Enumeration of NK cells in Patients of Type-II Diabetes Mellitus

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ABSTRACT

BACKGROUND: Diabetes is a metabolic disorder characterized by chronic hyperglycemia. Patients of diabetes mellitus are managed by two basic drug regimens. Generally type-I diabetes is managed by insulin therapy, while type-II diabetes is treated by oral hypoglycemic agents. Natural Killer (NK) cells being an important member of innate immunity have protective and pathogenic roles. A study was designed to enumerate NK cells in diabetes mellitus patients being treated with insulin and oral hypoglycemic therapy. **MATERIAL & METHODS:** It was a cross sectional study that comprised of 80 patients of diabetes mellitus. They were categorized as group-I (on oral hypoglycemic) and group-II (on insulin); 40 patients in each group. For enumeration of NK cells, blood sample was analyzed by four color FACS caliber, using CD16/CD56 PE, and CD45 PerCP monoclonal antibodies. **RESULT:** Mean±SD of percentage of NK cells of patients on oral hypoglycemic agents was high (11.10±5.66) compared to patients on insulin therapy (9.91±5.08) but on comparison it was not statistically significant (p=0.327). Mean±SD of percentage of NK cells of male diabetic patients was high (12.01±5.84) compared to female diabetic patients (9.48±4.83) and on comparison it was statistically significant (p=0.039).**CONCLUSION:** No significant difference was observed in the percentage of NK cells of diabetes mellitus patients on insulin and oral hypoglycemic agents. However percentage of NK cells was significantly high in male patients as compared to female patients.

Key words: NK cells, NKT cells, T cells, Diabetes mellitus, oral hypoglycemic agents

الملخص

خلفية: داء السكري هو عبارة عن اضطراب في التمثيل الغذائي يتميز بارتفاع مزمن لمستوي السكر في الدم . يتم علاج مرضي داء السكر بطريقتين، الطريقة الاولي وهي لعلاج النوع الاول من داء السكري ويتم العلاج بالأنسولين، في حين يتم التعامل مع داء السكري من النوع الثاني عن طريق الفم . تعتبر الخلايا القاتلة الطبيعية (NK) مكون مهم في المناعة الفطرية و لديها أدوار وقائية وقد تؤدي الي بعض الأدوار المسببة للأمراض. تم تصميم هذه الدراسة لتعداد الخلايا القاتلة الطبيعية في مرضى داء السكري الذين يعالجون بالأنسولين أو العلاج عن طريق الفم. المنهج: كانت الدراسة دراسة مقطعية لعدد ٨٠ مريضا بداء السكري. تم تقسيمهم الي مجموعتين بواقع ٤٠ مريضا في كل مجموعة ، المجموعة الاولي (تعالج عن طريق الفم) والمجموعة الثانية (تعالج بالأنسولين). لتعداد الخلايا القاتلة الطبيعية، تم تحليل عينة من الدم بطريقة (FACS) وهي طريقة تصنيف الخلايا بتنشيطها بالفلوروسين ، وذلك باستخدام الأجسام المضادة CD٥٦/CD١٦ (PE)، وCD٤٥ PerCP) . **النتيجة:** المتوسط ± الانحراف المعياري من نسبة الخلايا القاتلة الطبيعية (NK) لمرضى داء السكري الذين يعالجون عن طريق الفم مرتفع (١١,١٠ ± ٥,٦٦) مقارنة مع المرضى الذين يعالجون بالأنسولين (٩,٩١ ± ٥,٠٨) ولكنه ليس ذو دلالة احصائية (p = ٠,٣٢٧). المتوسط ± الانحراف المعياري للخلايا القاتلة الطبيعية (NK) لمرضى داء السكري الذكور مرتفع (١٢,٠١ ± ٥,٨٤) مقارنة مع مرضى السكري الإناث (٩,٤٨ ± ٤,٨٣) و كان الارتفاع ذو دلالة إحصائية (p = ٠,٠٣٩). **الخلاصة:** لا يوجد فرق ذو دلالة احصائية في نسبة الخلايا القاتلة الطبيعية لمرضي السكر من النوعين ، ولكن يوجد فرق ذو دلالة احصائية في نسبة الخلايا بين المرضي الذكور والمرضي الاناث .

