SMU
Sikkim Manipal University
Manipal
INSPIRED BY LIFE
SMU Medical Journal
ISSN : 2349 – 1604 (Volume – 2, No. 1, January 2015) Research article

Percentage of T Cells in Diabetes Mellitus Patients Managed with different Treatment Modalities

RabiaTufail[1], Nadeem Afzal[1], Khursheed Javeid[1], Faheem Shahzad[1] Saba Khaliq[1], Afia Abbas[1], Waqas Latif[2], Romeeza Tahir[1]

[1]Department of Immunology, University of Health Sciences Lahore Pakistan
[2]Department of Biostatistics, University of Health Sciences Lahore Pakistan

Corresponding author
Dr. Nadeem Afzal
Associate Professor
Head, Department of Immunology,
University of Health Sciences, Lahore, Pakistan
immunology@uhs.edu.pk ndmfzl@yahoo.com
92-321-4086-452, 92-42-99231304 ext 343 Fax 92-42-99230870

Manuscript received : 17.10.2014
Manuscript accepted: 25.11.2014

Abstract

Diabetes mellitus (DM) is managed by insulin (type-I diabetes) and oral hypoglycemics (type-II diabetes). Oral hypoglycemics prevent T cell proliferation. It was hypothesiszed that oral hypoglycemics decreases number of T cells.

A cross sectional study was undertaken to determine frequency of T cells in DM patients being treated with oral hypoglycemics and with insulin. Study included 80 subjects and their blood sample was analyzed for T cells by four color FACS caliber, using fluorescein isothiocyanate

tagged monoclonal antibodies against CD3 and PerCP against CD45.

Mean±SD of T cell percentage of patients on oral hypoglycemics was high (61.11±8.68 %) compared to insulin therapy (60.80±11.91 %). On comparison there was no statistically significant difference.

No significant difference in percentage of T cells but significant difference was observed in age, BMI, systolic and diastolic BP of DM patients on oral hypoglycemics and insulin therapy.

Key words: T cells, Diabetes mellitus, oral hypoglycemics, insulin

Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia with disturbances of carbohydrates, fats and protein metabolism. On the basis of insulin requirement, diabetes is differentiated into insulin dependent and non-insulin dependent.[1] Pakistan is categorized as a high prevalence area of diabetes with 6.9 million patients of diabetes that can be increased to 11.5 million by 2025.[2] Patients suffering from DM are managed by two basic drug regimens i.e. by insulin or by oral hypoglycemics. T cells are the components of adaptive immune system responsible for antigen specific immune response.[3] T cells express $CD3^+$ surface marker as a component of T cell receptor.[4] Type 1 diabetes mellitus (T1DM) results from autoimmune destruction of insulin producing beta cells.[5] In T1DM there is increased infiltration of lymphocytes, inflammatory cytokines and chemokines around pancreatic beta cells which may lead to their destruction. TNF-α and IFN-γ from NK cells and T cells are involved in the destruction of pancreatic beta cells. [6] T1DM patients treated with insulin exhibit a reduction in the population of T lymphocytes. Type 2 diabetes mellitus (T2DM) patients are normally treated with oral hypoglycemics. [7]

An oral hypoglycemic agents e.g. sulphonylurea prevents T cell proliferation whereas it stimulates insulin secretion.[8] Both oral hypoglycemics and diabetes decreases growth and proliferation of lymphocytes.[9,10,11,12] Therefore a study was designed to enumerate the number T cells and compare these cells in patients of DM being treated with insulin and oral hypoglycemic agents.

Materials and Methods

It was a cross sectional study comprising of eighty subjects of DM; grouped as 40 subjects on oral hypoglycemics (group-I) while 40 subjects on insulin therapy (group-II). These subjects were recruited from the Diabetes Management Center, Services Hospital Lahore. Immunophenotyping was performed by using fluorescein isothiocyanate (FITC) tagged MoA against CD3, peridinin-chlorophyll-protien (PerCP) tagged MoA against CD45. Lyse-wash method using whole blood for sample preparation was opted. Cells were analyzed with 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 was gated and data for $CD3^+$ 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 tests 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 demographic data of study subjects is summarized in Table 1. Mean±SD of percentage of T cells of patients on oral hypoglycemics was high (61.11±8.68) compared to patients on insulin therapy (60.80±11.91) and on comparison, there was no significant difference between two groups (p=0.541). Mean±SD of percentage of T cells was high in female patients (61.01±11.36) compared to male patients (60.88±8.80) and on comparison, there was no significant difference between male and female (p=0.956) (Table-2).

