Vitamin C and ubiquinone able to maintain sperm telomers length on infertile’s men: A clinical trial at Doctor Soetomo Regional General Hospital, Surabaya, Indonesia

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ABSTRACT

Background: Infertility is one of the clinical problems that affect medical and psychological conditions. An estimated 15% of couples in the world experience infertility and almost half occur on men. Excessive stress oxidation has been known responsible to the DNA damage and induces shortening of sperm telomere length. This study aims to determine the role of vitamin C and ubiquinone as antioxidants to maintain sperm telomers length among infertile men.

Methods: An experimental clinical trial with a pre and post-test control group design has been conducted among 30 infertile men respondents at Doctor Soetomo Regional General Hospital, Surabaya. The respondents were divided into three groups: the combination of Vitamin C and Ubiquinone (P1), Vitamin C and Placebo (P2), and Ubiquinone and Placebo (P3) administration. All three groups were examined for pre- and post-test of sperm telomere length. Data were analyzed using SPSS version 21 for Windows.

Results: Most of the respondents were origin from Surabaya (66.7%), never been treated (66.7%), normal BMI (46.7%), non-smoking (66.7%), non-alcohol consumption (90.0%), having 22-25 of IIEF-5 Score (60.0%), no heat exposure (90.0%), no chemical exposure (73.3%), and diagnosis with primary male infertility (90.0%). No significant difference of sperm telomere length was found among three treatment groups (p>0.05) using Post-Hoc Test.

Conclusion: There was no significant difference in sperm telomere length of infertile men among groups. All antioxidants used in this study have a similar ability to maintain or influence sperm telomere length in infertile men.

Keywords: Sperm Telomere Length, Vitamin C, Ubiquinone, Infertile Men

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INTRODUCTION

Infertility is a clinical problem that affects medical and psychological conditions in married couples.1 It is estimated that 15% of all married couples in the world experience infertility.1 Men contribute almost half of the conditions of infertility, women cause around 30% of cases, and the remaining 20% of cases are caused by problems that exist on both sides party.1,2 Many factors cause infertility disorders in men, including anatomical abnormalities, hormonal disorders, immunological problems, gene mutations, radiation, chemotherapy, sexual dysfunction, oxidative stress, and exposure to pollution from the environment.2 Today, many studies have begun to focus on oxidative stress reactions and their possible role as a cause of male infertility.3 Excessive production of Reactive Oxygen Species (ROS) causes oxidative stress reactions which cause damage to Deoxyribonucleic Acid (DNA) and induce shortening of sperm telomere length.3

It has been reported that the telomerase activity and sperm telomere length plays an important role especially in the stages of fertilization and embryo development.4 Reduced telomerase enzyme activity and shortening of the telomere spermatозоа length will trigger gene segregation errors, DNA fragmentation, apoptosis, reduce the number of spermatозоа and ultimately decrease the level of telomere male fertility.4

The primary treatment strategy that can be done to prevent the occurrence of oxidative stress above is by administering antioxidants.5 Many studies have examined the ability of antioxidants to balance the production of ROS to reduce oxidative stress and improve the quality of spermatозоа.5 Vitamin C (Ascorbic Acid) and Ubiquinone (Coenzyme Q10), both of which include antioxidant choices that are now often recommended and commonly prescribed in daily practice for the treatment of male infertility. The use of Vitamin C
has long been applied and a lot of literature states as a standard antioxidant in the treatment of male infertility cases for reasons of ease of obtaining drugs, low prices, minimal side effects, and good effectiveness.\(^2\) Whereas Ubiquinone has been found endogenously in the section sperm cell midpiece, and able to regenerate Vitamin E (Alpha Tocopherol).\(^2\)

Based on those mentioned above, this study aims to evaluate the effect of daily administration of Vitamin C and Ubiquinone on sperm telomere length in infertile men at Doctor Soetomo Regional General Hospital, Surabaya, Indonesia.

**METHODS**

A true experimental clinical trial study with a pre and post-test control group design was conducted in this study.\(^4\) The study sample was men with idiopathic infertility who visited the outpatient andrological outpatient unit of the Doctor Soetomo Regional General Hospital, Surabaya from July to December 2018.

Based on the formula, the minimum requirement of samples were 8 people.\(^6\) In order to anticipate the drop-out, the sample was enlarged so that each group consisted of 10 people. The total sample included in the study were 30 people who were divided into three treatment groups (P1, P2, and P3), without a control group. After undergoing the wash-out phase for 14 days, subjects in the P2 treatment group became P3, while the P1 group received one-time treatment.

