5 (2), 2019, 96-101
Received 2019-28-09; Revised 2019-22-10; Accepted 2019-28-10
Available online at : http://ejurnal-analiskesehatan.web.id

Quality of Genom in Type II Diabetes Mellitus Patients in Viewed of Temperature, Storage Duration, Number of Leukocyte

***Puspitasari[1], Evi Rinata[1], Rohman Dijaya[2], Siska Aprilia[1], Dina Trikumalasari[1], Livia Nur Azzah[1], Qilmia Fanani[1], Miftahul Mushlih[1,3], Andika Aliviameita[1], Dian Delta[3]**

[1]Faculty of Health Science, Universitas Muhammadiyah Sidoarjo, [2]Faculty Of Science And Technology, Universitas Muhammadiyah Sidoarjo Jl. Mojopahit No. 666, Sidoarjo, JawaTimur. [3]Indonesian Genetic and Biodiversity Community, Jl. Ikan Mujair No. 15, Tunjung Sekar, Lowok Waru, Malang *Email: puspitasari@umsida.ac.id
DOI: 10.31964/mltj.v%vi%i.229

Abstract: Type II Diabetes Mellitus is a disorder caused by genetic and environmental factors. Molecular analysis of T2DM abnormalities has been carried out. But the analysis in sample preparation especially the stages of DNA isolation has not been done much. The aims of this study are to investigate the effect of temperature, storage duration and level of white blood cell (WBC) with Genome Quality in T2DM. The treatment (n:10) which were divided into several tubes and then stored at 4 °C, 25 °C and 32 °C for 21 days. To determining storage duration effect, we use periodically isolation of DNA (3, 15 and 30 days) after sampling. The effect of WBC with DNA quality was carried out using 17 samples. DNA isolation was done by the DNA Isolation Kit manual without modification and then tested qualitatively and quantitatively. Based on this research, it can be concluded there is a correlation between the numbers of WBC with DNA quality. The higher the number of WBC, the higher DNA concentration (r: 0.818. p value: 0.000). The concentration of DNA at a temperature of 4°C (135.1 ± 165.2 ng / µl) was higher compared with the temperature treatment 25 ° C (29.7 ± 36.5 ng / µl) and 32 ° C (22.14 ± 7.13 ng / µl) ($p<0.05$). Increasing storage duration caused decreasing gradually amount of DNA concentration significantly ($p<0.05$) (3d: 104.58±39.4 ng/µl, 15d: 80.01±64.64ng/µl, 30d: 16.45±8.37ng/µl).
Keywords: type II diabetes mellitus; genom quality; white blood cell (WBC); temperature; storage duration

INTRODUCTION

Diabetes is divided into several categories: Type I Diabetes Mellitus, Type II Diabetes Mellitus, Gestational diabetes mellitus (GDM), Impaired glucose tolerance (IGT) and impaired fasting glycaemia (IFG) & Diabetes due to other causes (WHO, 2016). Type II Diabetes Mellitus is a metabolic disorder caused by a lack of the hormone insulin, insulin that does not work properly or effectively which is characterized by increased blood sugar levels (Suneja et al., 2018). It is 422 million adults were living with diabetes in 2014. Type II Diabetes Mellitus is characterized by increased fasting sugar levels> 126 mg / dL (7.0 mmol / L), random glucose values> 200 mg / dL (11.1 mmol / L), HbA1C> 6.5% (48 mmol / mol), or patients with symptoms of hyperglycemia or hyperglycemic crisis (WHO, 2016).

Corresponding Author: Puspitasari
Faculty of Health Science, Universitas Muhammadiyah Sidoarjo
Email: puspitasari@umsida.ac.id

It is well known that Type II Diabetes Mellitus is not a simple disease inherited as Mendelian's lows. But it is a complex diseases controlled by genetic and environmental factors (Shafee & Lowe, 2017;). Genetic factors are the factors that have been coded and inherited by parents of their offspring (Bonnefond & Froguel, 2015). While environmental factors are factors that arise from daily life habits including diet, types of food consumed, environmental areas and others (Besseling, Kastelein, Defesche, Hutten, & Hovingh, 2015). Same case type II Diabetes Mellitus is correlated with other metabolic failure such as Obesity (Leitner et al., 2017). The efforts to identify molecular genetic diseases in recent years have been intensively carried out using molecular methods (Wu, Ding, Tanaka, & Zhang, 2014). Including screening and identification of polymerofism (Lyssenko & Laasko, 2013). The method of molecular biology can identify accurately and be able to find out of gene related with type II Diabetes Mellitus (Ingelsson & McCarthy, 2018). The first common steps in molecular analysis including sample preparation and DNA Isolation (Tan & Yiap, 2009).