Age (yrs), BMI (lb/in^2), systolic and diastolic BP (mm/Hg) of patients on oral hypoglycemics was high (41.10±6.503, 27.35± 4.08, 119.25±16.70 and 77.25±9.33) compared to patients on insulin therapy (29.06 ±12.26, 21.16± 3.80, 110.50±14.84 and 73.62±9.60 respectively) and 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-2).

Mean±SD of blood sugar level (mg/dl) and duration of drug use (yrs) in diabetes patients on insulin therapy was high (241.18±110.49 and 2.86±2.52) compared to patients on oral hypoglycemics (227.25±69.12 and 2.82±2.12 respectively) and on comparison, there was no significant difference between two groups (p= 0.501 and 0.705 respectively) (Table-2).

Mean±SD of duration of diabetes (yrs) of patients on oral hypoglycemics was high (3.82±5.79) compared to patients on insulin therapy (3.01±2.55) and on comparison, there was no significant difference between two groups (p=0.581) (Table-2).

The present study comprised of 32 male (40%) and 48 females (60%). Mean±SD of age (yrs), systolic and diastolic BP (mm/Hg) was high in male patients (35.50±12.40 yrs, 117.19±16.49 mm/Hg and 76.72±10.12 mm/Hg) compared to female patients (35.47±11.18 yrs, 114.38±16.11 mm/Hg and 75.21±9.22 mm/Hg) respectively. On comparison, there was no significant difference between male and female among these parameters (p=0.991, 0.453 and 0.492 respectively) (Table-3).

Mean±SD of blood sugar level (mg/dl), body mass index (BMI) (lb/in^2), duration of disease (yrs) and duration of drug use (yrs) 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 among these parameters (p= 0.358, 0.233, 0.591 and 0.870 respectively) (Table-3).

Discussion

In the present study, mean±SD of percentage of T cells of patients on oral hypoglycaemics was high (61.11±8.68) compared to patients on insulin therapy (60.80±11.91). Mean±SD of percentage of T cells of female patients was high (61.01±11.36) compared to male patients (60.88±8.80). On comparison, there was no significant difference between two groups (p= 0.546 and 0.956 respectively).

The current study is in agreement with Manssor *et al* (2011)[13] who also documented oral hypoglycemic agents had no significant effect on leukocytes of male and female diabetic patients. The current study is in agreement with Buschard K *et al* (1983)[14], who also reported

that insulin therapy had no significant effect on T cell population. However, current study is not in agreement with Rungta *et al* (2008)[15] who reported higher number of T cells in males as compared to female (mean±SD of absolute numbers of CD4 and CD8 lymphocytes/microliter was 743.4±307.8 and 541.7±176.4 in males and 790.7±280.4 and 497.03±203.6 in females respectively). In fact, the current study should not be compared with Rungta *et al* (2008)[15] as they studied CD 4 cells; a subset of T cells, in patients suffering from human immunodeficiency virus whereas the current study reported T cells in male and female DM patients on insulin and oral hypoglycemics.

The current study is not in agreement with Mello *et al* (2011)[16] who reported T cells of DM patients on oral hypoglycemics had reduced proliferation, number and viability. However in the current study, there was no significant difference in the T cells of DM patients being treated with oral hypoglycemics or insulin therapy. Klotsas et al (2010)[17] reported that raised white blood cells was associated with high risk of T2DM. Klotsas et al (2010)[17] studied the total white blood cells population whereas present study considered T cells population only.

In the current study, age of the DM patients on oral hypoglycemics was high (41.10±6.503 years) compared to patients on insulin therapy (29.06±12.26 years) and on comparison, there was statistically significant difference between the two groups (p=0.000). It is in agreement with Salti *et al* (2001)[18] as they also reported higher age of the patients on oral hypoglycemics (54.0±11.0 years) compared to patients on insulin therapy (31.0±12.7 years).

In the current study, BMI of patients on oral hypoglycemics was high (27.35± 4.08 lb/in^2) compared to patients on insulin therapy (21.16± 3.80 lb/in^2) and on comparison, there was statistically significant difference between the two groups (p= 0.000). The current study is in partial agreement with Gimenez *et al* (2007)[19] who reported low BMI in patient of T1DM. The reason for partial agreement could be that Gimenez *et al* (2007)[19] compared T1DM with normal healthy population whereas current study compared DM patients taking two different treatments.

