

Interleukin-4 Gene Polymorphisms in Type 2 Diabetes Mellitus in Medan, Indonesia

Milahayati Daulay¹, Mutiara Indah Sari²

¹Department of Physiology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia

²Department of Biochemistry, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia

Keywords: Interleukin-4 Gene, Polymorphisms, Diabetes Mellitus.

Abstract: Chronic hyperglycemia in type 2 diabetes mellitus is associated with the production of proinflammatory cytokines such as Interleukin (IL)-1, Tumor Necrosis Factor (TNF), Interferon (IFN)- γ , IL-6, IL-12, and IL-18. This inflammatory condition triggers the formation of anti-inflammatory cytokines such as IL-4, IL-10, IL-13, and IFN- α that work antagonistically against the pro-inflammatory cytokines. Interleukin-4 gene polymorphisms are associated with inflammatory events arising from hyperglycemic conditions. This study aims to determine the polymorphism of IL-4 -33 C>T gene in diabetic patients. A total of 40 diabetic patients from Universitas Sumatera Utara Hospital, Medan were genotyped for IL-4 SNPs (single nucleotide polymorphisms) -33 C>T using restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR). Genotype frequencies were calculated in patients by direct gene counting. The most frequent genotype in diabetic patients from Universitas Sumatera Utara Hospital in Medan was TT in 20 patients (50%). The frequency of CT genotype was 18 (45%) and CC genotype was 2 (5%). Previous studies have shown differences in genotype frequencies in other regions indicating a genetic factor difference in diabetes mellitus. Based on the result of present study it may be concluded that the functional gene polymorphisms of IL-4 play an important role in type 2 diabetes mellitus.

1 INTRODUCTION

Epidemiological studies have shown a tendency to increase incidence rates and the prevalence of type 2 Diabetes Mellitus (DM) in different parts of the world. The World Health Organization (WHO) predicts an increase in the number of people with DM who become one of the global health threats. In Indonesia, WHO predicts an increase in the number of people with DM from 8.4 million in 2000 to about 21.3 million by 2030. This report shows an increase in the number of people with DM 2-3 times by 2035. The International Diabetes Federation (IDF) predicts an increase in the number of people with DM in Indonesia from 9.1 million in 2014 to 14.1 million by 2035 (PERKENI, 2015).

According to the American Diabetes Association (ADA) 2010, diabetes mellitus is a group of metabolic diseases with characteristic hyperglycemia that occurs because of abnormalities of insulin secretion, insulin resistance or both. Chronic hyperglycemia in DM is associated with the production of proinflammatory cytokines and

inflammatory cytokines through Toll-Like Receptors (TLRs) 2 and 4 activation resulting in destruction of pancreatic beta cells and pancreatic endocrine dysfunction in type 1 and 2 DM. This is characterized by changes in proinflammatory cytokine levels such as interleukin IL-1, Tumor Necrosis Factor (TNF), Interferon (IFN) - γ , IL-6, IL-12, and IL-18 in DM patients (Frances, 2013). This inflammatory condition triggers the formation of anti-inflammatory cytokines such as IL-4, IL-10, IL-13, and IFN- α that work antagonistically against the pro-inflammatory cytokines (Voehringer, 2013)

Several studies have shown that there is an association of IL-4 cytokine gene expression with metabolic abnormalities of diabetes mellitus. Interleukin-4 gene polymorphisms and changes in IL-4 levels are associated with inflammatory events arising from hyperglycemic conditions. (Tripathi, 2015) in his study showed the association of IL-4 genotypic polymorphism with type 2 DM in the Indian population. Type 2 DM is known to be associated with IL-4-590 C> T gene polymorphism with increased CT variant in type 2 diabetes mellitus

compared with healthy control (Alsaid, 2013). (Li, 2013) found an association between IL-10- 1082 G / A gene polymorphism with type 2 diabetes mellitus. (Bid, 2008) also found an association between IL-4 and IL-1RN (VNTR) gene polymorphisms with the incidence of type 2 diabetes mellitus. This study aims to determine the polymorphism of IL-4 -33 C> T gene in diabetic patients at Universitas Sumatera Utara Hospital, Medan.

