

CHARACTERISTICS OF PILI HEMAGGLUTININ PROTEIN AND ITS ROLE IN THE PATHOGENESIS OF URINARY TRACT INFECTION WITH UROPATHOGENIC ESCHERICHIA COLI

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ABSTRACT

Urinary tract infection (UTI) is one of the most common infectious diseases encountered in the community. The bacteria most frequently implicated as the causes of UTI are the Gram-negative bacteria, especially *Escherichia coli*. Early phase of the pathogenesis of the infection constitutes adhesion of bacteria onto the epithelial cells of urinary tract. SDS-PAGE examination was carried out to investigate molecule of hemagglutinin protein and hemagglutination (HA) test continued by adhesion and inhibited adhesion tests.

The observation of molecular weight of pili *E. coli* protein molecule by explorative examination using SDS-PAGE showed on *E. coli* strip that the most prominent molecular weights (MW) of the proteins were 61 kDa, 37 kDa, 30 kDa, and 20 kDa. Purification by electro-elution was done to proteins of the MW of 20 kDa, 37 kDa and 61 kDa. The HA test results indicated the 61 kDa, 37 kDa, and 20 kDa were hemagglutinin proteins and one which subsequently applied as sub unit proteins of Uropathogenic *E. coli* (UPEC) was the 37 kDa. Adhesion test of UPEC bacteria carried out on urinary bladder epithelial cells of rabbit showed a significant correlation between adhesion index and various doses of subunit proteins of UPEC 37 kDa as coat of urinary bladder epithelial cells. Spearman rank correlation test and regression/non regression analysis confirmed an exponential decrease of UPEC adhesion to urinary tract epithelial cells as a response to the increasing doses of coating protein. This finding points out that pili hemagglutinin protein subunit of UPEC 37 kDa was an adhesive molecule. Pili hemagglutinin protein subunit of UPEC 37 kDa is an adhesive molecule, which plays a role in adherence of UPEC to epithelial cells of urinary bladder at the early pathogenesis of urinary tract infection.

Key words: Uropathogenic *Escherichia coli*, molecule of hemagglutinin protein, adhesive index, urinary tract infection

INTRODUCTION

Urinary tract infection (UTI) is one of the most common infectious diseases encountered in both developing and developed countries.¹

The bacteria most frequently found as the causes of UTI are of the Gram negative group, especially of the E.coli. Uropathogen *Escherichia coli* (UPEC) is E.Coli that causes infection of urinary tract and produces adhesine that enhance adhesion of the E. coli bacteria onto the uroepithel cells.^{2,3}

In order to make colonization and invasion into the host cells, the bacteria should first adhere onto the surface of the cells.⁴ Thus, adhesion of the microbes onto the host cells is deem important first step in the process of infections of pathogenic bacteria.⁵ Adhesion ability is enhanced by adhesion molecule existing in the bacteria and a receptor existing in host cells The adhesion factors of UPEC can be different tiated according to their structural and chemical structures into pili or fimbrial adhesin and afimbrial adhesin or outer membrane protein (Omp).³

The characteristics of adhesin molecule can be studied based on its ability to agglutinate blood cells of mammals. The already known molecular weights (MW) of HA appear to vary from one type of bacteria to the other, such as that of *Bordetella* (B) *pertusis* with MW of 200 kDa and *Vibrio* (V) *parahaemolyticus* of 17 kDa.⁶ HA of *Klebsiella* (K) *pneumoniae* has MW of 29.5 kDa.⁷ Up to now, however, no researcher as yet has reported the true molecular weight of HA of UPEC. To determine which functions as the adhesin molecule it is a must to do a study on the adhesion of bacteria with surrounding urinary bladder's epithelial cells.⁸

MATERIALS AND METHODS

Before the experimental study was carried out to test adhesion of UPEC onto urinary bladder's epithelial cells of a rabbit, an explorative laboratory study was done to isolate hemagglutinin protein of bacterial pili of UPEC.

This study of adhesion test was an experimental study using Posttest Only Control Group Design.

Material for the explorative study used was UPEC bacteria isolated from mid-stream urine specimens of patients having UTI who were hospitalized at Sanglah Hospital, Denpasar.

Samples of the experimental study for testing adhesion were epithelial cells of urinary bladder of an experimental rabbit that were homogenous samples.