Several factors are thought to be successful in DNA isolation. Temperature and storage duration (Al-Griw et al., 2017), pressure and pH (Bergerová *et al.*, 2011) and sample condition (Samoo et al., 2017). Research on the quality of genomes from DNA isolation from samples diagnosed with abnormalities, especially type II Diabetes Mellitus has never been done. Huang et al (2017) used normal human blood samples. In this study was use several treatments to simulate the effects of external factor in Type II Diabetes Mellitus blood sample using the higher temperatures than before (Huang et al., 2017). This study also analyzed the correlation between the number of white blood cells (WBC) and genome concentration in Type II Diabetes Mellitus sample.

MATERIALS AND METHOD

The research method uses laboratory experiments. Ethical study was approved by the dental ethics commission, Airlangga University, Indonesia with no. 195 & 195 & 196 / HRECC.FODM/V/2019. A total of 27 samples of Sidoarjo Branch Wound Houses were used in this study (n: 10 for analyze the effect of storage duration and n: 27 for analyze correlation WBC with DNA Quality). Whole Blood Type II Diabetes Mellitus patients were taken macro sampling in venous blood as much as 3 cc into EDTA anticoagulant tubes. Furthermore, blood samples of Type II Diabetes Mellitus were labelled and then examined at 4 °C, 25 °C, and 32 °C for 21 days. To analyze the effect of storage time on DNA quality was gradually examined by 5, 15, and 30 days stored at 4 ° C. WBC was counted with Sysmex Kx-21N hematology analyzer. Gene Aid DNA isolation kit (Genomic DNA Mini Kit (Blood / Cultured Cell) No. Ver.02.10.17 protocol was followed without modification. DNA Quality Test is carried out qualitatively and quantitatively. The Quantitative Test uses a double beam UV-Vis spectrophotometer (thermo evolution 201) with 3 replications per sample (the ratio of DNA and ddH2O is 1: 8 / 9x dilution) using A 260 / A 280nm wavelength. Qualitative test of DNA isolation used 1% agarose gel electrophoresis. Gel visualized using UV Trans illuminator (302 nm). Statistical analysis using Shapiro Wilk to test data distribution (CI: 95%). The data is then processed using the one way anova and Kruskal Wallis to determine the differences and Pearson to determine the correlation.

RESULTS AND DISCUSSION

Diabetes mellitus is usually accompanied by severe infections. Diabetes mellitus induces immunity deficiency through several mechanisms. Increased sugar levels can cause disruption of the function of phagocytes of leukocyte cells that will accumulate in the site of inflammation. The higher the sugar level, the lower the number of leukocytes (Chodijah, Nugroho, & Pandelaki, 2013). Thus analysis needs to be related to molecular analysis especially for examination using blood samples. To find out the correlation between the number of leukocyte counts and the quality of DNA produced, 17 samples were used. The results of statistical analysis show the correlation between WBC counts and the quantity of DNA shows an r: 0.869, p: 0.000 (Figure 1). It is means, there is a significant correlation between of them. Huang et al., (2017) states that the correlation between WBC and DNA quality correlates 0.708. The use of diabetes samples with higher glucose assumption does not seem different with the quality of the genome produced.

