Original article

Differences in expression of interleukin-6 and tumor necrosis factor-alpha fallopian tubes non-patent and fallopian tubes patent

Uki Retno Budihastuti [1], Abdurahman Laqif [2], Eriana Melinawati [3], Teguh Prakosa [4], Hermawan Udiyanto [5], Heru Priyanto [6], Darto [7], Affi Angelia Ratnasari [8], Asih Anggraeni [9], Agung Sari Wijayanti[10], Devi Cynthia Dewi[11], Kresto Atalyo De Hann[12]*

Abstract:

Background: Fallopian tube infertility results from sexually transmitted infections and pelvic inflammatory diseases. The most common causes of infertility in women are infections caused by Chlamydia trachomatis and Neisseria gonorrhoeae. These bacterial infections initiate an inflammatory reaction, and ongoing inflammation leads to scarring, obstructing the reproductive tract, and infertility. The pro-inflammatory cytokines IL-6 and TNF-α are involved in the inflammation response triggered by Chlamydia trachomatis and Neisseria gonorrhoeae infections. The study aimed to investigate the connection between the expression of IL-6 and TNF-α in patent and non-patent fallopian tubes. ***Methods:*** Analytical observational research with a cross-sectional approach. The 30 subjects were divided into two groups. Group I consisted of 15 subjects diagnosed with non-patent fallopian tubes, and group II consisted of 15 subjects diagnosed with patent fallopian tubes. Sampling technique using consecutive sampling. The research was conducted at Dr. Moewardi General Hospital from January to June 2022. Both groups were assessed based on age, BMI, parity, level of education, occupation, type of infertility, and expression of IL-6 and TNF-α. ***Results:*** The expression levels of IL-6 and TNF-α were compared between non-patent and patent fallopian tubes, with statistical analysis indicating a significant difference between the two groups. The p-value for IL-6 was 0.008, and the p-value for TNF-α was 0.030. ***Conclusion:*** There was a significant difference in IL-6 expression between the non-patent fallopian tube and the patent fallopian tube, with the IL-6 expression being higher in the non-patent fallopian tube than in the patent fallopian tube. Similarly, the expression of TNF-α on the non-patent fallopian tube and patent fallopian tube shows a higher expression of TNF-α on the non-patent fallopian tube than the patent fallopian tube.

Keywords: infertility; fallopian tube; interleukin-6; tumor necrosis factor-α; chlamydia trachomatis; neisseria gonorrhoeae

Bangladesh Journal of Medical Science Vol. 23 No. 01 January'24 Page : 221-227
DOI: https://doi.org/10.3329/bjms.v23i1.70753

1. Uki Retno Budihastuti, Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Sebelas Maret/Dr. Moewardi Hospital, Surakarta, Jawa Tengah, Indonesia
2. Abdurahman Laqif, Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Sebelas Maret/Dr. Moewardi Hospital, Surakarta, Jawa Tengah, Indonesia
3. Eriana Melinawati, Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Sebelas Maret/Dr. Moewardi Hospital, Surakarta, Jawa Tengah, Indonesia
4. Teguh Prakosa, Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Sebelas Maret/Dr. Moewardi Hospital, Surakarta, Jawa Tengah, Indonesia
5. Hermawan Udiyanto, Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Sebelas Maret/Dr. Moewardi Hospital, Surakarta, Jawa Tengah, Indonesia
6. Heru Priyanto, Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Sebelas Maret/Dr. Moewardi Hospital, Surakarta, Jawa Tengah, Indonesia
7. Darto, Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Sebelas Maret/Dr. Moewardi Hospital, Surakarta, Jawa Tengah, Indonesia
8. Affi Angelia Ratnasari, Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Sebelas Maret/Dr. Moewardi Hospital, Surakarta, Jawa Tengah, Indonesia
9. Asih Anggraeni, Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Sebelas Maret/ UNS Hospital, Sukoharjo, Jawa Tengah, Indonesia
10. Agung Sari Wijayanti, Department of Obstetrics and Gynecology, Dr. Moewardi Hospital, Surakarta, Jawa Tengah, Indonesia
11. Devi Cynthia Dewi, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Jawa Tengah, Indonesia
12. Kresto Atalyo De Hann, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Jawa Tengah, Indonesia