2 METHOD

The study was conducted in Universitas Sumatera Utara Hospital and Integrated Laboratory of Medical Faculty Universitas Sumatera Utara. To be eligible for the study, patients had to give informed consent. The study protocol was reviewed and approved by the Research Ethical Committee Faculty of Medicine, Universitas Sumatera Utara. All participants provided written informed consent to participate in this study.

The type of this research is descriptive research with cross sectional study approach with the number of samples as much as 40 patients with type 2 diabetes mellitus at Universitas Sumatera Utara Hospital, Medan. Venous blood taken as much as 5 cc. DNA isolation using kit from Promega. Examination of interleukin-4 -33 C> T polymorphisms with PCR-RFLP technique using BSmAI restrictive enzyme and electrophoresis at 2% agarose. Primer F IL-4 -33 C> T Primer F (forward) CAAGTTACTGACAATCTGGTGT Primer R (reverse) CGGCACATGCTAGCAGGAA with a large PCR product of 223 bp. The genotype frequencies are calculated directly.

3 RESULTS AND DISCUSSIONS

Table 1 shows the characteristics of respondents. Most of the diabetic patients in Universitas Sumatera Utara Hospital in Medan are male (52.5%) and more common in the age between 40 -70 years as many as 33 patients (82.5%).

Table 1: Characteristics of respondents (n= 40).

Charateristics	n	%
Sex		
Male	21	52.5
Female	19	47.5
Age		
< 40	1	2.5
40-70	33	82.5
>70	6	15

Current study showed that type 2 diabetes mellitus occurs most commonly in men. According to (Ferguson, 2017), the high prevalence of diabetes in men can be caused by several factors such as higher visceral fat in males where visceral fat is associated with high fat in the liver and pancreas. This risk difference is influenced by the distribution of body fat. In men, fat accumulation is concentrated around the abdomen that triggers central obesity, which is more at risk of triggering metabolic disorders. In other words, men are more susceptible to diabetes than women. In addition, hormonal factors also play a role in high prevalence of diabetes in men. In this study also found that diabetes mellitus type 2 is most common in adults older than 40 years. The presence of decreased function of organs due to aging causes decreased work of the organ including the pancreas. According to Indonesian Society of Endocrinologists, age over 40 years is one of the risk factors for the occurrence of type 2 diabetes mellitus (PERKENI, 2015).

Table 2: Distribution of frequency of genotypes.

Genotypes	CC	CT	TT
n (%)	2 (5)	18 (45)	20 (50)

Analysis of the polymorphisms at -33 C>T of IL-4 by BSmAI restriction enzyme showed that the frequency of the TT genotype was 20 (50%) . The frequency of CT genotype was 18 (45%) and CC genotype was 2 (5%).

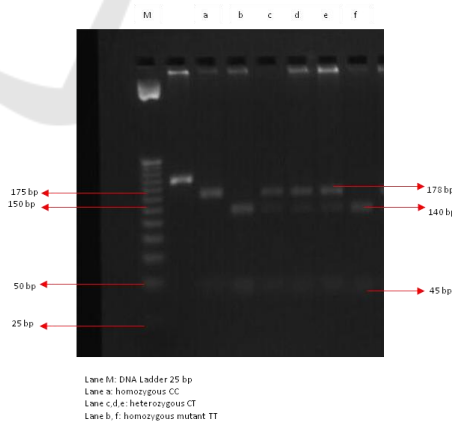


Figure 1: RFLP-PCR product of IL-4 gene -33 C>T.

Mechanisms of various diseases such as infection, autoimmune, malignant disease are affected by the production of proinflammatory and antiinflammatory cytokines (Bidwell, 1999). The difference in gene expression brought by differences in gene sequence

concludes how the mechanism and development of a disease (Sari and Sari, 2017). The presence of different cytokine profiles among individuals indicates the presence of gene polymorphisms that produce these cytokines. This study showed polymorphism of IL-4 -33 C>T gene in diabetic patients where the TT genotype has the largest frequency among people with type 2 diabetes mellitus in Medan, North Sumatera, Indonesia. This is in contrast to the results obtained by (Alsaid, 2013) in Egypt, where in the type 2 diabetes mellitus patients, most genotypes were CT and in Pakistan by (Micheal, 2013), the highest genotypes were CC in patients with asthma and allergic rhinitis. Meanwhile, in Iran, by (Arababadi, 2010) found the most genotype is CC in patients with type 2 diabetes mellitus. This indicates that the interleukin-4 gene polymorphisms have an effect on the occurrence of diabetes mellitus.