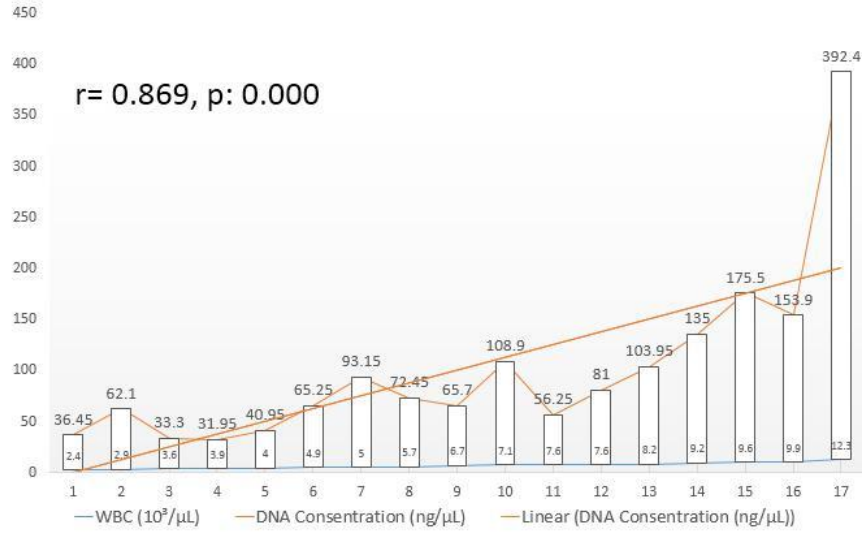

Figure 1. Correlation of Leukocyte Number with Genome Concentration in Type II Diabetes Mellitus samples

The success of DNA isolation is not only influenced by circumstances and methods. All samples are in the normal range. The successful of DNA isolation is closely related to the DNA source of sample. In this study using blood samples assuming blood is a sample that is usually taken and easy to store. However, the condition of the blood component, especially in the sample, is not stable depending on the patient's or environment condition. To find out the effect of temperature on DNA quality, 10 samples were used. Sampling was treated by using a temperature of 4 °C with general storage assumption. The temperature is 25 °C which is room temperature assumption while the temperature of 32 °C is the average temperature outside the room. Respondents from this study were 55 ± 7.6 years old. The average random blood sugar obtained was 300 ± 115.1mg / dl (n: 10). The results of genomic DNA concentrations are 135.1 ± 165.2 ng / µl at 4 °C, 29.7 ± 36.5 ng / µl at 25 °C and 22.14 ± 7.13 ng / µl at 32 ° C. The DNA concentration at the temperature of 4°C was higher and significantly differ compared with 25 °C and 32 °C (Kruskal-Wallis test. p value 0.008 / p<0.05).

To find out the effect of storage time on DNA quality, 4° C was used for storage. Then DNA is isolated periodically, ie 3 days, 15 days, 30 days. The quantity decreased from 104.58 ± 39.4 ng / µl to 80.01 ± 64.64 ng / µl in the treatment of 3 days to 15 days. Whereas at a delay of 30 days the average DNA concentration decrease to 16.45 ± 37 ng / µl (p value: 0.000). Decreasing DNA concentration is significantly caused by temperature due to storage duration in 4° C. Although DNA lasts for 15 days when stored in whole blood, the quantity of DNA indirectly decreases because of white blood cell lysis. Storage of whole blood for more than three days significantly changes the quality of DNA (Huang et al., 2017)