Correspondence: Uki Retno Budihastuti**,** Faculty of Medicine, Universitas Sebelas Maret / Department of Obstetrics and Gynecology, Dr. Moewardi General Hospital, Ir. Sutami 36 A, Kentingan, Surakarta, Jawa Tengah, Indonesia 57126**,** E-mail address: ukiretno@staff.uns.ac.id

Introduction:

Female infertility is a significant global health issue, including in Indonesia [1]. Studies have shown that a woman's likelihood of infertility increases with age [2]. In addition, the incidence of infertility caused by fallopian tube factors is higher in black women than in white women. Sexually transmitted infections and pelvic inflammatory diseases are common causes of infertility caused by fallopian factors [3]. Among the contributing factors to tubal infertility are infections from Chlamydia trachomatis (C. trachomatis), Mycoplasma, and Neisseria gonorrhoeae (N. gonorrhoeae) [4].

This bacterial infection may go unnoticed because it can be symptomless, leading to delayed and inadequate treatment. The infection also triggers an inflammatory response and chronic inflammation and can cause scarring, blocking the reproductive tract and causing infertility. In 2016, the World Health Organization (WHO) estimated that around 87 million people were infected globally, with an estimated rate of 20 cases per 1000 women and 26 cases per 1000 men. In women, gonorrhea may not produce symptoms, making early detection and treatment difficult and increasing the risk of cervicitis and pelvic inflammatory disease. In men, it has been associated with epididymitis, epididymal-orchitis, and chronic prostatitis [5–7].

The infections caused by C. trachomatis and N. gonorrhoeae directly lead to the loss of microvilli and cilia from the cells in the tubal epithelium, causing disruptions in epithelial tissue homeostasis and the spread of damage beyond the initially infected cells. In response to these fallopian tube infections, there is an increase in interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and other pro-inflammatory chemokines and cytokines [8,9]. These pro-inflammatory cytokines are involved in the inflammation response triggered by both the innate and adaptive immune systems. IL-6 and TNF-α have improved various obstetric cases, including infertility. They are produced in response to tissue damage and contribute to the body's defense mechanism. IL-6 plays an essential role in activating neutrophils, mediating oxidative reactions of respiration, and stimulating the degradation of tissue enzymes through metalloproteinase, serine proteinase, and its inhibitors. In addition, IL-6 also plays a crucial role in inflammation and infection. TNF-α is a multifunctional T-helper 1 (Th-1) cytokine that is a critical inflammatory cytokine produced by macrophages during inflammation and activated by endotoxin lipopolysaccharides (LPS) [8,10–12].

Materials and methods:

Study Design

Observational analytical research with a cross-sectional approach in non-patent fallopian tube patients. The study was conducted at Dr. Moewardi General Hospital for six months, from January – June 2022.

Population and Sample

The study enrolled patients who visited the Obstetrics and Gynecology clinic or treatment room at Dr. Moewardi General Hospital, Surakarta. Patients were diagnosed with non-patent or patent fallopian tubes using ultrasonography (ultrasound), hysterosalpingogram (HSG), and laparoscopy. Patients diagnosed with non-patent fallopian tubes underwent laparoscopy to determine the anatomical and functional abnormalities in the fallopian tubes, and salpingectomy was performed. A fallopian tube sample was then taken, and an immunohistochemistry examination was conducted to assess the expression of IL-6 and TNF-α, which was then compared to patients with patent fallopian tubes. The subjects were divided into two groups: group I comprised 15 patients diagnosed with non-patent fallopian tubes, and group II comprised 15 patients diagnosed with patent tubes. In both groups, assessments were carried out based on age, body mass index (BMI), parity, education, occupation, and expression of IL-6 and TNF-α.

Operational Definition of Variables

Non-patent fallopian tubes

Patients with anatomical and functional abnormalities in their fallopian tubes underwent HSG, ultrasound, and laparoscopy.