Independent variable: concentration of pili HA protein sub unit of UPEC 37 kDa, Dependent variable: adhesion index. Analysis of correlation was done by Spearman rank correlation test and regression analysis was done on its being linear or non-linear.

For analysis of data obtained in first stage of the study to determine if the pili molecular HA protein subunit UPEC 37 kDa was adhesion molecule, Spearman rank correlation test was used with adhesion index as dependent variable and dose of the coating (pili HA protein sub unit UPEC 37 kDa) as independent variable. Power of the correlation was indicated by the value of r^2 . In testing the hypothesis that the two groups of variables had a linear correlation, we referred to the value of p. The linear correlation between adhesion index and dose of coating protein was indicated by linear or nonlinear regression equation of $y = a + bx$, in which a is the intercep, showing the value of adhesion index if

the dose of coat was near to 0, while b is slope showing the increase of adhesion index in every increase of each unit of dose.

RESULTS

From the explorative study done previously by SDS-PAGE method, the molecular weights of pili UPEC proteins were identified as shown in Fig 1.

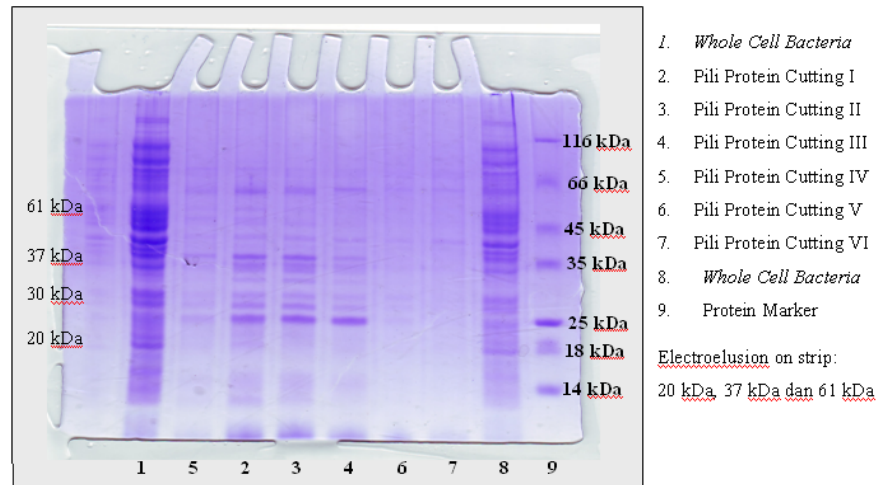


Fig 1. Profiles of molecular weights of pili UPEC protein by SDS-PAGE

The profiles of molecular weights of the UPEC proteins demonstrated by SDS-PAGE showed the prominent proteins of the no 1 strip with MW of 61 kDa, 37 kDa, 30 kDa and 20 kDa. The MW of 30 kDa seemed to be the least if compared with the other

proteins. Thus, the proteins chosen to be purified by electroelusion are the ones with MW of 20 kDa, 37 kDa and 61 kDa. Then HA testing was done on the purified proteins resulted from the electroelusion. Fig 2 shows results of the hemagglutination of pili UPEC proteins subunit of MW 61 kDa, 37 kDa, and 20 kDa

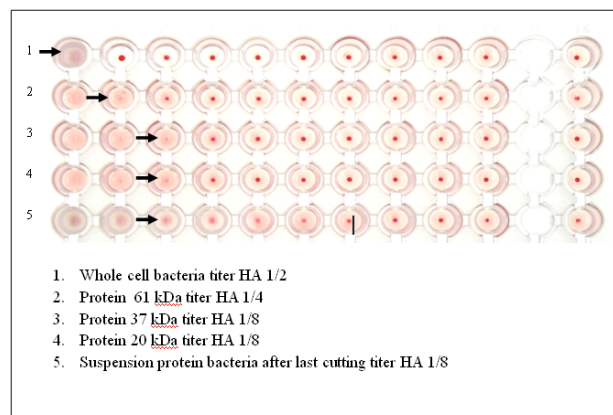


Fig 2. Profiles of hemagglutination of pili UPEC protein subunits using blood of mice