INTRODUCTION

Diabetes Diabetes is a metabolic disorder characterized by chronic hyperglycemia with disturbances of carbohydrates, fats and protein metabolism. On the basis of insulin requirement, diabetes can be differentiated into insulin dependent and non-insulin dependent [1]. Pakistan is categorized as high prevalence area with 6.9 million diabetes mellitus patients that can be increased to 11.5 million by 2025 [2]. Natural Killer (NK) cells being the first line of defense play a key role and express CD56+ and CD16+ surface markers [3]. In type-I diabetes mellitus (T1DM), there is an increased infiltration of lymphocytes, inflammatory cytokines and chemokines around pancreatic beta cells which may lead to their destruction. TNFα and IFNγ from

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NK and T cells are involved in the destruction of beta cells of pancrease, [4] therefore NK cells are mandatory in the progression and severity of T1DM [5]. T1DM is accelerated by an increased number of intra-pancreatic NK cells whereas depletion of NK cells arrest the progression of diabetes [6].

Patients on insulin therapy documented an increase of NK cells and their number was decreased in patients of low insulin [7] Patients of resent on-set of T1DM had low number of NK cells compared to patients of long-standing T1DM [8]. Another study documented decreased number of NK cells in T1DM patients compared to controls [9]. Further, patients on insulin treatment documented restoration of normal number of NK cells [10]. Moreover non-significant difference of percentages of NK cell among type 2 diabetes mellitus (T2DM) patients and healthy subjects had been reported [11] Oral hypoglycemic agents e.g. sulphonylureas blocks K channels and T cell proliferation whereas it stimulates insulin secretion [12]. Both diabetes and oral hypoglycemic decreases growth and proliferation of lymphocytes [10, 13, 14]. Therefore a study was designed to enumerate NK cells in diabetes patients on insulin and oral hypoglycemic therapy.

Material and Methods

It was a cross sectional study comprising of 80 diabetic subjects i.e. 40 on oral hypoglycemic agents (group-I) while 40 on insulin therapy (group-II). The study was approved by the Ethical Review Committee and Advanced Studies & Research Board of UHS and Services Hospital Lahore. After a written informed consent, patients were recruited from the Diabetes Management Center, Services Hospital Lahore. Immunophenotyping of NK cells was performed by using fluorescein isothiocyanate (FITC) tagged monoclonal antibody (MoA) against CD3, phycoerythrin (PE) tagged MoA against CD16/CD56 and peridinin-chlorophyll-protien (PerCP) tagged MoA against CD45. Lyse-wash sample preparation method using whole blood was used. Cells were analyzed by FACS Calibur 4-color analyzer (BD Biosciences, California USA). Two parameter-dot-plot of forward angle light scatter side scatter and SS CD45 was used and lymphocytes (CD45 brightest population with lowest side scatter) in SS CD45 dot plot were gated and data for CD16+/CD56+, CD3+ cells and CD16/+CD56+, CD3+ double positive cells was acquired. Data was analyzed using SPSS 20.0. Mean ± SD for quantitative variables while frequencies and percentages for qualitative variables were given. Kolmogorov-Smirnov and Shapiro-Wilk test for distribution of data, student t-test for normally distributed and Mann-Whitney test for not normally distributed data was used. A p-value of ≤ 0.05 was considered as statistically significant.

