ORIGINAL PAPER

Differential Expression of IL-10 Gene and Protein in Target Tissues of Rattus Norvegicus Strain Wistar Model Type 2 Diabetes Mellitus (T2DM)

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doi: 10.5455/aim.2018.26.87-92
ACTA INFORM MED. 2018 JUN; 26(2): 87-92
Received: Jan 27, 2018 • Accepted: Apr 16, 2018

ABSTRACT

Introduction: Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease caused by insulin resistance. Insulin resistance leads to hyperglycaemia that causes complication such as microangiopathy and macroangiopathy. The immune system of T2DM will be produce IL-10 as an anti-inflammatory cytokine role immune-stimulator and immunosuppressant in the organ system. This present study investigated of IL-10 gene profile and protein expression in the rat organ (*Rattus norvegicus*) strain Wistar model T2DM. **Material and Methods**: This research was used three of male rats group T2DM and three of male of normal rat as a control. The DNA tissues were isolated, amplified and sequenced by using IL-10 gene primer. The IL-10 protein profile and expression of rat tissues was analyzed using Experion-Pro260 gel and dot blotting using IL-10 antibody. **Results:** This study showed the differential expression of IL-10 gene profile among tissues among normal and T2DM groups. The IL-10 gene sequences, we found eight mutations in brain and twenty-seven mutations on gastric of T2DM group compare with control group, meanwhile there are no mutation in other tissues of both groups. The protein profile of all tissues in both groups was completely diverse as proper. Moreover, the level expression of IL-10 of heart, lung, gastric and kidney of T2DM group was lower than other tissues of both groups. **Conclusion:** This study concludes that T2DM animal model triggering mutation of IL-10 gene sequences of brain and gastric and induced the increasing level expression of IL-10 of ileum, brain and liver.

Keywords: hyperglycaemia, IL-10 gene, T2DM.

1. INTRODUCTION

The case of Type 2 Diabetes Mellitus (T2DM) in Indonesia has increased, the World Health Organization (WHO) reported the case of T2DM in Indonesia ranked 5th in the South-East Asia Region and continues to increase around 6% of the population by 2030 (1, 2). The T2DM is characterized by insulin resistance, which is causing hyperglycaemia. When the prolong onset of hyperglycaemia caused blood vessels damage and trigger abnormal metabolic activity resulting in diabetic ketoacidosis. In addition, the function of the heart as an organ that serves as blood circulation and kidneys as filtration blood up-regulated. Consequently the patient has chronic complications T2DM induces microangiopathy such as retinopathy, nephropathy, neuropathy and also macroangiopathy such as increased risk of cardiovascular disease and peripheral artery disease (PAD). T2DM also affects the damage other organs such as brain and digestive system (3-8).

Hyperglycaemia induces the inflammation by releasing pro-inflammatory (IL-1β, IL-6, TNF-α, etc) and anti-inflammatory cytokine (IL-4, IL-10, IL-11, IL-13, etc). The IL-10 is has important function as immune-stimulator and immunosuppressant to repair the damaged organ (9–11). Yaghini et al (12) reported the in serum IL-10 levels in T2DM patients lower than normal. Recently our study also shown the decreasing expression of IL-10 cause ileum destruction in rheumatoid arthritis animal model (13). We were also found the T2DM rats brain reduced cell proliferation and increased apoptosis in brain cells (14). Though, the cause of decreasing of IL-10 levels on T2DM rats brain still unclear. To examine the abnormality IL-10 gene sequence and IL-10 protein expression of different