In the current study, mean±SD of systolic and diastolic BP (mm/Hg) of patients on oral hypoglycemics was high (119.25±16.70 and 77.25±9.33 respectively) compared to patients on insulin therapy (110.50±14.84 and 73.62±9.60 respectively) and on comparison, there was

statistically significant difference in the two groups (p= 0.005 and 0.044 respectively). Current study is in agreement with Daousi *et al* (2006)[20] because they also reported increased systolic and diastolic BP of diabetes patients on oral hypoglycemic (144.9±23.7 and 74.1±12.6 mm/Hg) compared to patients on insulin (127.4±23.3 and 68.9±11.8 mm/Hg).

Conclusion

There was significant difference in the age, BMI, systolic and diastolic BP but no significant difference was observed in the percentage of T cells of DM patients on oral hypoglycemics and insulin therapy.

Acknowledgement

We acknowledge Higher Education Commission Pakistan and University of Health Sciences Lahore Pakistan for providing funds and research facilities.

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 (yrs) Mean ±SD	41.10±6.50	29.06±12.26
Body mass index (lb/in^2) Mean ±SD	27.35±4.08	21.16±3.80
Duration of diabetes (yrs) Mean± SD	3.82±5.79	3.01±2.55
Duration of drug use (yrs) Mean± SD	2.82±2.12	2.86±2.52

Table-2. Comparison and mean±SD of different variables in two groups

Variables	Group (n = 40)	Mean ± SD	*p*-value
Age (yrs)	Group-I	41.10±6.503	0.000*
	Group-II	29.06 ±12.26	
Body mass index (lb/in^2)	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 (yrs)	Group-I	2.82±2.12	0.705
	Group-II	2.86±2.52	
Duration of disease (yrs)	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	
T cells %	Group-I	61.11±8.68	0.541
	Group-II	60.80±11.91	

* statistically significant ($p \leq 0.05$)

Table-3. **Comparison and mean±SD of different variables between male and females**

Variables	**Gender**	**Mean± SD**	***p*-value**
Age (yrs)	Male	35.50±12.40	0.991
	Female	35.47±11.18	
Body mass index (lb/in^2)	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 (yrs)	Male	2.78±2.22	0.870
	Female	2.87±2.35	
Duration of disease (yrs)	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	
T cells (percentage)	Male	60.88±8.80	0.956
	Female	61.01±11.36	

* statistically significant ($p \leq 0.05$)

References

1. Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications. Report of a WHO Consultation. WHO/NCD/NCS/99.2, 1999.

2. Qidwai, W. and Ashfaq, T. Imminent Epidemic of Diabetes Mellitus in Pakistan, Issues and Challenges for Health Care Providers. *JLUMHS,* 9(3),112-113, 2010.

3. Rabb, H. The T cell as a bridge between innate and adaptive immune systems, Implications for the kidney. *Kidney International,* 61,1935–1946, 2002.

4. Yang, H., Parkhouse, R.M.E. and Wileman, T. Monoclonal antibodies that identify the CD*3* molecules expressed specifically at the surface of porcine γδ-T cells. Immunology, 115,189–196, 2005.

5. Yoon, J. W and Jun, H. S. Autoimmune Destruction of Pancreatic β Cells. *American Journal of Therapeutics*, 12,580–591, 2005.

6. Iwahashi, H., Itoh, N., Yamagata, K., Imagawa, A., Nakajima, H., Tomita, K., Moriwaki, M., Waguri, M., Yamamoto, K., Miyagawa, J., Namba, M., Hanafusa, T. and Matsuzawa, Y. Molecular mechanisms of pancreatic beta-cell destruction in autoimmune diabetes, potential targets for preventive therapy. *Cytokines Cell Mol Ther.* 4(1),45-51, 1998.

7. Eichner, H.L., Lauritano, A. A., Woertz, L. L, Selam, J. L., Gupta, S. and Charles, M. A. Cellular immune alterations associated with human insulin therapy. Diabetes Res, 8(3),111-5, 1998.