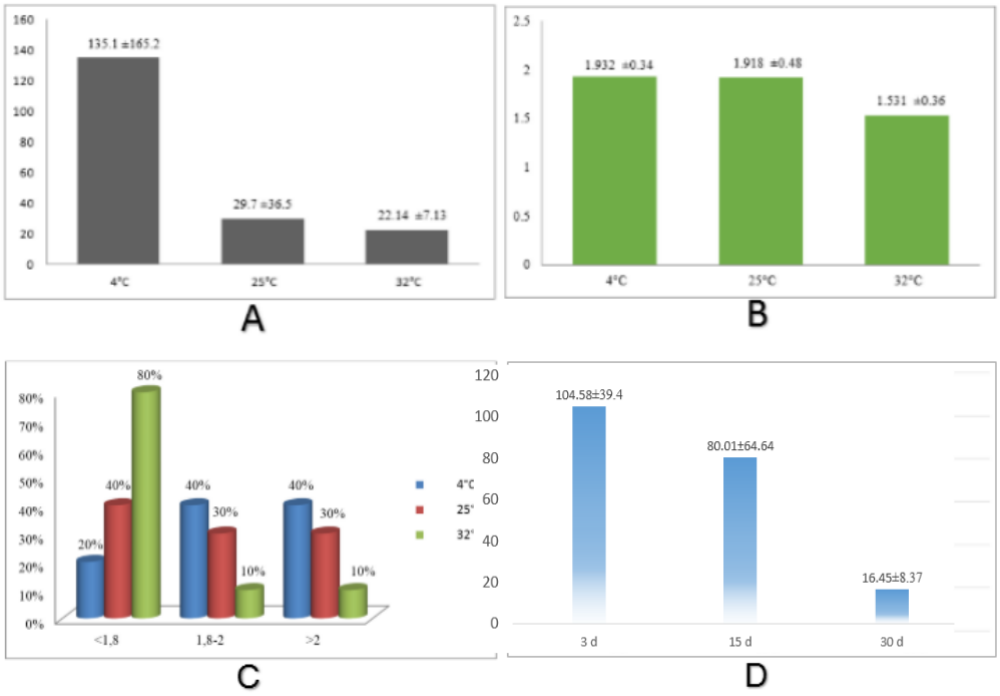

Figure 1. Effect of temperature and storage duration on DNA quality
A. DNA Concentration treated by temperature (ng/µl) (*Kruskal Wallis*<0.05). B. Purity of DNA (A 260/280) (one way anova, p>0.05). C. Percentage of Purity level of DNA influenced by temperature, D. DNA Concentration treated by storage duration (*Kruskal Wallis,* p<0.05)

Electrophoresis analyses shows there a degradation of band thickness in each treatment different temperature (Figure 2). The volume of electrophoresis inserted into the well identifies the presence of a decrease in amount of DNA obtained. Previous studies blood stored at -30 ° C did not change in quality within 15 years of storage(Chen et al., 2018). the success of DNA isolation in the blood is determined by the WBC,storage methods,sample condition (Huang et al., 2017), dan isolation method (Chen et al., 2018). Blood can be stored at temperatures (4 °C and -20 ° C) after DNA is extracted, but to have intact DNA with a higher molecular weight at -20 ° C it is recommended to pure DNA storage (AlRokayan, 2000).

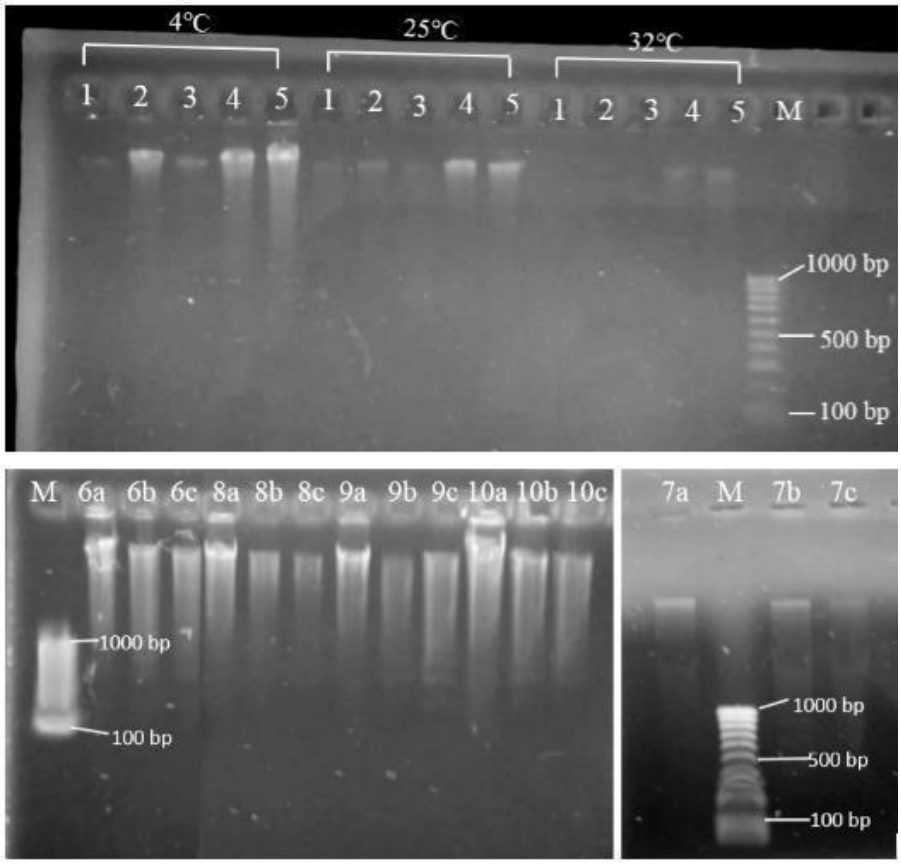

Figure 2. The differences of Genome Electroforesis based on temperature treatment (6-10 indicate sample, a: 4° C, b: 25° C, b: 25° C). Marker: 100 bp

CONCLUSION

Based on this study it can be concluded that the number of WBC has a positive correlation with the DNA produced. The recommended number of WBC is more than 4 (10^3/µl). The concentration of DNA stored at a temperature of 4°C was higher compared with the temperature treatment 25 ° C and 32 ° C, so the recommended temperature is 4° C. Increasing storage duration caused a decreasing amount of DNA concentration gradually. In this study, the recommended storage duration not more than 3 days.