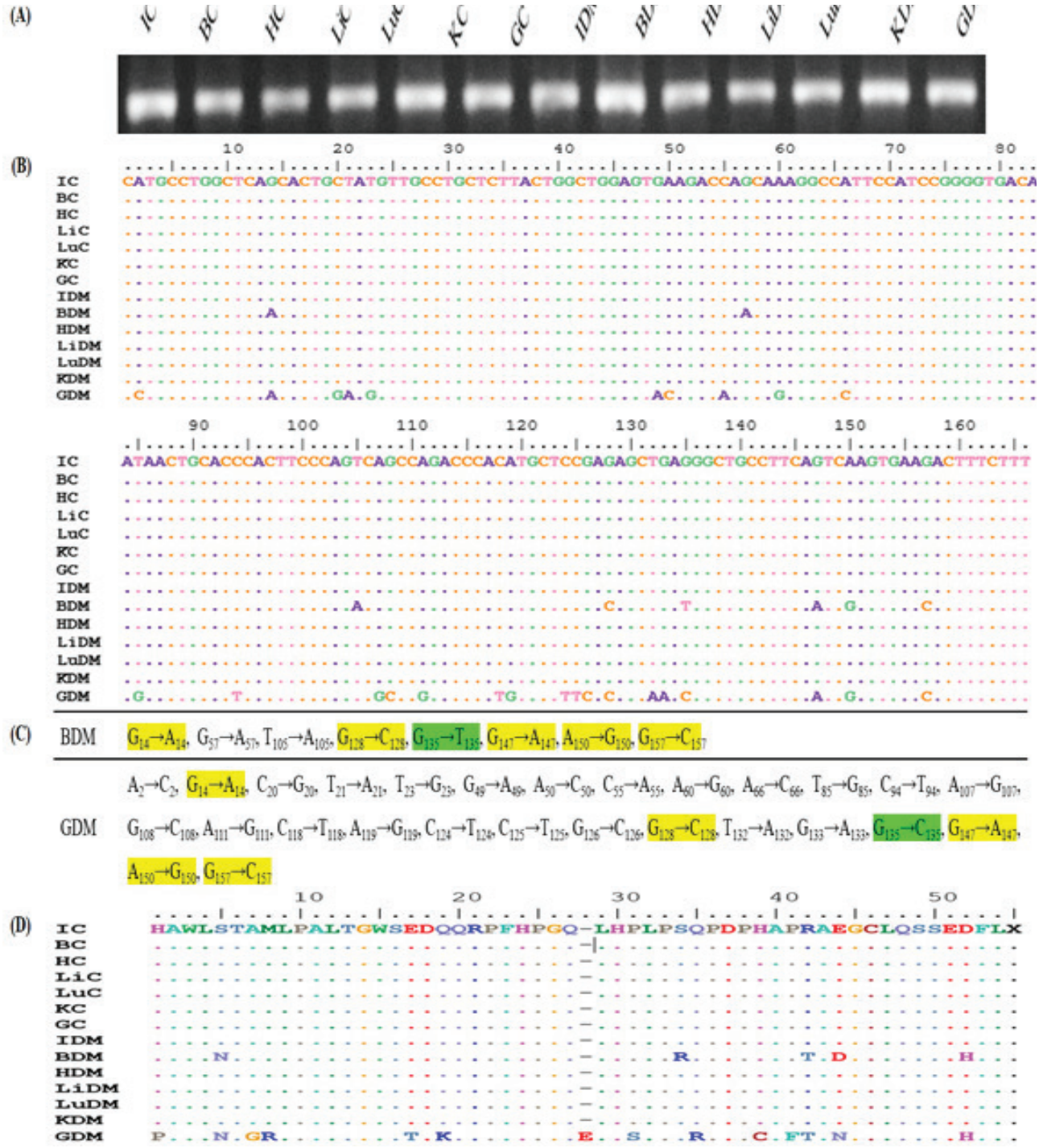


Figure 1. Profile IL-10 gene Control rats Group and T2DM rats Group. (A) Electrophoresis 1.5% PCR product using primer IL-10 470bp. (B) Alignment sequencing of IL-10 gene using the bioedit software. (C) Mutation in T2DM rats group at the BDM and GDM organ. Yellow colour showed same mutation in BDM and GDM, green colour showed mutation in same number base nucleotide but different nucleotide. (D) Aligment Profile amino acid from IL-10 gene. Control rats Group (ileum (IC), brain (BC), heart (HC), liver (LiC), lung (LuC), kidney (KC), gastric (GC)) and T2DM rats group (ileum (IDM), brain (BDM), heart (HDM), liver (LiDM), lung (LuDM), kidney (KDM), gastric (GDM)).

tissues, this study prepared T2DM rat animal model and control rat group. This study focused to investigate the differential profile of IL-10 gene sequence and IL-10 protein expression level in target tissues of rat model T2DM.