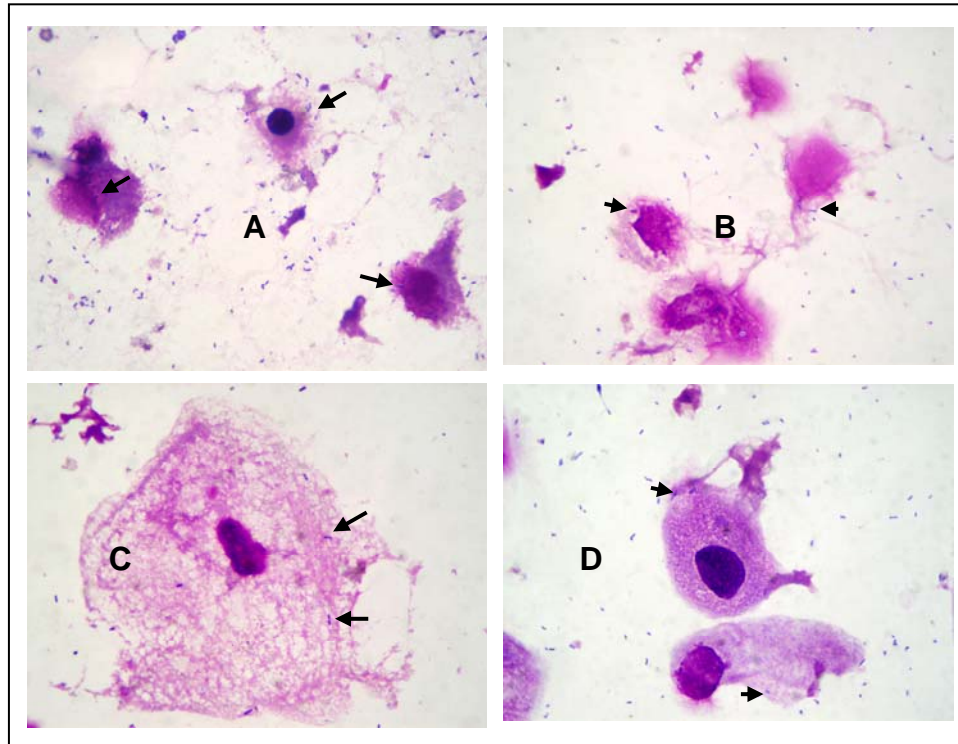


Fig. 3 Showing adhesion of UPEC onto epithelial cells of rabbit's urinary tract coated with HA protein of pili UPEC subunit of 37 kDa in a dose of 25 ug (A), 50 ug(B), 100 ug (C) and 400 ug(D).

From Fig 2 it is clear that the three chosen pili UPEC proteins of the MW of 20 kDa, 37 kDa and 60 kDa were proved to be HA proteins. The next pili UPEC protein to be used was that with MW of 37 kDa, on consideration that besides the fact it was HA protein, in bacteria other than UPEC the adhesin molecules were located around MW 37 kDa. In the next step the pili protein subunit was tested to

assess whether it was an adhesion molecule that could adhere onto to epithelial cells of rabbit's urinary bladder. Result of the latter test is presented in Fig 3 showing the number of UPEC bacteria adhered onto host epithelial cells of rabbit's urinary bladder.

The numbers of adhering bacteria shown in Figures 5.2.4 A, B, C, and D were at the averages of 745, 615, 419, and 230.

A table 1 shows the counts of UPEC bacteria adhering onto epithelial cells of rabbit's urinary bladder in different doses of pili HA protein subunits of UPEC 37 kDa

Table 1. Levels of adhesion indexes when epithelial cells of rabbit's urinary tract were coated with several doses of pili HA protein subunit of UPEC 37 kDa

No	Coating dose	No UPEC bacteria adhering onto 100 epithelial cells of urinary bladder; 3 times replication			
		Replication 1	Replication 2	Replication 3	Average
1	0 ug	1120	1023	1002	1048
2	10 ug	898	820	872	863
3	25 ug	738	752	746	745
4	50 ug	589	623	634	615
5	100 ug	398	436	424	419
6	200 ug	388	379	387	304
7	400 ug	205	265	302	230

Based on the data of Table 1, statistical analyses were performed for correlation and linear/nonlinear regression using

Spearman rank correlation test, the result of which is shown in a curve diagram presented in Fig 4.

