

CROSS-SECTIONAL STUDY

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Physical Activity Maintain Immune Response Through TLR-2/TLR-4 Gene Expression in Type-2 Diabetes Mellitus Patient at Medan City

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ABSTRACT

Background: The Increasing in type-2 diabetes mellitus (T2DM) needs to solve comprehensively and holistically. Patients with T2DM should have self-coping due to lifestyle modification. Abdominal fat accumulation can release pro-inflammatory cytokine that leads TLR-2 and TLR-4 to the response. These two kinds of toll-like receptors exist on the monocyte surface membrane which is an innate immunity cell. **Objective:** The aims of this study were to get the profile of physical activity, metabolic state, and mononuclear cell response to the expression of the TLR2 and TLR4 genes in T2DM patients. **Methods:** It was a descriptive-analytic study with a cross-sectional study design. Thirty-two eligible patients with inclusion criteria participated as subjects. All subjects answered questions by IPAQ, and checked metabolic state with body composition analysis. The TLR2 and TLR4 gene expression was determined with quantitative Real- Time PCR. **Results:** This study result found that most T2DM patients were in a highly active category in which most of their activity was walking (light intensity). The average abdominal circumferences were 91.81 ± 15.4 cm, body fat percentage was $29.5 \pm 8.8\%$, and fasting blood sugar was 187.07 ± 67.03 mg/dl. Mononuclear cells number were normal. The expression of the TLR2 gene was lower by 0.71 fold and TLR4 gene expression was lower by 0.9 fold compared with non-DM ($p < 0.05$). By chi-square test, there was a positive correlation between TLR2 gene expression with fasting blood glucose ($p = 0.011$), and a positive correlation between the abdominal circumference and TLR4 gene expression ($p = 0.011$). **Conclusion:** Type-2 Diabetes mellitus patients in primary health care keep walking as their physical activity to maintain blood glucose. Patients need to do moderate to vigorous exercise regularly to reduce body fat percentage especially abdominal fat to reduce Toll-like receptor gene expression, so insulin resistance and blood glucose level might decline to normal.

Keywords: physical activity, metabolic state, TLR2, TLR4, DM type-2.g.

1. BACKGROUND

The prevalence of type-2 Diabetes mellitus (T2DM) enhance in most of Asian countries and become one of health problem that should be solving holistically and comprehensive. The increasing of this prevalence related with lifestyle changes in physical activity and energy imbalance between energy output and intake. Energy imbalance between diet and physical activity promote cell metabolism impairment in skeletal muscle lead to insulin resistance (1).

Previous studies found that insulin resistance has correlation with immune response (2). Visceral fat accumulation increase numbers of free fatty acid in plasma. This increasing is detected by trans-membrane's receptor which extend at immunity cells such as monocyte, macrophage, granulocyte, natural killer cell, lymphocyte B and T that is toll like receptor-2 (TLR-2) and toll like receptor-4 (TLR-4). Activating TLRs will activated monocyte or macrophage as natural immune response (innate immunity) to release pro-inflammatory cytokine such as TNF- α , IL-1 β and IL-6. Invading macrophage to adipose tissue can also much produce much of Nitric Oxide Synthase 2 (NOS2) which can reduce Insulin receptor-1 on skeletal muscle and lead to insulin signaling disturbance (3).

Mechanism of insulin resistance due to physically inactive, promote mitochondrial dysfunction. Moving skeletal muscle actively through physical

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activity or exercises regularly improve mitochondrial biogenesis progenitor such as PGC-1 alpha and AMPK in skeletal muscle (4). Chronic adaptation change mitochondrial size and volume, to do it function properly. In addition, T2DM patient who is physically active found in increasing insulin receptor density on skeletal muscle, so insulin resistance was decline (5). It proved that exercise could prevent macrophage pro-inflammatory infiltration to adipose tissue. But so far, there are not many research had been done to find out how was the physical activity, expression of TLR2 gene, TLR4 gene and mononuclear cell as natural immune response to T2DM patients (6).

2. OBJECTIVE

The aim of the study was to get the figure of physical activity, metabolic status, and mononuclear cells response through the expression of TLR2 and TLR4 on T2DM patients.

3. PATIENTS AND METHODS

Participants

It was a descriptive analytic study with a cross-sectional design. Data were taken during covid-19 pandemi on October 2021 when activity restriction regulation were applied. Thirty two T2DM patients from Glugur Darat public health Medan, North Sumatera who were eligible as respondents followed this study. The selected research subjects were those who met the inclusion criteria: had been diagnosed with type-2 DM and were willing to participate in the study, with the exclusion criteria: currently experiencing an infection and having limitations in movement. Before the procedure started, all respondents had been given informed concern about the research purposes and procedures and assigned the form agreement. Each T2DM patient was measured blood pressure, height, body weight, abdominal circumference, and got body composition analysis. To assess physical activity, all participants answered the International Physical Activity Question (IPAQ). Five cc blood from cubital vein were taken by expert analyst to check fasting blood glucose, routine blood, gene expression of TLR2 and TLR4. Gene expression were assessed by using quantitative real time PCR.