2. MATERIAL AND METHOD

Experimental Animal

The experimental animals were using *Rattus norvegicus* strain Wistar obtained from the Laboratory of Experimental Animal, Technical Implementation Units, Integrated Research and Testing Laboratory Gadjah Mada University Yogyakarta, Indonesia. The animals divided into two groups with 3 control rats (C) and 3 T2DM rats (DM). All animal obtained were acclimatized for one week. The T2DM rats group were established from normal rat that fed by high cholesterol food for 2 months and then injected with a single dosage by streptozotocin 25mg/BW a week after the rat positive hypercholesterolemia. Samples were collected from the control rats group (ileum (IC), brain (BC), heart (HC), liver (LiC), lung (LuC), kidney (KC), gastric (GC)) and T2DM

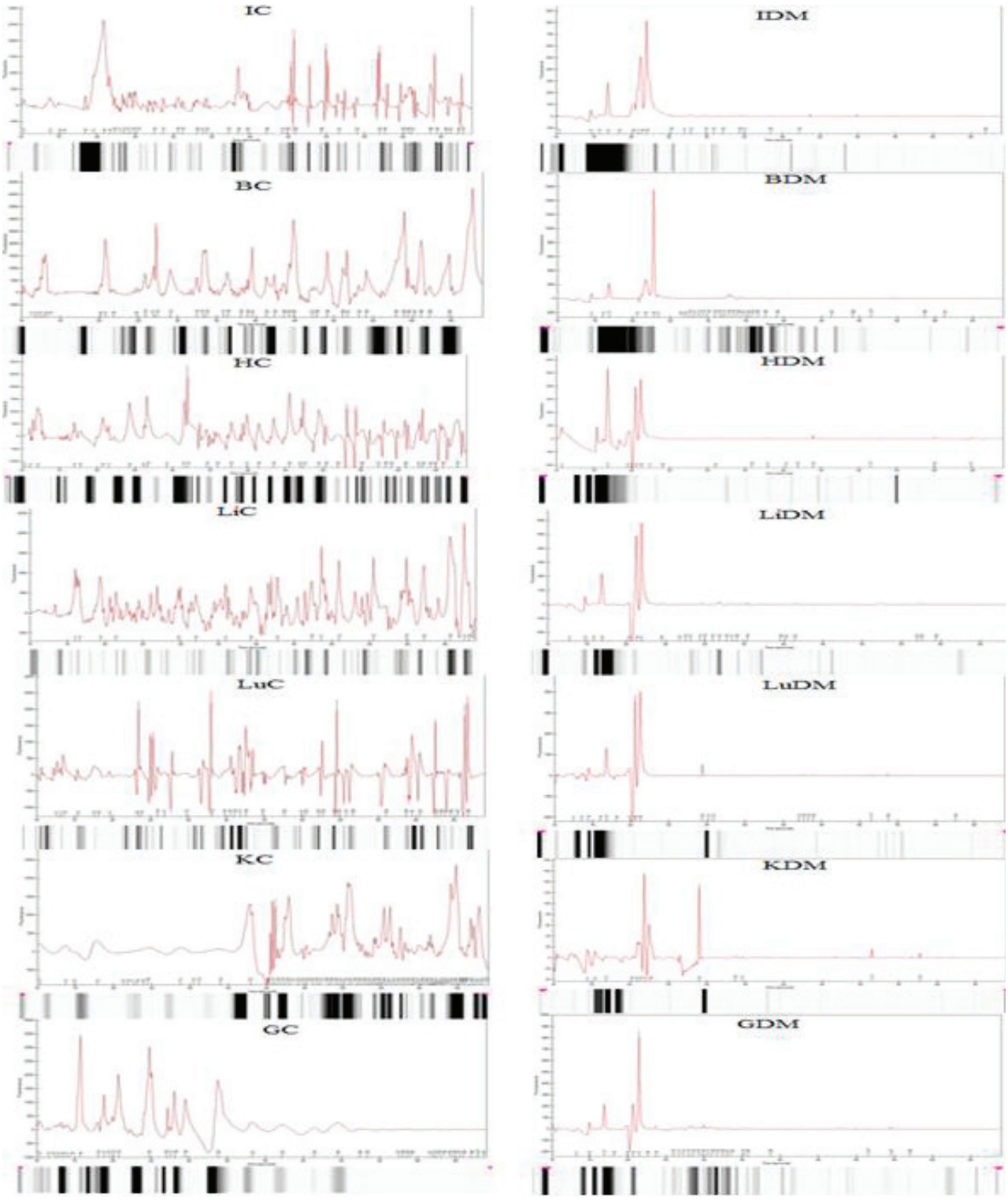


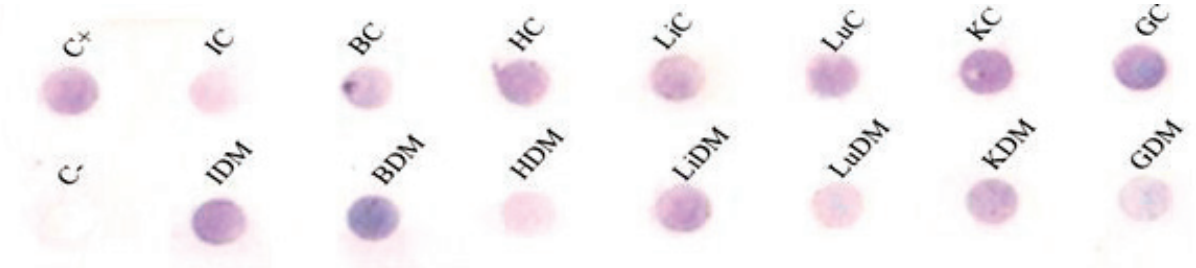
Figure 2. Profile of protein based on molecular weight of protein using analysis experion pro260. The profile of Control rats Group (ileum (IC), brain (BC), heart (HC), liver (LiC), lung (LuC), kidney (KC), gastric (GC)) and T2DM rats group (ileum (IDM), brain (BDM), heart (HDM), liver (LiDM), lung (LuDM), kidney (KDM), gastric (GDM)).

rats group (ileum (IDM), brain (BDM), heart (HDM), liver (LiDM), lung (LuDM), kidney (KDM), gastric (GDM)). This research study has been evaluated and approved by the Ethics Commission of Brawijaya University Malang, East Java (Certificate number, 417-KEP-UB Year 2015).

DNA Isolation, Amplification, and Sequencing

