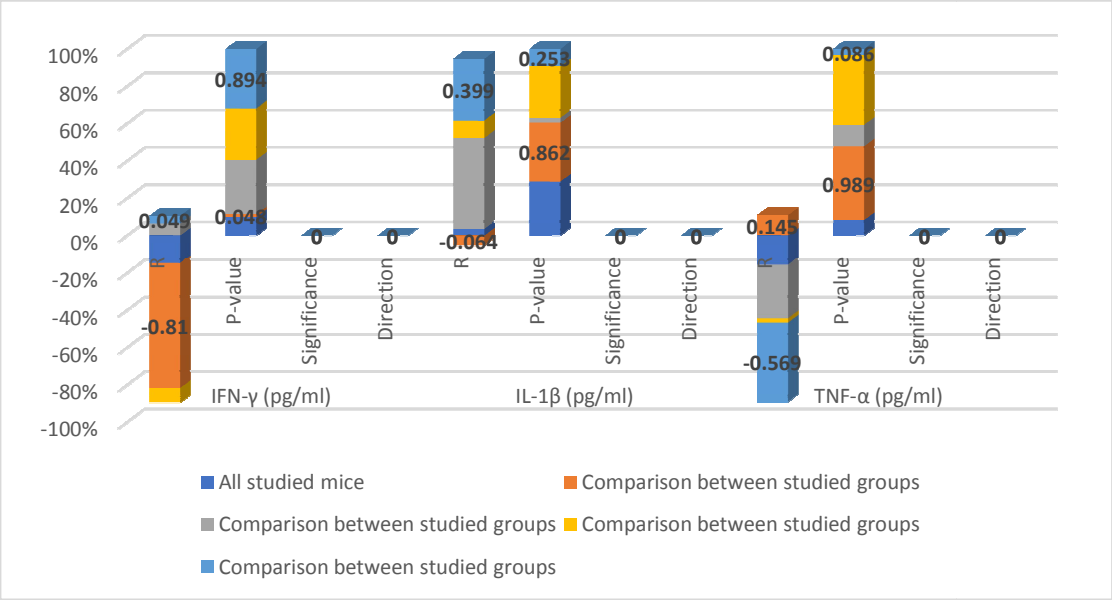

Fig. 1. Correlation of studied cytokines level with Weight in diabetic and control group

Table 5. Correlation of IFN-γ level with glycaemic profile in diabetic and control group:

			Comparison between Studied Groups			
		All studied mice N= 40	**M-Control N= 10**	**F-Control N= 10**	**M-STZ N= 10**	**F-STZ N= 10**
Glucose (mg/dl.)	**R**	0.671	0.047	0.620	0.274	0.002
	P-value	0.001	0.897	0.056	0.444	0.995
	Significance	HS	NS	NS	NS	NS
	Direction	**+**	+	+	+	+
Insulin (µu/L)	**R**	-0.656	0.518	-0.294	0.604	-0.196
	P-value	0.001	0.125	0.410	0.064	0.587
	Significance	HS	NS	NS	NS	NS
	Direction	**-**	+	-	+	-

*Statistical analysis was carried out using Spearman's correlation analysis. r= Spearman's rank correlation coefficient, *p-value ≤0.05 is considered significant. HS; highly significant; NS; not significant*

Correlation between TNF-α and glycaemic profile (glucose, insulin) in diabetic and control groups revealed a significant linear correlation (had high strong positive correlation with blood glucose level and negative correlation with insulin level) (Table 7). The correlation of IFN-γ level with lipid profile in diabetic and control groups showed a significant linear correlation with lipid profile (cholesterol, LDL, HDL), however; no significant correlation with TG level. IFN-γ level had slight positive correlation with cholesterol level, moderate correlation with LDL and HDL levels. In the M-control group, there was a significant linear correlation with cholesterol level, while in the F-control group, there was a significant linear correlation with TG level (Table 8). When correlating IL 1-β level with lipid profile in diabetic and control group, we detected a significant linear correlation with LDL in diabetic and control mice, however; no significant correlation with cholesterol, HDL and TG levels. In the M-control group, there was a significant linear negative correlation with TG level. In the F-control group; there was a significant linear negative correlation with LDL level as well as a significant linear negative correlation with HDL level and a significant linear negative correlation with cholesterol level (Table 9). In the correlation of TNF-α level with lipid profile in diabetic and control groups, there was a significant linear correlation with HDL in diabetic and control mice, however; no significant correlation with cholesterol, LDL and TG levels. In the F-STZ group; there was a significant linear negative correlation with TG level (Table 10).