4 CONCLUSIONS

In this study, we found the presence of interleukin-4 gene polymorphism in type 2 diabetes mellitus in Medan, North Sumatera, Indonesia. The functional gene polymorphisms of interleukin-4 play an important role in type 2 diabetes mellitus. Further research is needed to compare interleukin-4 gene polymorphisms with the control group.

ACKNOWLEDGEMENTS

Authors are grateful to the Ministry of Research and Technology and Higher Education of Republic Indonesia. This work was supported by research grants from TALENTA USU 2018 contract number 2590/UN5.1.R/PPM/2018 date 16 March 2018.

REFERENCES

- Alsaid, A., El-Missiry, M., Hatata, E., Tarabay, M., Settin., M. 2013. Association of IL-4-590 C>T and IL-13-1112 C>T Gene Polymorphisms with the Susceptibility to Type 2 Diabetes Mellitus. *Disease Markers*, 35(4), 243-247
- American Diabetes Association (ADA). 2010, Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care*, 37(1), 81–90
- Arababadi, M. K. , 2010. Interleukin-4 gene polymorphisms in type 2 diabetic patients with nephropathy. *Iranian Journal of Kidney Diseases*, 4(4), 302-306
- Bid, H. K., Konwar, R., Agrawal, C. G., Banerjee, M. 2008. Association of IL-4 and IL-1RN (receptor antagonist) gene variants and the risk of type 2 diabetes mellitus: a study in the north Indian population. *Indian J Med Sci.*, 62(7), 259-266
- Bidwell, J., Keen, L., Gallagher, G., Kimberly, R., Huizinga, T., McDermott, M. F., Oksenberg, J., McNicholl, J., Pociot, F., Hardt, C., Alfonso, S. D. 1999. Cytokine gene polymorphism in human disease: on-line databases. *Genes and Immunity*, 1, 3–19
- Francés, D. E., Ingaramo, P.I., Ronco, M. T., Carnovale, C. E. 2013. Diabetes, an inflammatory process: Oxidative Stress and TNF-alpha involved in hepatic complication. *J. Biomedical Science and Engineering*, 6, 645-653
- Ferguson, L. D., Celis-Morales, C., Ntuk, U. E., Mackay, D. F., Pell, J. P., Gill, J. M. R., Satta, N. 2017. Men across a range of ethnicities have a higher prevalence of diabetes: findings from a cross-sectional study of 500000 UK Biobank participants. *Diabetes Medicine*, 1-16
- Li, J., Wu, S., Ming-Rui, W., Ting-Ting, W., Ji-Min, Z. 2013. Association of the interleukin-10 – 592A/C, – 1082G/A and – 819T/C gene polymorphisms with type 2 diabetes: A meta-analysis. *Gene*, (5)21, 211-216
- Micheal, S., Minhas, K., Ishaque, M., Ahmed, F., Ahmed, A. 2013. IL-4 Gene Polymorphisms and Their Association With Atopic Asthma and Allergic Rhinitis in Pakistani Patients. *J Investig Allergol Clin Immunol*, 23(2), 107-111
- Perkumpulan Endokrinologi Indonesia (PERKENI). 2015, Pengelolaan dan Pencegahan Diabetes Mellitus Tipe 2 di Indonesia. PB. PERKENI. Jakarta, 5th edition
- Sari, M. I., Sari, D. I. 2017. Nutrient Intake Apolipoprotein A5 -1131T>C Polymorphism and Its Relationship with Obesity. IOP Conference Series: Materials Science and Engineering.180. 012094
- Tripathi, A. K., Smriti, S., Jitendra, K. T., Rishabh, D. S., Srikant, K., Pranav, M., Ugam, K. C., Manoj, I. 2015. Association of Genetic Polymorphism of Inflammatory Genes (IL-1 β and IL-4) with Diabetes Type 2. *Journal of Genetic, Molecular and Cellular Biology*, 2(1), 1-9
- Voehringer, D., Reese, T. A., Huang, X., Shinkai, K., Locksley, R. M. 2006. Type 2 immunity is controlled by IL-4/IL-13 expression in hematopoietic non-eosinophil cells of the innate immune system. *Journal of Experimental Medicine*, 203(6) 1435–1446