DNA Isolation method according Sambrook et al (15) with some modifications. DNA was amplified using the IL-10 primer from GenBank NC_005112.4. The primer was designed from exon 1 in IL-10 gene sequence, IL-10F 5'-ATAAAAGGGGGACACCGGGC-3' and IL-10R 5'-CTCATAACCCATGGCTTGGC-3'. Amplification products PCR program hot denaturation 94°C for 5 minutes (1 cycle), denaturation 94°C for 45 seconds, annealing 57°C for 45 seconds, extension 72°C for 45 seconds (35 cycles), and post extension 72°C for 7 min. The PCR products were measured qualitatively using 1.5% agarose gel. DNA sequencing was using ABI 3730xl DNA Sequencer (Koeln, Germany). Alignment

(A)



(B)

Sample	IC	BC	HC	LiC	LuC	KC	GC
Density (INT/mm2)	2.553.996,48	2.649.776,71	3.176.825,78	2.613.062,42	2.627.342,82	2.946.701,58	2.754.359,06
Sample	IDM	BDM	HDM	LiDM	LuDM	KDM	GDM
Density (INT/mm2)	2.759.354,29	2.808.508,06	2.296.954,44	2.941.045,60	2.345.950,05	2.556.843,24	1.619.154,67

Figure 3. Level of IL-10 epression in different tissues of T2DM and Normal Rats. (A) Level IL-10 protein was identified by dot blot analized using IL-10 antibody. (B) Statistic Analysis of dot blot. Positive control (C+) from LiC, negative control (C-) using PBS, Control rats Group (ileum (IC), brain (BC), heart (HC), liver (LiC), lung (LuC), kidney (KC), gastric (GC)) and T2DM rats group (ileum (IDM), brain (BDM), heart (HDM), liver (LiDM), lung (LuDM), kidney (KDM), gastric (GDM)).

was analyzed by *the Bioedit* software ver. 7.2.