Table 6. Correlation of IL 1-β level with Glycaemic profile (Glucose, insulin):

			Comparison between studied groups			
		All studied mice N=40	**M-Control N =10**	**F-Control N =10**	**M-STZ N =10**	**F-STZ N =10**
Glucose (mg/dl)	R	0.723	0.114	0.001	-0.382	-0.119
	P-value	0.001	0.752	0.997	0.276	0.743
	Significance	HS	NS	NS	NS	NS
	Direction	+	+	+	-	-
Insulin (µu/l)	R	-0.755	-0.365	-0.433	0.341	-0.017
	P-value	0.001	0.299	0.211	0.335	0.963
	Significance	HS	NS	NS	NS	NS
	Direction	-	-	-	+	-

*Statistical analysis was carried out using Spearman's correlation analysis. r= Spearman's rank correlation coefficient, *p-value ≤0.05 is considered significant. HS; highly significant; NS; not significant*

Table 7. Correlation of TNF-α level with glycaemic profile in diabetic and control group

			Comparison between studied groups			
		All studied mice N=40	**M-Control N =10**	**F-Control N =10**	**M-STZ N =10**	**F-STZ N =10**
Glucose (mg/dl)	R	0.625	0.348	-0.090	0.390	0.198
	P-value	<0.001	0.325	0.805	0.265	0.583
	Significance	HS	NS	NS	NS	NS
	Direction	+	+	-	+	+
Insulin (µu/l)	R	-0.506	0.124	-0.136	0.457	0.069
	P-value	<0.001	0.733	0.708	0.185	0.489
	Significance	HS	NS	NS	NS	NS
	Direction	-	+	-	+	+

*Statistical analysis was carried out using Spearman's correlation analysis. r= Spearman's rank correlation coefficient, *p-value ≤0.05 is considered significant. HS; highly significant; NS; not significant*

Table 8. Correlation of IFN-γ level with Lipid profile in 4 groups of mice)

			Comparison between Studied Groups			
		All studied mice N= 40	**M-Control N= 10**	**F-Control N= 10**	**M-STZ N= 10**	**F-STZ N= 10**
Ch (mmol/L)	R	0.389	0.691	0.157	0.064	0.206
	P-value	**0.013**	**0.027**	0.666	0.861	0.567
	Significance	S	NS	NS	NS	NS
	Direction	+	+	+	+	+
LDL (mmol/L)	R	0.422	0.367	0.027	-0.123	0.568
	P-value	**0.007**	0.296	0.940	0.734	0.087
	Significance	S	NS	NS	NS	NS
	Direction	+	+	+	-	+
HDL (mmol/L)	R	0.401	0.312	0.172	-0.096	0.336
	P-value	**0.010**	0.379	0.634	0.793	0.343
	Significance	S	NS	NS	NS	NS
	Direction	+	+	+	-	+
TG (mmol/L)	R	0.154	-0.257	0.736	-0.084	0.218
	P-value	0.344	0.474	**0.015**	0.818	0.546
	Significance	NS	NS	S	NS	NS
	Direction	+	-	+	-	+

*Statistical analysis was carried out using Spearman's correlation analysis. r= Spearman's rank correlation coefficient, *p-value ≤0.05 is considered significant. S; significant; NS; not significant*

Table 9. Correlation of IL 1-β level with Lipid profile in diabetic and control group