RNA Isolation

Blood was centrifuged at 3000rpm for 15 minutes. Then put into a tube 1 ml of Tripure reagent and added with 10 x 10⁶ Buffy Coat. Pipette until homogeneous. After that the extraction results were incubated for 5 minutes at room temperature. Then 200µl of Chloroform was added and shaken for 15 seconds, then incubated again for 10 minutes at room temperature. After that it was centrifuged at 12,000 RCF for 15 minutes at 4oC. The supernatant was put into a new tube and added 500µl of Isopropanol (tube cap, back and forth until mixed), incubated for 5-10 minutes at room temperature, and centrifuged again at 12,000

RCF for 10 minutes at 4o C, discarding the supernatant. Then add 1 ml of 75% ethanol, vortex. Centrifuge again at 7,500 RCF for 5 minutes at 4oC, discarding the

supernatant. After that the tube was dried for 30 minutes. Finally, add DEPC. Water as much as 50-100 µl, and incubated again for 10-15 minutes at 55-60oC.

Quantitative Real Time PCR

To obtain isolate RNA, researchers used Tripure Isolation Reagent (Roche) Cat No. 11 667165 001 and follow the available procedures for RNA preparation. The RNA obtained is converted into cDNA by adding cDNA synthase (Sigma Aldrich) as much as 4µl into the Eppendorf tube. the working solution is prepared by mixing KAPPA green 10µl, primer Forward, primer reverse, RNA 2 ° L and NF water, mixed in a 0.2 ml PCR tube. The Cycling threshold is then assessed with the rotor gene. For gene reference used GADPH gene:

All collected data were tested for normality with Shapiro wilk test. Univariate data were presented in table distribution frequency. To analyse the changes of TLR2 and TLR4 gene expression DMT2 patient compared to Non DM, we used livask formula. And the difference cycling threshold between DM and Non DM, we analysed using independent t – test.

Ethical Approval

Animal handling and treatment of experimental animals is accordance with the Declaration of Helsinki with due observance of the principles of reduction, refinement and replacement. This study had been approved by the medical faculty of Universitas Sumatera Utara and Adam Malik general hospital committee ethic No. 263/KOMET/FK USU/2016

4. RESULTS

The characteristic of respondent based on age, body composition analysis, and fasting blood glucose

The average of body composition analysis and fasting blood glucose and be seen at Table.1

Table.1 showed that the averages of abdominal and arm circumferences T2DM patients were higher than normal and so is Fasting blood glucose respondent ≥ 126 mg/dl. It means that there was accumulation fat abdomen to T2DM patient and the blood glucose was not reaching normal level even though patient consumed anti diabetic drug.

Frequency distribution of respondent nutritional status

Nutritional status from T2DM patient who came to Glugur Darat public health services can be seen at Table 2. From Table. 2 we showed that most of T2DM patient at Glugur Darat Public health service were obese.

Description of Physical activity of people with T2DM

We assessed physical activity patient by using International Physical Activity Questioner (IPAQ). From the results of the study it is known that most of the activities carried out are walking activities, with average of running time is 302.74 minutes / week. While for those who do moderate activities during free time there are about 3 people with an average time of 17.42 minutes/week. As for strenuous activities, performed by 4 people with an average time used 263.22 minutes / week . Category of T2DM patient's physical activity can be seen by the fig-

n= 30	Mean	SD	Min	Maks
Age (years)	56.2	7,14	35	72
Body Composition				
abdominal circumference (cm)	91.81	19.4	33	163
arm circumference (cm)	30.38	31.5	24	38
Bone mass	9.73	2.16	6.1	14.1
% Body fat	29.5	8.8	14.1	54.0
Muscle mass	32.8	6.33	21.1	43.3
Hydration	48.79	6.8	32.6	59.1
Calori (cal)	1812.69	273.83	1084	2343
Fasting Blood Glucose (mg/dl)	187.07	67.03	101	331

Table 1. The characteristic of respondent based on age, body composition and blood glucose

Nutritional status	Frequency (n)	Percentage (%)
Underweight	0	0
Normoweight	9	28.1
Overweight	8	25.0
Obesity	15	46.9
Total	32	100