Profiling protein and Dot Blotting

Protein isolation was conducted based method on Fatchiyah, et al (16). The protein concentration was measured by using Nanodrop spectrophotometer. Profile protein analysis used Experion Pro260 kit (Catalog Bio-Rad©, Hercules, CA). Detection of IL-10 protein expression level using dot blotting based on Rohmah et al (17) with some modifications. Primer antibody was using mouse anti IL-10 (1:1500 Santa Cruz Biotechnology, Inc) and Anti-mouse IgG labeled with Alkaline Phosphatase conjugated as secondary antibody. Density of IL-10 reaction measured quantitatively by ChemiDoc Gel Imaging (BioRad) and Quantity One program and analyzed by Microsoft Excel.

3. RESULTS

IL-10 Gene Profile

The IL-10 gene amplification (Figure 1A) was successfully demonstrated with 1.5% gel agarose electrophoresis of 470bp. The sequence target DNA exon 1 of IL-10 gene size 166bp. Alignment using *Bioedit* showed a mutation were two organs, in the brain (BDM) were eight bases, (Figure 1C) and twenty-seven bases of gastric (GDM). The similar type of mutation that occurs are five mutations that change $G_{14}\rightarrow A_{14}$, $G_{128}\rightarrow C_{128}$, $G_{147}\rightarrow A_{147}$, $A_{150}\rightarrow G_{150}$, $G_{157}\rightarrow C_{157}$, this mutation induced the amino acid (Ser-Arg-Asp) also changed into Asn-Thr-His. Besides that, the other mutation in brain (BDM) are $G_{57}\rightarrow A_{57}$, $T_{105}\rightarrow A_{105}$, $G_{135}\rightarrow T_{135}$, induced amino acid Ser into Arg and in gastric (GDM) are $A_{2}\rightarrow C_{2}$, $C_{20}\rightarrow G_{20}$, $T_{21}\rightarrow A_{21}$, $T_{23}\rightarrow G_{23}$, $T_{48}\rightarrow A_{48}$, $G_{49}\rightarrow A_{49}$, $C_{54}\rightarrow A_{54}$, $A_{60}\rightarrow G_{60}$, $A_{66}\rightarrow C_{66}$, $T_{85}\rightarrow G_{85}$, $C_{94}\rightarrow T_{94}$, $A_{107}\rightarrow T_{107}$, $G_{108}\rightarrow C_{108}$, $A_{111}\rightarrow G_{111}$, $C_{118}\rightarrow T_{118}$, $A_{119}\rightarrow G_{119}$, $C_{124}\rightarrow T_{124}$, $C_{125}\rightarrow T_{125}$, $G_{126}\rightarrow C_{126}$, $T_{131}\rightarrow A_{131}$, $G_{132}\rightarrow A_{132}$, $G_{135}\rightarrow C_{135}$, induced the amino acid (His-Ala-Met-Glut-Gln-Pro-Gln-His-Pro) to be Pro-Gly-Arg-Thr-Lys-Ser-Arg-Cys-Phe (Figure 1D). In GDM we found new amino acid in number twenty-eight is Glut as absent in other tissues both of group.

Protein Profile

The protein profiles found in the control rats group (C) and T2DM rats group showed different results (Figure 2). In organ Ileum (IC) found 37 bands, brain BC) found 42 bands, heart (HC) found 36 bands, liver (LiC) found 18 bands, lung (LuC) found 40 bands, kidney (KC) found 63 bands, gastric (GC) found 39 bands, ileum (IDM) found 19 bands, brain (BDM) found 26 bands, heart (HDM) found 17 bands, liver (LiDM) found 25 bands, lung (LuDM) found 19 bands, kidney (KDM) found 13 bands, gastric (GDM) found 28 bands. Protein profile in the control rats group (C) and T2DM rats group showed different amounts of protein level, the number of protein bands in the normal group was higher than T2DM.

Identification of IL-10 protein expression using specific antibody showed that blue-purple visualization on spots of positive control and proteins from protein sample. Binding of proteins and antibody specific showed that control positive was higher of mean density than T2DM (Figure 3A). In this study we found different amount of density (Figure 3B). The density of IL-10 in IDM, BDM, HC, LiDM, LuC, KC and GC higher than among of tissues.

4. DISCUSSION

The IL-10 gene mutations that occur in BDM and GDM cause different effect on organ function. Mutations in BDM are a type of substitution mutation, in which there is a change of base in some parts replaced with another base (Figure 1C). This mutation accounts for about 5% of the total DNA sequence of IL-10 and the mutation leads to increased pro-inflammatory cytokines as mediators of damaged organ. The IL-10 gene mutations occurring in the brain will cause the increased performance of the IL-10 as anti-inflammatory. The inflammation in T2DM microgial cells brain increasing immunocompetent cells has potent and diverse effects on essentially all hematopoietic cells that infiltrate the brain following injury (14, 18).

