# Quantitation of human Telomerase Reverse Transcriptase (hTERT ) level in normospermic man using ELISA method

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Abstract—Human Telomerase Reverse Transcriptase (hTERT) has a role to maintain the integrity of telomeres at the ends of chromosome which actively proliferated. This enzyme is found in somatic cells and germ cells, one of which is in human sperm cells. The purpose of this study was to measure the quantity of hTERT level in normospermic man. The method used in this research is quantitative descriptive which used ELISA to determine the level of hTERT in human sperm cells. The results showed that the lowest standard concentration of 0pg/mL produced an average absorption value of 0.000. while the lowest standard d with a concentration of 5000pg/mL produced the highest average absorption of 0.088. In addition, the value of hTERT level in human sperm was 2.5pg/mL . Through the determination of hTERT level in sperm, further analysis can be done regarding the quality and infertility in men.

Keywords—aging, cell death, germ cells, spermatozoa, telomerase

## I. INTRODUCTION

Telomeres are non-gene fragments at the ends of chromosomes that prevent shortening of chromosomes. This fragment length approximately 5-15kb in each chromosomes ends[1]. Over the life span of the cells, telomeres will become shorter and cells will age (senescent) after the minimum telomere length size has been reached[2]. During DNA replication, the nitrogen bases do not replicate correctly in the lagging strand. This occurs because DNA polymerase replicate the DNA in lagging strand through different mechanism so it leaves a blank fragment at the end of 3' DNA lagging strand[3]. Telomere presences to complete this blank fragment.

To maintain the integrity of telomeres at the ends of chromosomes, the role of telomerase is needed. This enzyme works by adding a repeating DNA sequence ("TTAGGG" to all vertebrates) at the end of 3' DNA strands on the telomeres[4]. As a ribonucleoprotein complex, telomerase is composed of RNA and protein. In its active form, it consists of two main subunits, namely telomerase reverse transcriptase (TERT and hTERT in humans) and RNA telomerase that contain template sites for DNA elongation. Each subunit has a specific biological function, while hTERT is a catalytic part that acts as a limiting factor for telomerase activity and telomere extension[5].

Telomerase in human is commonly found active in germ cells during embryogenesis, in adult stem cells and in active immune cells[6]. This enzyme is absent or expressed in very low level in adult cells that have undergone differentiation and inactive immune cells[7]. Male germ cells are unique in terms of enzymatic activity. The highest telomerase activity occurs in the early germ cells or spermatogonia[8]. In the spermatocyte stage, the telomerase level is high. Spherical spermatids have the same telomerase level at the stage of spermiogenesis, but telomerase level decrease along with cells that are not actively transcribed during chromatin[9].

Examination of sperm DNA integrity is indeed commonly done as an additional examination to support the examination of semen analysis in enforcing male infertility. There are many techniques that can be carried out for the examination, but in general requires a long time, equipment and quite expensive cost. That is why the researchers added new innovations to analyze immunologically by calculating hTERT level in human sperm cells which used ELISA technique.

## II. METHOD

This research was done under ethical clearance from Faculty of Medicine, Universitas Indonesia No.: KET-872/UN2.F1/ETIK/PPM.00.02/2019 protocol number 19-07-0924. The method of this research was quantitative descriptive, to determine the human Telomerase Reverse Transcriptase (hTERT) level in normospermic man which used ELISA technique. The 3mL of sperm sample was obtained from healthy man of productive age. Sperm sample was taken after the respondent had not ejaculated for 3-5 days and the sample was stored in a collecting tube.

**Sample preparation.** Sperm cells are inserted into a 1.5mL microtube, then centrifuged at 1000xg for 5 minutes. After the centrifugation, the supernatant was taken and washed for 3 times using cold PBS as much as 150-250μL. Next, the centrifugation was carried out at 1500xg at 4o C for 10 minutes and the supernatant was taken. The next step is Freeze-thaw, which was done by incubating the sample at -80o C until it frozen, then the sample was incubated at the room temperature until it thawed, then incubated at -80$^{o}$C.

**Reagent preparation.** All reagents are stored at room temperature (18-25°C) for 15 minutes. Wash Buffer was made by diluting 30mL Aquades. The standard solution was prepared by dissolving 5000pg/ mL standard powder which diluted in stages as follows: 5000; 2500; 1250; 625; 312.5; 156.25; 78.13; 0 pg/mL.

**hTERT quantitation.** The sample was prepared by ELISA kit then was analyzed at 450 nm wavelength using the ELISA Reader (Biosystem) machine. The absorbance values which obtained from ELISA Reader were processed in Microsoft Excel 2010 to make a standard curve. This curve showed the correlation between the concentration of solutions (x-axis) and the absorbance ( y-axis ). The curve also showed the regression equation y = ax + b, which means the slope of the line (a); a constant (b); the level of hTERT (x).

## III. RESULTS AND DISCUSSION

This studi aimed to determine the level of telomerase in human sperm cells as a follow-up research after a study of human Telomerase Reverse Transcriptase (hTERT) level in lymphocytes. This follow-up study used human sperm cells as fresh biological samples in order to determine the hTERT level immunologically which can determine the sperm quality. Human sperm are used as samples because the cells always regenerate rapidly. Telomerase in normal cells can be found in germ cells, lymphocytes,and hepatocytes because these cells have unlimited proliferation[10].