Table 2 Nutritional status of T2DM patient

Sitting time	
Seat working (min/day)	149.193
Seat leisure (min/day)	109.19
Total sitting time (min/week)	964.35
Average sitting time (day/hour)	2.296

Table 3. Description of sedentary activities (sitting in work and sitting in leisure time) T2DM patients

	Mean (%)	SD	Min	Maks
Limfosit (20- 40%)	31	6.32	19.3	44.8
Monosit (2 – 8 %)	6.62	1.41	4.1	9.9

Table 4. The average of lymphocyte and monocyte patient with T2DM.

ure.1 and the description of sedentary activities (sitting in working and sitting in leisure time T2DM Patients can be seen at Table 3. From the Figure 1 we can see that the intensity of physical activity in T2DM patient at Glugur Darat public health service mostly light intensity and have sitting time 2.3 hour per day.

Mononuclear cells and TLR2/4 Expression

The average number of lymphocyte and monocyte T2DM patients was normal. Eventhough few patients have lymphocyte and monocyte above normal, which are maximum 44.8% for lymphocyte and 9.9% for monocyte (Table 4).

From the table here we found that T2DM patients in a good immune condition.

By using real time PCR, we found that the average of cycling threshold mRNA TLR2 1.21 (non DM) and 1.71 (DM) (p=0.000) and TLR4 3.19 (non DM) and 3.32 (DM) (p =0.000) Cycling threshold inverted with gene expression.

Using livask method obtained that patients with Type-2 diabetes decreased TLR2 expression by 0.71 fold and decreased TLR4 by 0.9 fold compared to normal individu (Non DM).

Differences in the expression of TLR2 and TLR4 in patients with Type-2 diabetes with normal individuals were statistically significant (p<0.05).

The correlation between Blood Glucose level and TLR2/4 gene expression

By using pearson correlation test, there was a significant correlation between fasting blood glucose with the expression of TLR2 gene (p=0.011) but there was not a correlation with TLR4 gene expression (p=0.089).

The correlation between abdominal circumferences with TLR2/4 gene expression By using pearson correlation test, there was no significant correlation between abdominal circumferences and TLR2 gene expression (p=0.054) but has significantly correlated with TLR4 gene expression (p=0.011).

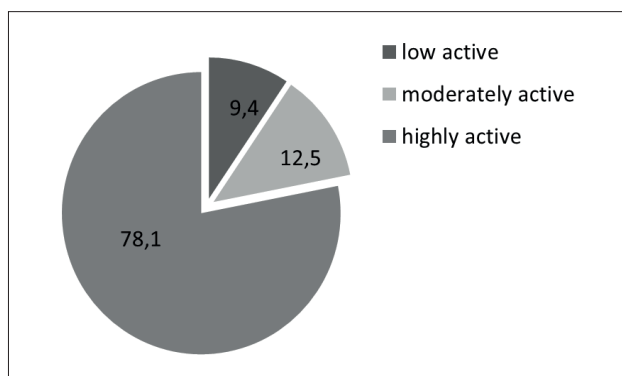


Figure 1. Physical activity category among T2DM patients

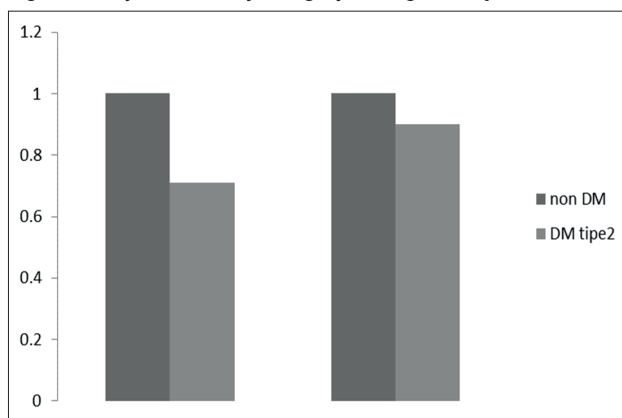


Figure 2. The Expression difference of TLR 2 gene and TLR4 gene between DMT2 and Non DM.

5. DISCUSSION

Adults can reduce their risk of type 2 diabetes and improve insulin sensitivity and glucose uptake through regular and adequate levels of physical activity and healthy. WHO has developed recommendations on healthy diet and physical activity that, if implemented, can reduce an individual’s risk of type-2 diabetes. Physical activity in addition to maintain fitness can also lose weight and reduce insulin resistance, so people with so people with DM are recommended to stay actively moving (7, 8).