Patent fallopian tubes

Fertile patients used the women's operative method of contraception (MOW).

Age

The lifetime of the subjects from birth until this study was conducted.

Body Mass Index (BMI)

A person's weight (in kg) is divided by their height (in meters) squared to determine whether their weight is ideal.

Education

The formal education level is based on a person's last academic diploma.

Employment

The main activity for generating income.

Interleukin-6 expressions

The accumulation of percent IL-6 expression is indicated by immunohistochemistry staining of the entire field of view at a magnification of 40x.

TNF-α expression

The accumulated percent of TNF-α expression is shown with immunohistochemistry staining of the entire field of view at a magnification of 40x.

Study Instruments

Research instruments to support this research are medical records, immunohistochemistry staining of IL-6 and TNF-α, as well as samples of non-patent fallopian tubes and patent fallopian tubes.

Data Analysis

Characteristics tests were conducted using the independent t-test, Mann-Whitney, and chi-square test to compare age, BMI, education, and occupation between the non-patent and patent fallopian tube groups. The independent t-test was used to compare normally distributed numerical data, while the Mann-Whitney test was used to compare ordinal categorical data. The Chi-square/Fischer exact test was used to compare ordinal categorical data. A p-value <0.05 was considered statistically significant, indicating a significant difference between the two groups. All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 21.0 for Windows.

Ethical Clearance

Ethical research practices include obtaining informed consent, ensuring anonymity, and obtaining ethical clearance. The Research Ethics Committee at Dr. Moewardi General Hospital, Surakarta, Indonesia, approved the ethical clearance for this study under reference number 763/VI/HREC/2022.

RESULTS

Table 1. Characteristics of the Research Subjects

Characteristics	Group		p
	Patent Fallopian Tubes	Non-patent Fallopian Tubes	
Age (year)			
<35	8 (53.3%)	11 (73.3%)	0.256
≥35	7 (46.7%)	4 (26.7%)	
BMI (kg/m^2)			
<30	11 (73.3%)	12 (80.0%)	1.000
≥30	4 (26.7%)	3 (20.0%)	
Education			
Primary school	4 (26.7%)	4 (26.7%)	1.000
High school-Collage	11 (73.3%)	11 (73.3%)	
Employment			
Unemployed	9 (60.0%)	9 (60.0%)	1.000
Employed	6 (40.0%)	6 (40.0%)	

BMI: body mass index

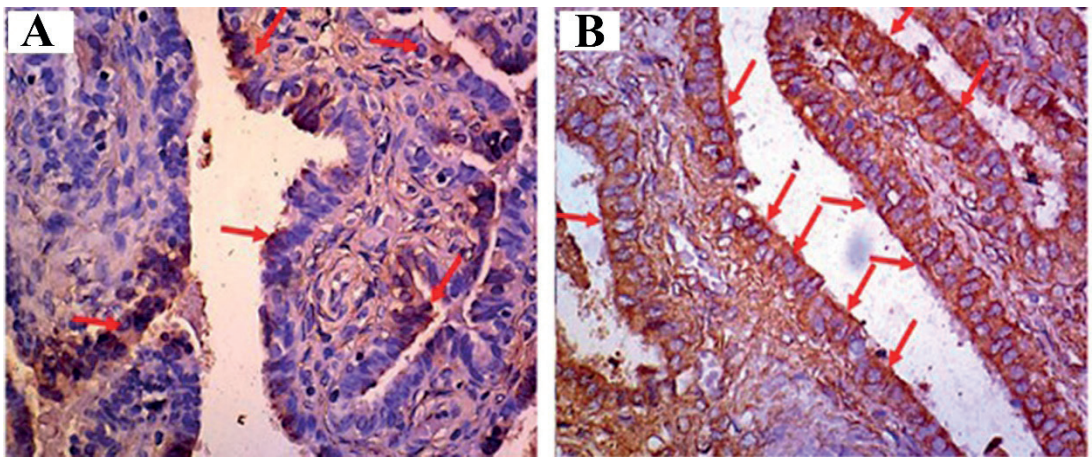

Figure 1. IL-6 expression in the fallopian tube. The red arrows indicate a patent fallopian tube (A); and a non-patent fallopian tube (B).