**Table 1. The value of absorbance**

| Concentration of solution (pg/mL) | U1 | U2 | Mean |
|---|---|---|---|
| Standard 1 (0) | 0.000 | 0.000 | 0.000 |
| Standard 2 (78.13) | 0.005 | 0.006 | 0.006 |
| Standard 3 (156.25) | 0.016 | 0.015 | 0.016 |
| Standard 4 (312.50) | 0.019 | 0.013 | 0.016 |
| Standard 5 (625) | 0.022 | 0.023 | 0.023 |
| Standard 6 (1250) | 0.045 | 0.035 | 0.040 |
| Standard 7 ( 2500) | 0.080 | 0.086 | 0.083 |
| Standard 8 (5000) | 0.086 | 0.090 | 0.088 |

Note: U1 was the absorbance on the first test, U2 was the absorbance of the second test. The mean is the average absorbance between the first and second tests.

The ELISA method used in this study is the ELISA Sandwich, which a method carried out by placing a sample between the first and second antibodies so that it is like a sandwich. The use of the ELISA method in this study is based on its high sensitivity, specifications, and flexibility[11]. In addition, this method is easy and fast to do because it involves specific antigens. Every well that has been provided in the kit has been given anti-TE (Telomerase Enzyme) antibodies. The first antibody (known as a catching antibody) is coated on the well, the anti-TE antibody. This method generally requires the use of suitable antibody pairs for the epitope part of the antigen molecule. In this study, the antigen was telomerase.

Telomerase bound with anti-TE antibodies and Bio-tynalated Ab, as a second antibody, was given to make the bond stronger. In the next step, Avidin-HRP was added as peroxidase enzymes which bound to the per- oxidase reagent substrate then bound to the reagent substrate. After adding a coloring reagent substrate to the well, the solution turned on to blue as an indicator of specific antibodies in the sample. Then, this enzymatic reaction was stopped using a stop solution and the solution turned on to yellow[12].

Table 1 was the absorbance data according to the concentration of standard solutions which diluted in stages. The Standardization procedure aimed to find out whether the value from this study had no significant difference with the value from the actual data. The table showed that standard 1 with the lowest concentration of 0pg/mL produced the lowest mean of the absorbance value, which is equal to 0.000. In contrast, standard 8 with a concentration of 5000pg/mL produced the highest mean of the absorbance value. The absorbance data showed that the higher the concentration of the solution, the greater the absorbance value produced.

Figure 1 was a telomerase standard curve from the absorbance of standard solutions data. R2 was a number whose value is between 0 to 1 which showed how close the estimated value is to the regression analysis that represents the actual data. The regression analysis was reliable when the determinant value which obtained on standard measurements was equal to or close to 1. From the results of the standard protein curve, a determinant value was 0.759, this value was used in the calculation of hTERT level in the cells. The equation Y = 0.0023x + 0.008 with a regression value of 0.759 was obtained from the standard curve. If y is the absorbance value of the sample and x is the hTERT level, then the absorbance value depends on the telomerase content. A regression value of 0.759 means that there was a very strong relationship between the concentration of the solution and the absorbance value of the solution.

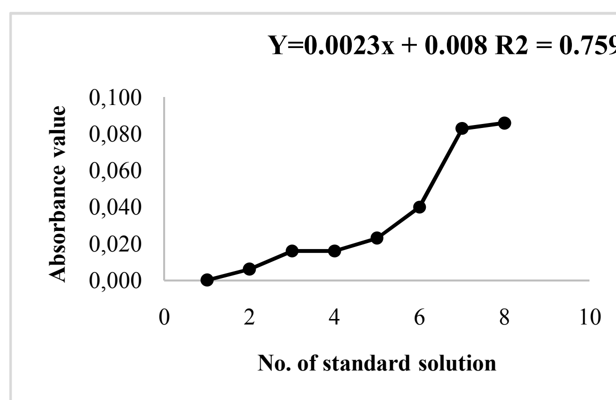


Figure 1. The standard curve of hTERT level in the sperm cells.

Based on the measurements of hTERT level in the sperm cells, the results obtained are 2.5pg/mL. Where the pg/mL unit of hTERT level means that the enzyme level needed to change 1mmol substrate or produce 1mmol of product within 1 minute, in temperature and pH of certain environments. The hTERT level will determine the telomere length, but this relation also depends on the frequency of telomerase-telomere level and the number of

telomere repeats added to each telomere upon recruitment[1]. Telomeres will shorten progressively when the telomerase activity is absence [13].

In contrast with this result, another study stated that telomerase activity gradually decreases from spermatogonia to spermatozoa[8]. Spermatogonia need telomerase to maintain their telomere length during DNA replication in spermatogenesis. The activity of telomerase is higher in this form of the cell to ensure the sperm quality and quantity, also to prevent DNA fragmentation in mature sperm cells[14]. When the cells have differentiated to spermatozoa, telomerase no longer exists[15]. Besides the germinal cells, telomerase also exists in progenitor cells, stem cells, and activated lymphocytes[16]. In some normal cells which lacking telomerase, the telomere length is maintained by the alternative lengthening of telomeres (ALT) pathway[17]. This pathway also occurs in human sperm instead of increasing telomerase activity to maintain telomere rejuvenation[18].

However, the quantitation of hTERT in sperm cells is still possible. Recent study found that the sub-fertile sperm has lower percentage cells contained hTERT level than the fertile[19]. This proves that there is hTERT level in sperm cells that can be a supporting examination of male fertility. Moreover, the telomere length also importantly needed[20].

Nevertheless, this research is certainly still lacking. The hTERT level was successfully estimated, but its function in sperm cells remains unknown. Hence, further study is needed to analyse the function of telomerase in mature sperm cells.

## Conclusion

hTERT level in human sperm cells was 2.5pg/mL. Unfortunately, there is no evidence of the function of hTERT presence in mature sperm cells. Further study is needed to analyse this issue.

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