Results

The present study comprised of 80 diabetic patients that included 32 (40%) male and 48 (60%) females. Group-I (on oral hypoglycemic agents) included 14 (35%) male and 26 (65%) females, while group-II (on insulin) had 18 (45%) male and 22 (55%) females. Demographic data of the study subjects is summarized in Table 1. Mean ± SD of age (years), systolic and diastolic blood pressure (BP, mm/Hg) was high in male patients (35.50±12.40, 117.19±16.49 and 76.72±10.12) compared to females (35.47±11.18, 114.38±16.11 and 75.21±9.22) respectively. On comparison of these parameters, there was no significant difference between male and female (p=0.991, 0.453 and 0.492 respectively) (Table 2).

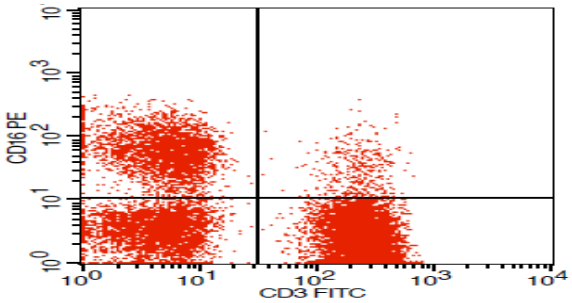

Figure 1: Two parameter histogram showing distribution of CD3 and CD16/56 (x-axis:CD3 FITC; y-axis:CD16/56 PE)

Table 1: Demographic data of the studied subjects

Variables	Group-I	Group-II
Male (n, %)	14, 35	18, 45
Female (n, %)	26, 65	22, 55
Age (years) Mean ±SD	41.10±6.50	29.06±12.26
Body mass index (lb/in2) Mean ±SD	27.35±4.08	21.16±3.80
Duration of diabetes (years) Mean ±SD	3.82±5.79	3.01±2.55
Duration of drug use (years) Mean± SD	2.82±2.12	2.86±2.52

Mean ± SD of blood sugar level (mg/dl), body mass index (BMI) (lb/in2), duration of disease (years) and duration of drug use (years) was high in female patients (241.98±87.38, 24.90± 4.62, 3.10±2.37 and 2.87±2.35) compared to male patients (222.56±98.41, 23.86±4.86, 2.81±2.25 and 2.78±2.22 respectively). On comparison, there was no significant difference between male and female (p= 0.358, 0.233, 0.591 and 0.870 respectively) (Table 2).

Table 2: Comparison of different variables between male and females

Variables	Gender	Mean± SD	p value
Age (years)	Male	35.50±12.40	0.991
	Female	35.47±11.18	
Body mass index (lb/in2)	Male	23.86±4.86	0.233
	Female	25.11±4.35	
Blood sugar level (mg/dl)	Male	222.56±98.41	0.358
	Female	241.98±87.384	
Duration of drug use (years)	Male	2.78±2.22	0.870
	Female	2.87±2.35	
Duration of disease (years)	Male	2.81±2.25	0.591
	Female	3.10±2.37	
Systolic BP (mm/Hg)	Male	117.19±16.49	0.453
	Female	114.38±16.11	
Diastolic BP (mm/Hg)	Male	76.72±10.12	0.492
	Female	75.21±9.22	
NK cells (%)	Male	12.01±5.84	0.039*
	Female	9.48±4.83	
*statistically significant p≤0.05			

Mean ± SD of percentage of NK cells was high in male (12.01±5.84) compared to female patients (9.48±4.83). On comparison, there was significant difference between male and female patients (p= 0.039) (Table 2).

Age (years), BMI (lb/in2), systolic and diastolic BP (mm/Hg) of patients on oral hypoglycemic agents was high (41.10±6.50, 27.35± 4.08, 119.25±16.70 and 77.25±9.33) compared to patients on insulin (29.06 ±12.26, 21.16± 3.80, 110.50±14.84 and 73.62±9.60 respectively). On comparison, there was statistically significant difference in age, BMI, systolic and diastolic BP of two groups (p= 0.000, 0.000, 0.005 and 0.044 respectively) (Table 3).