Table 2. Differences in IL-6 Expression between Non-Patent Fallopian Tubes and Patent Fallopian Tubes

Variable	Group		p
	Patent Fallopian Tubes	Non-patent Fallopian Tubes	
IL-6 expression			
Mean ±SD	5.47 ±4.45	9.80 ±3.03	0.008*
Median (Min-Max)	4.00 (1.00- 12.00)	12.00 (3.00-12.00)	

The non-paired group difference test of numerical data did not pass the normality requirement (Mann-Whitney). *: Significant p<0.05.

Table 2 shows that the mean expression of IL-6 in the patent fallopian tube group was 5.47 with a standard deviation of 4.45 (5.47 ±4.45), and the median value was 4.00 with a minimum of 1 and a maximum of 12. Meanwhile, the mean expression of IL-6 in the non-patent fallopian tube group was 9.80 with a standard deviation of 3.03 (9.80 ±3.03), and the median value was 12.00 with a minimum of 3 and a maximum of 12.

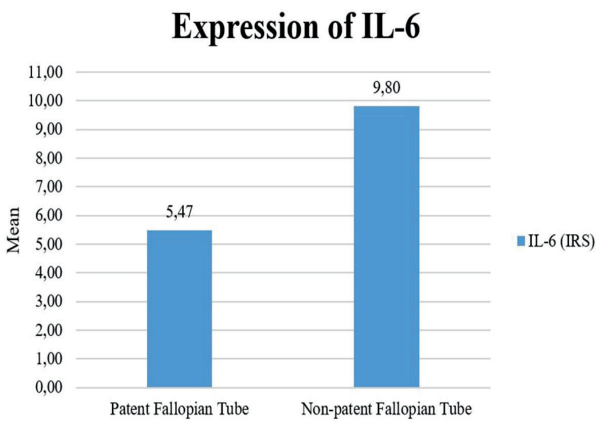

Figure 2. IL-6 expression between the non-patent fallopian tube and patent fallopian tube

According to the results of the statistical tests, there is a significant difference in IL-6 expression between non-patent and patent fallopian tubes, with a p-value of 0.008 (p<0.05). This means that subjects with non-patent fallopian tubes have higher expressions of IL-6 compared to patent fallopian tubes.

Table 3. Differences in TNF-α Expression between Non-Patent Fallopian Tubes and Patent Fallopian Tubes

Variable	Group		p
	Tuba Fallopian Patent	Non-Patent Fallopian Tube	
TNF-α expression			
Mean ±SD	3.60 ±4.31	6.87 ±4.50	0.030*
Median (Min-Maks)	2.00 (0 .00-12.00)	8.00 (0 .00-12.00)	

The non-paired group difference test of numerical data did not pass the normality requirement (Mann-Whitney). *: Significant p<0.05.

Based on Table 3, it is revealed that the mean expression of TNF-α in the patent fallopian tube group was 3.60 with a standard deviation of 4.31 (3.60 ±4.31). Furthermore, the median value of TNF-α in the patent fallopian tube group was 2.00, with a minimum value of 0 and a maximum of 12. On the other hand, the mean expression of TNF-α in the non-patent fallopian tube group was 6.87 with a standard deviation of 4.50 (6.87 ±4.50), and the median value of TNF-α in the non-patent fallopian tube group was 8.00 with a minimum value of 0 and a maximum of 12.

The statistical analysis showed a significant difference in TNF-α expression between non-patent and patent fallopian tubes (p=0.030, p<0.05). The results indicate that subjects with non-patent fallopian tubes had a higher TNF-α expression than those with patent fallopian tubes.

Discussion:

Several factors can lead to infertility related to the fallopian tubes, such as tubal obstruction or occlusion that can occur at different locations (proximal, distal, unilateral, or bilateral). Other potential causes may involve endometrial disorders, peri-adnexal adhesions, pelvic inflammatory diseases, endometriosis, ectopic pregnancy, using certain contraceptives, and undergoing induced abortion [13].