		Comparison between Studied Groups				
		All studied mice N= 40	**M-Control N= 10**	**F-Control N= 10**	**M-STZ N= 10**	**F-STZ N= 10**
Ch (mmol/L)	r	0.229	-0.204	-0.757	0.010	0.149
	P-value	0.156	0.571	**0.011**	0.977	0.682
	Significance	NS	NS	S	NS	NS
	Direction	+	-	-	+	+
LDL (mmol/L)	r	0.330	-0.288	-0.656	-0.062	0.304
	P-value	**0.037**	0.420	**0.039**	0.864	0.393
	Significance	S	NS	S	NS	NS
	Direction	+	-	-	-	+
HDL (mmol/L)	r	0.272	0.035	-0.668	-0.310	0.172
	P-value	0.090	0.924	**0.035**	0.383	0.634
	Significance	NS	NS	S	NS	NS
	Direction	+	+	-	-	+
TG (mmol/L)	r	0.095	-0.642	-0.237	0.122	0.287
	P-value	0.561	**0.045**	0.509	0.738	0.422
	Significance	NS	S	NS	NS	NS
	Direction	+	-	-	+	+

*Statistical analysis was carried out using Spearman's correlation analysis. r= Spearman's rank correlation coefficient, *p-value ≤0.05 is considered significant. S; significant; NS; not significant*

Table 10. Correlation of TNF-α level with Lipid profile in diabetic and control group

		Comparison between Studied Groups				
		All studied mice N= 40	**M-Control N= 10**	**F-Control N= 10**	**M-STZ N= 10**	**F-STZ N= 10**
Ch (mmol/L)	R	0.220	-0.253	0.266	0.077	-0.488
	P-value	0.173	0.481	0.457	0.832	0.153
	Significance	NS	NS	NS	NS	NS
	Direction	+	-	+	+	-
LDL (mmol/L)	R	0.237	0.141	0.075	-0.316	0.354
	P-value	0.141	0.697	0.837	0.373	0.316
	Significance	NS	NS	NS	NS	NS
	Direction	+	+	+	-	+
HDL (mmol/L)	R	0.312	0.229	0.359	-0.016	-0.197
	P-value	**0.050**	0.525	0.308	0.965	0.586
	Significance	S	NS	NS	NS	NS
	Direction	+	+	+	-	-
TG (mmol/L)	R	0.004	0.349	0.322	-0.079	-0.774
	P-value	0.978	0.323	0.364	0.828	**0.009**
	Significance	NS	NS	NS	NS	S
	Direction	+	+	+	-	-

*Statistical analysis was carried out using Spearman's correlation analysis. r= Spearman's rank correlation coefficient, *p-value ≤0.05 is considered significant. S; significant; NS; not significant*

4. DISCUSSION

Type 1 diabetes is a metabolic disorder resulting from an unexplained auto-immune state in which the immune system is activated to destroy the pancreatic beta cells. There is no cure nor preventive measures [17,18] though attempts are immune modulation are being made. It is one of the most common chronic diseases of childhood [19,20].

Fasting blood glucose testing is commonly used for diagnosis of diabetes mellitus [21-23]. In healthcare practices, mice are commonly used mammalian models in medical research for better understanding of the disease processes that carry risks to humans, but there exist key differences between mice and humans, which could affect the generatability of results to human disease [24,25] and streptozotocin induced diabetes is obviously different from the

autoimmune based diabetes of human type 1DM though both destroy pancreatic beta cells and result in type 1 diabetes. That is why we can not get a solid evidence from animal experimental work and apply it to human disease process, but it can be a good guide for more research in humans.

Our study aimed to determine the serum level of IFN-γ, IL-1β and TNF-α cytokines following induction of diabetes with streptozotocin in mice (10 males: M-STZ and 10 females: F-STZ compared to 10 males and 10 females controls) and to relate these levels to lipid and glycaemic profiles.