Mean ± SD of blood sugar level (mg/dl) and duration of drug use (years) in patients on insulin was high (241.18±110.49 and 2.86±2.52) compared to patients on oral hypoglycemic (227.25±69.12 and 2.82±2.12 respectively). On comparison, there was no significant difference between two groups (p= 0.501 and 0.705) (Table 3).

Table 3: Range and Mean±SD of different variables in two groups

Variables	Treatment Modality (n = 40)	Mean± SD	p value
Age (years)	Group-I	41.10±6.503	0.000*
	Group-II	29.06 ±12.26	
Body mass index (lb/in2)	Group-I	27.35 ± 4.08	0.000*
	Group-II	21.16 ± 3.80	
Blood sugar level (mg/dl)	Group-I	227.25±69.12	0.501
	Group-II	241.18±110.49	
Duration of drug use (years)	Group-I	2.82±2.12	0.705
	Group-II	2.86±2.52	
Duration of disease (years)	Group-I	3.82±5.79	0.581
	Group-II	3.01±2.55	
Systolic BP (mm/Hg)	Group-I	119.25±16.70	0.005*
	Group-II	110.50±14.84	
Diastolic BP (mm/Hg)	Group-I	77.25±9.33	0.044*
	Group-II	73.62±9.60	
NK cells (%)	Group-I	11.10±5.66	0.327
	Group-II	9.91±5.08	
*statistically significant p≤0.05			

Mean ± SD of duration of diabetes (years) and percentage of NK cells of patients on oral hypoglycemic agents was high (3.82±5.79 and 11.10±5.66) compared to patients on insulin (3.01±2.55 and 9.91±5.08 respectively). On comparison, there was no significant difference between two groups (p=0.581 and 0.327 respectively) (Table 3).

DISCUSSION

In the current study, age of the diabetes patients on oral hypoglycemic agents was high (41.10±6.503 years) compared to patients on insulin (29.06±12.26 years) and on comparison, there was significant

difference between the two groups (p=0.000). The present study is in agreement with Salti et al (2001) [16] as they also reported higher age of the patients on oral hypoglycemic agents compared to patients on insulin therapy. In the current study, BMI of patients on oral hypoglycemic was high (27.35± 4.08 lb/in2) compared to patients on insulin therapy (21.16± 3.80 lb/in2) and on comparison, there was significant difference between two groups (p= 0.000). The current study is in partial agreement with Gimenez et al (2007) [17] who reported low BMI in patient of T1DM. The reason for partial agreement could be that Gimenez et al (2007) [17] compared T1DM with normal healthy population whereas current study compared diabetes patients on two different treatments.

In the current study, mean ± SD of systolic and diastolic BP (mm/Hg) of patients on oral hypoglycemic agents was high (119.25±16.70 and 77.25±9.33) compared to patients on insulin therapy (110.50±14.84 and 73.62±9.60 respectively) and on comparison, there was significant difference in two groups (p= 0.005 and 0.044 respectively). Current study is in agreement with Daousi et al (2006) [18] because they also reported increased systolic and diastolic BP of diabetes patients on oral hypoglycemic agents compared to patients on insulin. The current study is also in agreement with Chih et al (2010) [19] who reported lower systolic and diastolic BP in patients on insulin therapy.

In the current study, mean ± SD of percentage of NK cells of male patients was high (12.01±5.84) compared to female patients (9.48±4.83) and on comparison significant difference was observed (p=0.039). The current study is not in agreement with Timmons et al (2006) [20] who reported no gender difference of NK cells in normal population. The probable reason for this disagreement could be that in the current study diabetes patients taking two different treatments were compared whereas Timmons et al (2006) [20] compared NK cells of healthy male and females.

Conclusion

Significant difference between age, BMI, systolic and diastolic BP of patients on oral hypoglycemic agents and insulin therapy was observed but there was no significant difference in the percentage of NK cells of diabetes patients on these two different treatments. The percentage of NK cells was significantly high in male diabetes patients compared to female diabetes patients.

Conflicting Interest

None of the authors have financial or other interest in the products used for this study.

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