In the United States, N. gonorrhoeae and C. trachomatis are among the most frequently reported sexually transmitted infections (STIs) [5,14,15]. N. gonorrhoeae is a significant public health concern because of its resistance to various drugs [16]. Unfortunately, this bacterial infection can go unnoticed and lead to

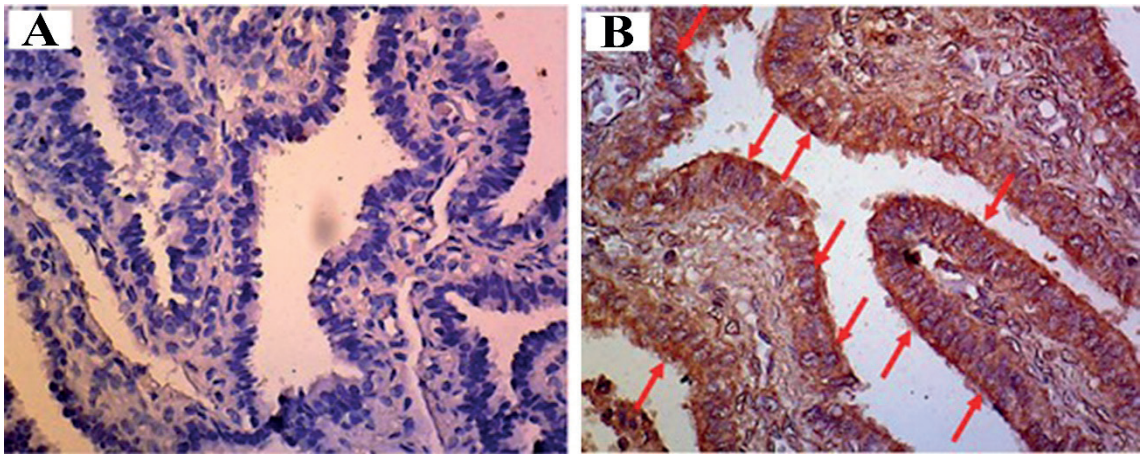

Figure 3. TNF-α expression in the fallopian tube. (A) patent fallopian tube; and (B) the red arrows indicate a non-patent tube.

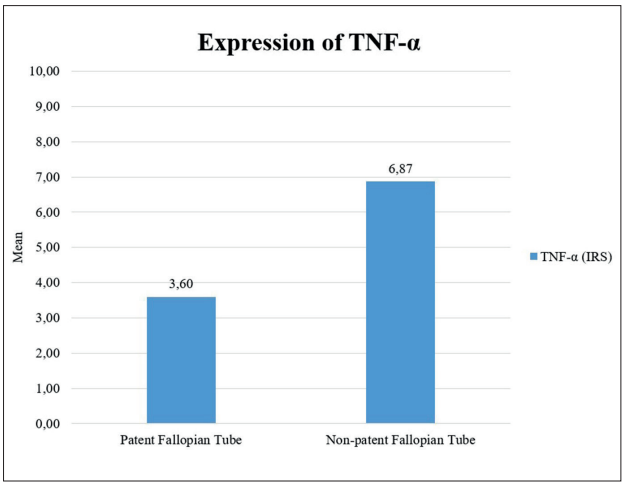

Figure 4. The TNF-α expression between the non-patent fallopian tube and patent fallopian tube

inadequate or delayed treatment. As a result, it can trigger chronic inflammation, scarring, blockage of the reproductive tract, and infertility [6]. C. trachomatis infection can also provoke an inflammatory response involving various pro-inflammatory cytokines from both the innate and adaptive immune systems [17]. One of these cytokines, IL-6 and TNF-α, showed significant improvement in the inflamed areas compared to non-inflamed areas of the fallopian tubes, with p-values of 0.008 and 0.030, respectively. These markers indicate the degree of inflammation in the non-patent fallopian tubes, where blockages can occur.