8. Mello, K.F., Lunardelli, A., Donadio, M.V.F., Caberlon, E., de Oliveira, C.S.A., Bastos, C.M.A., Pires, M.G.S., Nunes, F.B. and de Oliveira, J.R. Immunomodulatory effects of oral antidiabetic drugs in lymphocyte cultures from patients with type 2 diabetes. *J Bras Patol Med Lab*, 47(1),43-48, 2011.

9. Nunes, F. B. Immunomodulatory effect of fructose-1,6-bisphosphate on T-lymphocytes. *International Immuno pharmacology*, 3, 267-272, 2003.

10. Otton, R., Soriano, F.G., Verlengia, R. and Curi, R. Diabetes induces apoptosis in lymphocytes. *J Endocrinol,* 182**:**145–156, 2004.

11. Towler, M.C. and Hardies D.G. AMP-activated protein kinase in metabolic control and insulin signaling.*Circulation*, 100,328-41,2007.

12. Foss-Freitas, M.C., Foss, N.T.E.A., Donadi, E.A. and Foss, M.C. Effect of the glycemic

control on intracellular cytokine production from peripheral blood mononuclear cells of type 1 and type 2 diabetic patients. *Diabetes res clinpr*, 82, 329– 334, 2008.

13. Manssor, A.R.J. and Rajab, H.K. The effects of anti-diabetic agents on white blood cells. *Tikrit Journal of Pure Science.*16 (4),1-5, 2011.

14. Buschard, K., Röpke, C., Madsbad, S., Mehlsen, J., Sørensen, T.B. and Rygaard, J. Alterations of peripheral T-lymphocyte subpopulations in patients with insulin-dependent (type1) diabetes mellitus. *J Clin Lab Immunol*, 10(3),127-131, 1983.

15. Rungta, A., Hooja, S., Vyas, N., Rishi, S., Rao, A. and Gupta, S. Enumeration of CD4 and CD8 T lymphocytes in healthy HIV seronegative adults of northwest India, a preliminary study. *Indian J Pathol Microbiol*, 51(1),127-9, 2008.

16. Mello, K.F., Lunardelli, A., Donadio, M.V.F., Caberlon, E., de Oliveira, C.S.A., Bastos, C.M.A., Pires, M.G.S., Nunes, F.B. and de Oliveira, J.R., Immunomodulatory effects of oral antidiabetic drugs in lymphocyte cultures from patients with type 2 diabetes. *J Bras Patol Med Lab*, 47(1),43-48, 2011.

17. Klotsas, E. G., Ye, Z., Cooper, A.J., Sharp, S.J., Luben, R., Biggs, M.L., Chen, L.K., Gokulakrishnan, K., Hanefeld, M., Ingelsson, E., Lai, W. A., Lin, S.Y., Lind, L., Lohsoonthorn, V., Mohan, V., Muscari, A., Nilsson, G., Ohrvik, J., Qiang, J.C., Jenny, N.S., Tamakoshi, K., Kurktschiev, T.T., Wang, Y.Y., Yajnik, C,S., Zoli, M., Khaw, K.T.,Forouhi, N.G., Wareham, N.J. and Langenberg,C.,. *PLoS ONE*. 5(10),1-8, 2010.

18. Salti, I., B'enard, E.,Detournay, B., Bianchi-Biscay, Monique., Le Brigand, Corinne., Voinet, C. and Jabbar, A. A Population-Based Study of Diabetes and Its Characteristics during the Fasting Month of Ramadan in 13 Countries.*Diabetes Care,* 27,2306–2311, 2008.

19. Gim'enez, M., Aguilera, E., Castell, C., De Lara, N., Nicolau, J. and Conget, I. Relationship between BMI and age at diagnosis of Type 1 diabetes in a mediterranean area in the period of1990–2004.*Diabetes Care*, 30(6),1593-1594, 2007.

20. Daousi, C., Casson, I.F., Gill, G.V., MacFarlane, I.A., Wilding, J.P.H. and Pinkney, J.H. Prevalence of obesity in type 2 diabetes in secondary care, association with cardiovascular risk factors. *Post grad Med J*, 82,280–284, 2006.

Authors Column

Dr Nadeem Afzal working as Associate Professor, Head Department of Immunology, University of Health Sciences Lahore Pakistan. He is a medical graduate (MBBS), MSc (Medical Immunology) from the University of London (UK) and PhD (Immunology) from University of Health Sciences, Lahore, Pakistan

SMU Medical Journal, Volume – 2, No. – 1 , January, 2015,

PP. 139 - 149 .