In this study, we found that most of physical activity among participants was walking with the avarage time for sitting hours is 2.296 per day. Patient’s abdominal cir-

cumferences was 91.81 cm and around 50% of them were obese. This condition was followed by blood glucose level in average 187.07 mg/dl which was still on abnormal level of blood glucose. Fowler JR et al (2020) mentioned in his study that waist size related with physical activity level and insulin resistance. Insulin resistance declined when physical activity increased, physically active individual tends to have smaller waist than physically inactive person (9).

From this study we knew that more than 70% of T2DM in Glugur Darat public health service were overweight, which 65% of them are obese. Based on Martina (2019), an obese person has risk for DM 1,74 times more than non obese, however physical activity can decline the risk as much as 38%. Obesity as an important variable for T2DM, from her research, she found that people who not physically active but non obese has normal blood glucose level (10). It also well known that physically inactive people with obesity can increase risk of diabetes mellitus compared with the active people. According to Colberg in Fealy (2018), physical activity was became the focus of non pharmacology therapy and prevention for T2DM and so does other non communicable diseases (11). ADA care and WHO recommended physical activity for T2DM was moderate intensity (40-60% VO₂max), aerobic training, 3-5 times/week and can be followed by resistance training 2-3 times/week. The combination of these to type of exercise has better benefit than if do just one type (7, 11). Machrina, 2022 found that interval training both slow interval and fast interval can be the alternatif model of training to reach moderate-vigorous intensity impact without burdening the patient (12).

Previous study from Sola et.al (2016) said that as light physical activity, walking was a kind of activity that easy to do and could be done in the daily live. So, it can be as an ideal and practical adjuvant for controlling blood glucose level in diabetes. From his study, he found that patient who walked more than 10000 steps a day with 300 – 400 Kcal/day has a good controlling in blood glucose level (13). Controlling blood glucose level was important to prevent chronic hyperglycemia promote immune response. The increasing of blood glucose while decreasing insulin secretion indirectly influence cell immune function especially macrophage and lymphocyte. Impairment of chemotactic function, phagocytosis and antigen presenting cell activation lead T2DM patients were easier to get secondary infection (14). Macrophage and monocyte were the key component of innate immune system. Both were also has an important role of initiation regulation, development and inflammatory disturbance (15).

Diabetes is associated with increased monocyte and macrophage inflammatory status. The increasing number of cytokines, such as IL-1 β , TNF- α , and IL-6 (likely through activation of NF- κ B), as well as increased expression of toll-like receptors TLR2 and TLR4. (16, 17). TLRs (Toll Like Receptors) was trans-membrane protein which detected microorganism during infection and has important role in innate immunity response

(18). They play pivotal roles in the recognition of damage-associated molecular patterns (DAMPs), which occur during type 2 diabetes (19).

Previous study showed that TLRs expression on cell immune correlated with pathogenesis of diabetes especially TLR2 and TLR4 induced insulin resistance. Pro inflammatory pathway from TLR2/4 which release persistent increase in cytokines and leads to progression of insulin resistance. Experimental studied and also clinical studied found that activity of TLR2/4 enhance in diabetes patients (20).

In this study, we found that the expression of TLR2 decline 0.71 fold and TLR4 0.99 fold This reduction probably because T2DM patient mostly keep physically active even though in light intensity. Another research in higher activity shown that Physical activity such as high-intensity interval training (HIIT) with short-term moderate-intensity continuous training significantly reduced monocyte/lymphocyte TLR4 expression and lymphocyte TLR2 expression in a group of sedentary adults at risk of developing T2D (21-23). Exercise-induced reductions in TLR expression and signalling may be of particular relevance to inflammation in T2D because mechanistic studies have found that hyperglycemia can increase TLR2 and TLR4 expression in monocytes and both TLR2 and TLR4 are implicated in the pathogenesis of insulin resistance.

Several of literature studies on human and animal models mentioned that exercise or physical activity developed various type of response on mRNA TLR, protein and cell receptor expression. TLRs expression changes showed the improvement of chronic pro-inflammation, which related to enhancing of insulin sensitivity. The effect of exercise or physical activity on TLR expression and inflammation response depend on modes and duration (24, 25).

6. CONCLUSION

Physical activity is important to maintain blood glucose level and metabolic status of T2DM patient. Even light intensity such walking and reduce time sitting, can reduce the expression of TLR2 and TLR4 that has an important role to immune response. The reduction of blood glucose levels will also provide a good response to the immune system. Therefore we can conclude that T2DM patients at Glugur Darat public health service have a good immune response. A good immunity is important for T2DM patient against secondary infection

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- **Author's contribution:** YM design the study, analyse data and write manuscript. DA, YSP collect data and test laboratory procedure. NSH write manuscript. GP proof read V.P. and N.Ž.S.
- **Conflict of interest:** There are no conflicts of interest..

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