The inclusion criteria in the study include men who have idiopathic infertility either primary or secondary, spermatozoa concentration of more than 5 million/ml, aged 20-40 years, married and still living with his wife, free from previous antioxidant therapy (more than 14 days), blood sedimentation rate 0-15 mm/hour, and signed informed consent and information for consent letter. The exclusion criteria include organic and other metabolic disorders, a history of physical trauma or surgery in the genital area, and active and chronic inflammatory diseases.

While the Drop-Out criteria, namely not taking drugs for 7 days in a row and not coming on time for control again.

The independent variable in this study used a 500 mg Vitamin C preparation with the trade name Blackmores\(^*\) product from Kalbe Farma and Ubiquinone 100 mg with the trade name Ubesco\(^*\) product from Escolab in the treatment group. Each study subject was given in a dose once a day orally, in the morning after breakfast for 35 days.

The sperm telomere length is a dependent parameter used in this study as an indicator of assessing spermatozoa conditions and the success of natural fertilization and assisted reproductive technology.\(^2\) The telomere length of a cell is a relative telomere length with unit times expressed as a T/S ratio, i.e. T means the number of telomeres and S is the number of copies of a single gene. Then with a technique developed by O’Callaghan and Fenech in 2011 was able to get the absolute length of telomeres in kilos of base pairs (kbp).\(^8,9\)

The examination method used to assess the sperm telomere length is a one-step quantitative reverse transcriptase-polymerase Chain Reaction (qRT-PCR) with a real-time MyGo\(^*\) Mini (IT-IS Life Science, UK) machine with a kit from GoTaq\(^*\) qPCR Master Mix (Promega, United States). Stages of DNA isolation and extraction using the QIAamp\(^*\) DNA Mini Kit package (QIAGEN, Germany). The consumables and other instruments used during the study were prepared by the Institute of Tropical Diseases (ITD) Laboratory, Airlangga University, Surabaya, who was invited to work together.

The research flow begins with taking the data source on the research sample in accordance with the method used and filtering the data source according to the agreed inclusion and exclusion criteria. Determination of the sample into the treatment groups P1, P2 and P3 was carried out randomly and double-blinding by the hemodialysis pharmacy officer at Doctor Soetomo Regional General Hospital, Surabaya. Subjects who agree to participate in the study must sign an Informed Consent and Information for Consent letter. The procedure is carried out after the sample provided counselling and an explanation of the research.

After that, the identity of the study sample, examination of vital signs, history taking, general physical examination, andrological special examination, blood sedimentation rate examination, semen analysis, and main laboratory examination in the form of sperm telomere length with qRT-PCR were recorded. This sample inspection stage is carried out twice as many assessments with pretest and post-test sessions in each group. Ejaculate samples that have been collected from research samples are immediately sent to a laboratory and stored in a refrigerator (Thermo Fisher Scientific, China) minus 80°C. The following procedure was entering the stage of checking the length of the spermatozoa telomeres starting from the process of isolation and extraction of DNA and ending the qRT-PCR amplification.\(^9\)

Finally, the structuring of the data from the examination of sperm telomere length from the research sample and statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS) program 21.0 for Windows™.
(SPSS Inc., United States). Then the stage of completion of data analysis by making conclusions in the form of a table and its narrative.

RESULTS

The recent findings suggest that the average age of respondents was 32.6 years old. Most of the infertile men respondents were working in the private sector (83.3%), domicile from Surabaya (66.7%), never been treated (66.7%), normal BMI (46.7%), no smoking history (66.7%), no history of alcohol abuse (90.0), having IIEF-5 score of 22-25 (60.0%), diagnoses with primary male infertility (90.0%), and no history of heat (90.0%) or chemical (73.3%) exposure (Table 1).

The Shapiro-Wilk test has been conducted to all pre-test and post-test data among groups. The results obtained were normally distributed on the pre-test and post-test data (p>0.05) only in the group P2. However, from the Levene test, data were all homogeneous in pre-test and post-test group (p>0.05).

Based on the normality test, the bivariate analysis was conducted using the Wilcoxon Test in group P1 and P3. The group P1 has 7.30 ± 1.47 kbp in pre-test and 6.32 ± 1.13 kbp in post-test results, but not statistically significant (p=0.333). Similar results were also found in the group P2 and P3 whereas the pre-test and post-test evaluation of sperm length telomere not statistically significant (p>0.05) (Table 2).

Furthermore, the multivariate analysis was conducted using an One-way ANOVA test to compare the results of the pre-test between groups and obtained not statistically significant (p>0.05) (Table 4). Meanwhile, similar results were also found in the post-test between groups by Kruskal-Wallis test (p > 0.05) (Table 5). Post-Hoc Least Significant Differences (LSD) method, as shown in Table 5, was conducted to determine the relationship between groups. The results also exhibit similar findings, whereas no significant difference was found between the intervention groups (p>0.05) (Table 5).