Mutations occurring in GDM are a type of substitution mutation with a mutation count of about 16% of the total DNA sequence. Mutations occurring in the GDM will cause decreased effectiveness of IL-10 performance in gastricas an anti-inflammatory cytokine resulting in increased pro-inflammatory mediation TNF-α exacerbates damaged organ in the T2DM.Mutation of IL-10 in gastricdoes not regulate the homeostasis of the gastric mucosa and induce the development of mucosal metaplasia. Therefore, further investigation on the role of epithelial IL-10 in gastric tissue is needed (19, 20). Kryukov, et al (21) concluded that 20% of new mutations in humans result in loss of function, while 53% had adverse effects and 27% were neutral effectively related to phenotype.

Mutation occurring in sequence of IL-10 gene in organ BDM and GDM shows that T2DM may lead to frameshift mutation same amino acid (Ser→Asn, Arg→Thr, Asp→His), but mutation in amino acid number fourty-four has different amino acid result, BDM (Glu→Asp), GDM (Glu→Asn). Other frameshift mutations in BDM were (Ser→Arg), GDM (His→Pro, Ala→Gly, Met→Arg, Glu→Thr, Gln→Lys, Pro→Ser, Gln→Arg, His→Cys, Pro→Phe) (Figure 1D).

Bands in the normal rats group (C) and the T2DMrats group (DM) showed different amounts of bands, as a whole, the number of protein bands formed in the normal group was higher than that of the protein band under T2DM. Differences of bands density showed that in organs with T2DM losing some protein when compared with normal. One of the causes of flooding of activated protein differences is due to the condition of insulin resistance in patients with T2DM. Pareire et al (22) resulted that men with T2DM had insulin resistance against protein metabolism. Insulin-resistant, impaired energy inhibits stimulation of protein synthesis. Their study indicated that the clinical entity of T2DM involves defective protein metabolism impaired insulin plus amino acid stimulated protein synthesis in T2DM men may be of clinical importance.

Spots with blue-purple visualization on PVDF membrane indicated that primer antibody and secondary antibody had positive reaction with recombinant IL-10 protein. In this research we evaluated the expression of IL-10 protein by the primary antibody dotblot assay and did not use separated protein according to their molecular weight (23). The dot blot assay also showed colour intensity to determine titer of antigen antibody binding. In the previous study, Rohmah et al (13) showed that the ileum destruction was also related with alteration of inflammatory cytokines, the increasing of cytokine pro-inflamtionIL-17 and decreasing of cytokine anti-inflammation IL-10 in Rheumatoid Arthitis model with inflammation response. Interestingly, in this study we found that IL-10 on IDM, BDM, and LiDM (Figure 3B) indicate opposite performance, where earlier inflammatory conditions may provide different performance of IL-10 function. Interestingly on organ IDM, BDM, and LiDM the performance of IL-10 expression increased compare with normal. The increased expression of IL-10 protein correlated with dendritic cell signal transduction in IDM, BDM, and LiDM. IL-10 signaling is targeted toward surplus STAT1 activation, different with STAT3. Signaling from STAT1 causes IL-10 triggered pro-inflammatory responses, supports Th1-like inflammation, processes that favor apoptosis and control of tumour growth leading to increased inflammation in inflammatory diseases (24–26).

5. CONCLUSION

Based on our result, the T2DM animal model cause mutations on IL-10 gene and amino acid sequences of brain and gastric tissues. Those mutations induced the increasing of IL-10 expression level in ileum, brain, liver, but decreasing of IL-10 expression level in heart, lung, kidney and gastric.

- Acknowledgments: This research was supported by DGHE, Ministry of Science, Technology and Hinger Education research grant 2017 and LPDP 2017 Indonesia. Thanks to Rista N.R for kindness manuscript correction and discussion, thanks to Biosains Institute UB for providing the laboratory equipments.
- Conflict of interest None.
- Authors contributions: YB, APWM, TS and FF designed and data analysis the research, data interpretation and drafting & correcting the manuscript.

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