Previous research by Sukatendel et. al found a link between C. trachomatis infection and non-patent fallopian tubes, which a prevalence of 66.6% compared to 13.2% for normal fallopian tubes [1]. Another study by Papathanasiou et. al suggests that IL-6 can serve as an inflammatory marker and directly impact the transport function of the fallopian tubes [18]. Research also shows that women with ectopic pregnancy have higher levels of IL-6 than those with normal pregnancy [19].

An increase in the concentration of TNF-α is directly associated with a decrease in ciliary activity [14]. Increased TNF- α has several effects on C. trachomatis infections, including IFN-g inhibiting host cell metabolism through an increase in indoleamine 2, 3-dioxygenase (IDO) activity, which limits the growth of Chlamydia. TNF- α also impacts the survival of Chlamydia in vivo by inducing host cell apoptosis, which provides favorable conditions for the organism to grow [20]. The release of TNF-α can also lead to direct damage to nearby ciliated cells, causing a loss of ciliary activity and tissue damage, leading to scarring as a tissue repair mechanism [16].

Research has shown that IL-6 levels in both plasma and cervical mucus are higher in women experiencing idiopathic infertility compared to mRNA expression of IL-6 in fertile women. However, the secretion of soluble glycoprotein (sgp) is reduced in the mid-secretory phase of the endometrium of infertile women, causing a low ratio between inhibitory sgp-130 and IL-6. These findings suggest that the ratio between IL-6 and sgp-130 is crucial in preparing the endometrium for implantation and is a better indicator of fertility than just IL-6 levels alone [21].

The limitation of this study is that it did not measure hormonal levels or examine the possibility of fungal, bacterial, or viral infections as potential causes of C. trachomatis infection or inflammation.

Conclusion:

There is a significant difference in the expression of IL-6 between the non-patent fallopian tube and the patent fallopian tube, with the expression of IL-6 being higher in the non-patent fallopian tube than in the patent fallopian tube. Similarly, the expression of TNF-α on the non-patent fallopian tube and patent fallopian tube shows a higher expression of TNF-α on the non-patent fallopian tube than the patent fallopian tube.

Acknowledgment

The author would like to thank the director of Dr. Moewardi General Hospital for supporting this research.

Conflicts of Interest

There are no conflicts of interest.

Contribution of authors:

Conception and design: Uki Retno Budihastuti, Eriana Melinawati, Agung Sari Wijayanti

Acquisition of data: Eriana Melinawati, Abdurahman Laqif, Teguh Prakosa

Analysis and Interpretation of data: Hermawan Udiyanto, Heru Priyanto, Darto, Devi Cynthia Dewi, Kresto Atalyo De Hann.

Drafting and submission of the manuscript: Uki Retno Budihastuti

Statistical analysis: Agung Sari Wijayanti, Teguh Prakosa, Devi Cynthia Dewi, Kresto Atalyo De Hann.

Review and editing: Uki Retno Budihastuti, Abdurahman Laqif, Affi Angelia Ratnasari, Asih Anggraeni

All authors have agreed to be so listed and have read and approved the manuscript.

Funding Sources

This work is supported by non-Anggaran Pendapatan dan Belanja Negara (APBN) funds (number: 254/UN27.22/PT.01.03/2022).

References:

1. Sukatendel K, Mayniar TE, Aboet A, Adela CA, Lumbanraja S, Ichsan TM, et al. Relationship between chlamydia trachomatis infection with patency tubal and non-patency tubal occurrence in infertile women. *Open Access Maced J Med Sci*. 2019;**7**(20):3437–42.
2. Walker MH, Tobler KJ. Female Infertility. Matthew H Walker, Kyle J Tobler . 2022 May;
3. Anyalechi GE, Wiesenfeld HC, Kirkcaldy RD, Kissin DM, Haggerty CL, Hammond KR, et al. Tubal Factor Infertility, In Vitro Fertilization, and Racial Disparities: A Retrospective Cohort in Two US Clinics. *Sex Transm Dis*. 2021 Oct;**48**(10):748–53.
4. Egbe TO, Nana-Njamen T, Elong F, Tchounzou R, Simo AG, Nzeuga GP, et al. Risk factors of tubal infertility in a tertiary hospital in a low-resource setting: a case-control study. *Fertil Res Pract*. 2020;**6**(1):1–9.
5. Chemaitelly H, Majed A, Abu-Hijleh F, Blondeel K, Matsaseng TC, Kiarie J, et al. Global epidemiology of Neisseria gonorrhoeae in infertile populations: Systematic review, meta-analysis and metaregression. *Sex Transm Infect*. 2021;*97*(2):157–69.
6. Mascellino MT, Boccia P, Oliva A. Immunopathogenesis in Chlamydia trachomatis Infected Women . ISRN Obstet Gynecol. 2011;2011:1–9.
7. Lovett A, Duncan JA. Human immune response and the natural history of neisseria gonorrhoeae infection. Front Immunol. 2019;**10**(FEB).
8. Cunningham K, Stansfield SH, Patel P, Menon S, Kienzle V, Allan JA, et al. The IL-6 response to Chlamydia from primary reproductive epithelial cells is highly variable and may be involved in differential susceptibility to the immunopathological consequences of chlamydial infection. BMC Immunol. 2013;**14**(1).
9. Fifer H, Natarajan U, Jones L, Alexander S, Hughes G, Golparian D, et al. Failure of Dual Antimicrobial Therapy in Treatment of Gonorrhea. *N Engl J Med*. 2016;**374**(25):2504–6.
10. Hafner LM. Pathogenesis of fallopian tube damage caused by Chlamydia trachomatis infections. *Contraception*. 2015;**92**(2):108–15.
11. Murphy KM, Janeway CA, Travers P, Walport MJ. Janeway's Immunobiology. 8th ed. New York: W. W. Norton & Company; 2012. 868 p.
12. Omar J, Shafii N, Zainan AE, Sirajudeen KNS, Abdullah MR. Evaluation of wound healing biomarkers of interleukin 6 (IL-6), vascular endothelial growth factor (VEGF) and matrix metalloproteinases 9 (MMP-9) in post lower segment caesarean section (LSCS) patients consuming channa striatus extract. *Bangladesh J Med Sci*. 2020;**19**(3):520–6.
13. Briceag I, Costache A, Purcarea VL, Cergan R, Dumitru M, Briceag I, et al. Current management of tubal infertility: from hysterosalpingography to ultrasonography and surgery. J Med Life. 2015;**8**(2):157–9.
14. Lenz JD, Dillard JP. Pathogenesis of neisseria gonorrhoeae and the host defense in ascending infections of human fallopian tube. Front Immunol. 2018;9(NOV):1–18.
15. Fyle-Thorpe O. Chlamydia and Gonorrhea: An Update. J Nurse Pract. 2019;**15**(6):424–8.
16. Rodas PI, Pérez D, Jauffret C, González Y, Carreño C, Tapia C V., et al. Modified profile of matrix metalloproteinase 2 and 9 production by human fallopian tube epithelial cells after infection in vitro with neisseria gonorrhoeae. *J Infect Dis*. 2017;**215**(3):452–5.
17. Hafner LM. Pathogenesis of fallopian tube damage caused by Chlamydia trachomatis infections. *Contraception*. 2015;**92**(2):108–15.
18. Papathanasiou A, Djahanbakhch O, Saridogan E, Lyons RA. The effect of interleukin-6 on ciliary beat frequency in the human fallopian tube. *Fertil Steril*. 2008;**90**(2):391–4.
19. Balasubramaniam ES, Van Noorden S, El-Bahrawy M. The expression of interleukin (IL)-6, IL-8, and their receptors in fallopian tubes with ectopic tubal gestation. *Fertil Steril*. 2012;**98**(4):898–904.
20. Xiang W, Yu N, Lei A, Li X, Tan S, Huang L, et al. Insights Into Host Cell Cytokines in Chlamydia Infection. *Front Immunol*. 2021;**12**(May):1–14.
21. Prins JR, Gomez-Lopez N, Robertson SA. Interleukin-6 in pregnancy and gestationadisorders. *J Reprod Immunol*. 2012;**95**(1–2):1–14.