DISCUSSION

Description of the characteristics of research subjects and their spouses are included in the adult category with more than 30 years of age. The fact is that the majority of research subjects and their spouses tend to be late in seeking treatment for infertility problems with a duration of more than four years, and only a third have sought prior treatment. Added to the risk factors found in the form of some subjects, overweight and one third are active smokers.

Vitamin C is proven to be able to maintain sperm telomere length due to as one of the primary antioxidants in the body that can act as a scavenger of various ROS. The concentration of vitamin C in plasma seminal is almost ten times higher than the concentration in the blood whereas mostly secreted

Table 1  Baseline Characteristics of Respondents

| Variable                        | Respondents (N=30) |
|---------------------------------|--------------------|
| Age of Respondents (years)(mean)| 32.6               |
| Work (%)                        |                    |
| Government Employee             | 16.7               |
| Private                         | 83.3               |
| Domicile (%)                    |                    |
| Surabaya                        | 66.7               |
| Other                           | 33.3               |
| Mean Age (years)                |                    |
| Spouse                          | 30.7               |
| Length of Marriage              | 4.6                |
| Length of Infertile             | 4.3                |
| Medical History (%)             |                    |
| Ever treated                    | 33.3               |
| Never treated                   | 66.7               |
| Body Mass Index (BMI)(%)        |                    |
| Normal (18.4-24.9)              | 46.7               |
| Overweight (25.0-29.9)          | 40.0               |
| Obesity (>30.0)                 | 13.3               |
| Smoking History (%)             |                    |
| Yes                             | 33.3               |
| No                              | 66.7               |
| Alcohol History (%)             |                    |
| Yes                             | 10.0               |
| No                              | 90.0               |
| IIEF-5 Score                    |                    |
| 17-21                           | 40.0               |
| 22-25                           | 60.0               |
| Diagnosis (%)                   |                    |
| Primary Male Infertility        | 90.0               |
| Secondary Male Infertility      | 10.0               |
| Heat Exposure (%)               |                    |
| Yes                             | 10.0               |
| No                              | 90.0               |
| Chemical Exposure               |                    |
| Yes                             | 26.7               |
| No                              | 73.3               |
by the seminal vesicles. Increases in some markers of oxidative stress and decreased activity of antioxidant enzymes (superoxide dismutase, glutathione peroxidase, and glutathione reductase) are found to be correlated with shortening telomere length in male rats.

Vitamin C is the primary antioxidant in the seminal male plasma, contributing as a breaker to more than 65% of the oxidative reaction chain and is often used in vitro in many IVF clinics. Vitamin C at a dose of 1 gram daily can help increase the potential for fertility in men with positive spermatozoa agglutination. Provision of vitamin C in the form of ascorbic acid phosphoric ester magnesium salt (APM) can reduce oxidative stress on embryonic cells and human gametes, prevent telomere attrition and increase replication to prolong cell life. Study in-vivo at a dose of 1 gram of vitamin C per day is known to increase motility and decrease DNA fragmentation in spermatozoa.

Ubiquinone has also been shown to be able to maintain sperm telomere length in this study. It is naturally found in the mid-piece of spermatozoa and can reproduce vitamin E, control pro-oxidant capability, and be involved in energy production. Ubiquinone holds a fundamental role in the cellular bioenergy process as an electron transport chain cofactor from the mitochondria (respiratory chain) and is essential for the production of Adenosine Triphosphate (ATP). The tail structure of spermatozoa which are susceptible to oxidative damage can be protected by Ubiquinone so that the tail size can always be maintained and increase spermatozoa motility.

This study also proved that the combination of vitamin C and Ubiquinone was able to maintain sperm telomere length. However, the author failed to find scientific literature that directly examined the administration of a combination of antioxidants of vitamin C and Ubiquinone.

### Table 2
A statistical comparison of pre- and post-test of sperm telomere length among groups

| Variable | n  | Pre-Test (kbp) | Post-Test (kbp) | Mean Difference | p      |
|----------|----|---------------|----------------|----------------|--------|
| Vitamin C and Ubiquinone (P1) | 10 | 7.30 ± 1.47   | 6.32 ± 1.13   | 0.98 ± 0.34   | 0.333* |
| Vitamin C and Placebo (P2)   | 10 | 8.16 ± 0.46   | 8.91 ± 1.96   | -0.75 ± -1.5  | 0.687* |
| Ubiquinone and Placebo (P3)  | 10 | 10.30 ± 1.59  | 7.71 ± 1.94   | 2.59 ± -0.35  | 0.169* |