ACKNOWLEDGEMENT

The author would like to acknowledge to Universitas Muhammadiyah Sidoarjo for providing the applied research fund 2019. Thanks for Molecular Biology laboratory and Rumah Luka Sidoarjo for providing the facilities.

REFERENCES

Al-Griw, H. H., Zraba, Z. A., Al-Muntaser, S. K., Draid, M. M., Zaidi, A. M., Tabagh, R. M., & Al-Griw, M. A. (2017). Effects of storage temperature on the quantity and integrity of genomic DNA extracted from mice tissues: A comparison of recovery methods. *Open Veterinary Journal*, *7*(3), 239–243.

AlRokayan, S. A. (2000). Effect of storage temperature on the quality and quantity of DNA extracted from blood. *Pakistan Journal of Biological Sciences*, *3*(3), 392–394.

Bergerová, E., Godálová, Z., & Siekel, P. (2011). Combined effects of temperature, pressure and low pH on the amplification of DNA of plant derived foods. *Czech Journal of Food Sciences*, *29*(4), 337–345.

Besseling, J., Kastelein, J. J. P., Defesche, J. C., Hutten, B. A., & Hovingh, G. K. (2015). Association between familial hypercholesterolemia and prevalence of type 2 diabetes mellitus. *JAMA - Journal of the American Medical Association*, *313*(10), 1029–1036.

Bonnefond, A., & Froguel, P. (2015). Rare and common genetic events in type 2 diabetes: What should biologists know? *Cell Metabolism*, *21*(3), 357–368.

Chen, W. C., Kerr, R., May, A., Ndlovu, B., Sobalisa, A., Duze, S. T., … Babb De Villiers, C. (2018). The Integrity and Yield of Genomic DNA Isolated from Whole Blood Following Long-Term Storage at -30°C. *Biopreservation and Biobanking*, *16*(2), 106–113.

Chodijah, S., Nugroho, A., & Pandelaki, K. (2013). Hubungan Kadar Gula Darah Puasa Dengan Jumlah Leukosit Pada Pasien Diabetes Mellitus Dengan Sepsis. *Jurnal E-Biomedik (EBM)*, *volume 1*, 602–606.

Huang, L. H., Lin, P. H., Tsai, K. W., Wang, L. J., Huang, Y. H., Kuo, H. C., & Li, S. C. (2017). The effects of storage temperature and duration of blood samples on DNA and RNA qualities. *PLoS ONE*, *12*(9), 1–13.

Ingelsson, E., & McCarthy, M. I. (2018). Human Genetics of Obesity and Type 2 Diabetes Mellitus: Past, Present, and Future. *Circulation. Genomic and Precision Medicine*, *11*(6), e002090.

Leitner, D. R., Frühbeck, G., Yumuk, V., Schindler, K., Micic, D., Woodward, E., & Toplak, H. (2017). Obesity and type 2 diabetes: Two diseases with a need for combined treatment strategies - EASO can lead the way. *Obesity Facts*, *10*(5), 483–492.

Lyssenko, V., & Laasko, M. (2013). Genetic Screening for the Risk of Type 2 Diabetes. *Diabetes Care*, *36*, 120–126.

Organization, W. H. (2016). Global Report on Diabetes. *Isbn*, *978*, 92–94.

Samoo, I. T., Khatri, P., Bhutto, B., Tariq, M., Chandio, I., Soomro, M., … Shams, S. (2017). *Effect of Temperature and Storage Time on DNA Quality and Quantity from Normal and Diseased Tissues*. 203–206.

Shafee, T., & Lowe, R. (2017). Eukaryotic and Prokaryotic Gene Structure. *Ssrn*, (January).

Suneja, S., Christian, Y., & Chandra, N. C. (2018). Milieu of Diabetes in the 2nd Decade of 21st Century. *Journal of Diabetes & Metabolism*, *09*(09), 1–14.

Tan, S. C., & Yiap, B. C. (2009). *DNA , RNA , and Protein Extraction : The Past and The Present*. *2009*.

Wu, Y., Ding, Y., Tanaka, Y., & Zhang, W. (2014). Risk factors contributing to type 2 diabetes and recent advances in the treatment and prevention. *International Journal of Medical Sciences*, *11*(11), 1185–1200.