Our main finding is the significantly higher level of serum IFN-γ, IL- 1β, and TNF-α among diabetic mice. Accumulation of activated innate immune cells in metabolic tissues results in the release of inflammatory mediators that promote systemic insulin resistance (IR) and β-cell damage, including inflammatory cytokines [26]. Previous studies have shown that particular pro and/or anti-inflammatory cytokines can interfere with the absorption of insulin-responsive glucose and promote insulin resistance [27].

In our study we confirmed the development of fasting hyperglycaemia (of more than 200mg/dl) and hence diabetes by the statistically significant high fasting blood glucose level in the diabetic group of mice compared to the controls. Moreover, insulin blood level was significantly lower among diabetic mice. Our results were in line with that mentioned by Chengxin Sun et al., 2016 [28] where they stated that a daytime fast would be more appropriate to testing of blood glucose. The same results were confirmed by Hitoshi Ando et al., 2015 [29], as they mentioned that fasting blood glucose (FBG) and hepatic glucose production are regulated according to a circadian rhythm. In addition, our findings are consistent to the results obtained by Ahmad A. Al Ghamdi, et al., 2015, who reported that diabetes in mice was associated with hyperglycaemia and significant decreases in the insulin level and the lymphocyte count [30].

Regarding lipid profile, (cholesterol, LDL, HDL & TG levels), diabetic male mice showed significantly higher lipid profile as compared with M-control group. An unexplained finding is the significantly higher cholesterol level among F-control as compared with M-control group, this might be due to the effect of sex hormone or another unexplained factor, but unfortunately, we did not consider the oestrous cycle in the female group of mice, to be able to relate lipid profile abnormality to hormone changes. LDL was significantly higher among M-STZ group as compared with other studied mice groups.

HDL was significantly higher in diabetic mice, and TG was significantly higher among M-STZ group as compared with M-Control group.

Glycemic profile (glucose, insulin) had significant linear correlation in IFN-γ, IL 1-β, and TNF-α level. IFN-γ level had high strong positive correlation with blood glucose level and negative correlation with insulin level. Gamal Allam et al., 2014 [31] agreed with the present study in which children with Type I diabetes 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.

Correlation of IL 1-β level and TNF-α with Lipid profile in diabetic and control groups of mice showed significant linear correlation of IL 1-β level with LDL in 4 groups of mice, however; no significant correlation with cholesterol, HDL and TG levels. In the M-control group; there was a significant linear negative correlation of IL 1-β level with TG level. In the F-control group; there was a significant linear negative correlation in IL 1-β level with LDL level as well as a significant linear negative correlation with HDL level and a significant linear negative correlation in IL 1-β level with cholesterol level. Furthermore for, TNF-α there was a significant linear correlation in TNF-α level with HDL in 4 groups of mice, however; no significant correlation with cholesterol, LDL and TG levels.

In the F-STZ group; there was a significant linear negative correlation of TNF-α level with TG level. The pattern of correlation of IL-1β was similarly reported by Kamil Jonas et al., 2019 [32] that Patients with increased TG/HDL-C ratio (>3) as compared to patients with TG/HDL-C ≤3 were characterized by higher levels of IL-1β, MCP-1, and IL-6. TG level was correlated with IL-1β, IL-6, TNF-α, and MCP-1. IL-1β was also inversely correlated with HDL-C and this is contradicting to our results for HDL, for which we did not demonstrate correlation with IL-I β.

5. CONCLUSION

As a conclusion our study showed that, the levels of IFN-γ, IL 1-β and TNF-α were significantly higher among diabetic mice as compared with the control group and the induced diabetes in mice with streptozotocin showed variable

correlations with cytokines levels and blood lipid and glycaemic profiles.

DISCLAIMER

We as authors declare that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

The animals were maintained in accordance with King Abdul-Aziz University's policy and the international ethical guidelines on the care and use of laboratory animals. The ethical approval was obtained from the research ethics committee in the Faculty of Medicine at King Abdul-Aziz University.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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