*aPaired T-Test; *bWilcoxon Test; kbp: kilobase pairs; statistically significant if p-value less than 0.05

### Table 4
The multivariate analysis of pre-test and post-test group by One-way ANOVA and Kruskal-Wallis Test Result

| Variable | n  | Mean ± SD | p     |
|----------|----|-----------|-------|
| Pre-Test (kbp) |     |           |       |
| Vitamin C and Ubiquinone (P1) | 10 | 7.30 ± 1.47 | 0.248* |
| Vitamin C and Placebo (P2) | 10 | 8.16 ± 0.46 |       |
| Ubiquinone and Placebo (P3) | 10 | 10.30 ± 1.59 |       |
| Post-test (kbp) |     |           |       |
| Vitamin C and Ubiquinone (P1) | 10 | 6.32 ± 1.13 | 0.585* |
| Vitamin C and Placebo (P2) | 10 | 8.91 ± 1.96 |       |
| Ubiquinone and Placebo (P3) | 10 | 7.71 ± 1.94 |       |

*One-Way ANOVA test; *Kruskal-Wallis test; SD: standard deviation; kbp: kilobase pairs; p-value statistically significant if less than 0.05

### Table 5
Post-Hoc Test Result by using Least Significant Differences (LSD) method

| Method  | Intervention Groups                  | p     |
|---------|--------------------------------------|-------|
| LSD     | Vitamin C and Ubiquinone (P1) vs Vitamin C and Placebo (P2) | 0.296 |
|         | Vitamin C and Placebo (P3) vs Vitamin C and Ubiquinone (P1) | 0.572 |
|         | Ubiquinone and Placebo (P3) vs Vitamin C and Placebo (P2) | 0.625 |
|         | Vitamin C and Placebo (P2) vs Ubiquinone and Placebo (P3) | 0.625 |

*Pairwise comparison using the LSD method.
there is quite a lot of literature that reports research on vitamin C combined with vitamin E and as is well known that Ubiquinone can regenerate vitamin E. Vitamin C supplementation with vitamin E has been shown to reduce DNA spermatozoa fragmentation which then significantly increases the clinical pregnancy ratio and implantation after Intracytoplasmic Sperm Injection (ICSI). Statistical analysis proves the administration of a single or a combination of antioxidants between vitamin C and Ubiquinone has the same ability to maintain sperm telomere length (p>0.05). So far, no literature has been found that discusses the comparative strength of antioxidants to maintain sperm telomere length, especially those related to vitamin C, ubiquinone, and their combinations.

Vitamin C and Ubiquinone are mentioned in the systematic summary of various libraries as an antioxidant option for handling idiopathic oligoasthenoteroatozoospermia cases, in addition to vitamin E, selenium, and N-acetyl-cysteine. Besides, vitamin C also has a significant effect related to prevention of spermatozoa DNA fragmentation. In this study, a vivid picture of the pretest and post-test data for the average sperm telomere length showed an increase only in the P2 group, i.e. subjects given 1000 mg vitamin C and placebo daily for 35 days. Also added from the results of the statistical analysis of the comparison between multiple groups it was found that P2 group had the most considerable average difference between groups (alpha) so that it could be concluded that P2 was the best treatment group. However, there were no significant differences between treatment groups.

In addition to considering other aspects outside the research, such as vitamin C preparations that are relatively easy to find in the market, affordable price variants, variations in dosage forms and dosages of use, and the inclusion of vitamin C as a standard choice drug in clinical practice guidelines, conclusions can be drawn that the provision of Vitamin C is the first choice drug to maintain sperm telomere length in infertile men. However, further studies are still needed to prove again and even clarify the effect of vitamin C and Ubiquinone on sperm telomere length.

CONCLUSION

The administration of 1000 mg vitamin C, 100 mg of ubiquinone, and oral combination of both drugs every day for 35 days have been shown having the same ability to maintain or influence sperm telomere length in infertile men.

ETHICAL CLEARANCE

This clinical trial has received information on ethical conduct from the Komite Etik Penelitian Kesehatan (KEPK) or Health Research Ethics Committee of Doctor Soetomo Regional General Hospital, Surabaya with number 0413/KEPK/VII/2018. Besides also having a Material Transfer Agreement (MTA) from the Institute of Tropical Diseases (ITD), Airlangga University, Surabaya with number 116/14418.1/301/2018.

CONFLICT OF INTEREST

Authors have declared there is no conflict of interest regarding all aspects of this study.

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AUTHOR CONTRIBUTIONS

First author has performed all research procedures in the frame of his brevet and wrote all the content of this case report manuscript under the supervision of other authors. All authors already read and approved the final manuscript.

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