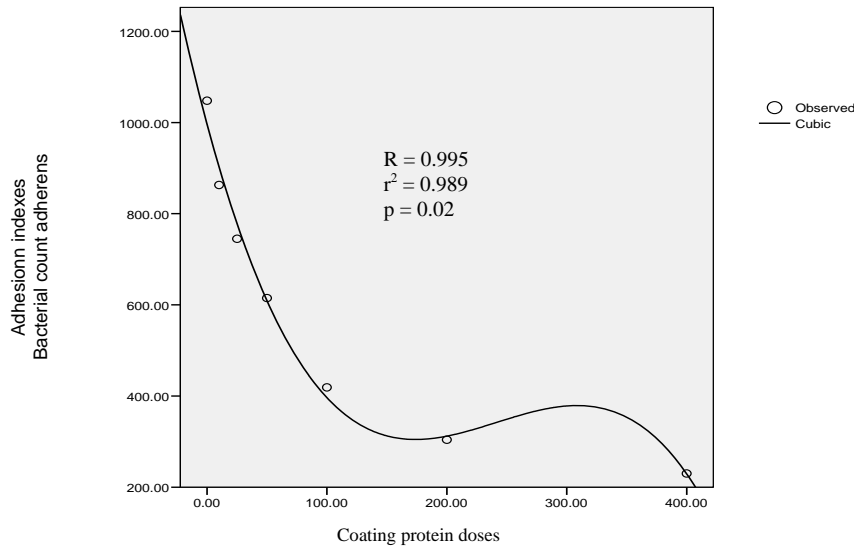


Fig 4. Curve diagram showing regression equation between molecules of pili HA protein subunits of UPEC 37 kDa in several doses of epithel coating, with adhesion indexes on epithelial cells of rabbit's urinary bladder.

From the above diagram, it appears that the adhesion index and dose of pili protein subunit of UPEC 37 kDa as the coating did not indicate a linear correlation. After being analyzed by nonlinear equation, the strongest correlation was evidenced in the cubic equation.

From above correlation analysis, it was found that there was a strong and significant correlation ($R = 0.995$; $r^2 =$

0.989; $p = 0.02$) between adhesion index and dose of pili protein subunit of UPEC

37 kDa as the coating protein.

The above correlation could be shown by cubic equation where adhesion index

$$= 9.96 - 9.79(\text{dosis penyalut}) + 0.04 (\text{dosis penyalut})^2 - 6.1E - 0.05(\text{dosis penyalut})^3$$

(p=0,005) (p=0,019) (p=0,034)

This finding indicates that pili HA protein subunit of UPEC 37 kDa is adhesion molecule (therefore the hypothesis has been proven true, meaning pili HA protein subunit UPEC 37 kDa possesses hemagglutination property and it is an adhesion molecule).

DISCUSSION

The study results showed: firstly, pili protein subunit of UPEC 37 kDa was HA molecule. The characteristic of adhesion molecule can be identified from the ability of the protein to agglutinate human erythrocytes, whose surface biological structure is similar to that of receptor of epithelial cells. Secondly, the correlation between adhesive index and various doses of pili protein subunit of UPEC 37 kDa as coat of rabbit's urinary bladder epithelial indicated an exponential decrease in response to the increase of coated protein dose. The most interesting finding of this study was after the dose of coating protein of 100 μg , the adhesion index pattern became somewhat flat. This finding seems to need further study for its explanation.

It is concluded from the above results that pili protein subunit of UPEC

of the MW 37 kDa is adhesion molecule, in which its protein can inhibit adherence of UPEC bacteria onto urinary bladder epithelial cells (host cells). Through close observation we found that the type of adhesion of the UPEC bacteria onto the urinary bladder epithelial cells was a local and aggregating type. In the local type of adhesion, there was a bacterial colonization clustering on the receptor cells, while in the aggregating type there was bacterial growth in the form of stacked-brick both on the urinary bladder epithelial cells and the bottom of the object glass. As a conclusion, results of the adhesion study confirmed that pili hemagglutinin subunit of UPEC 37 kDa was adhesion molecule of the aggregating type, hence the bacteria being studied are pathogenic or UPEC.

The already known molecular weights (MW) of HA appear to vary from one type of bacteria to the other, such as that of *Bordetella (B) pertussis* with MW of 200 kDa and *Vibrio (V) parahaemolyticus* of 17 kDa.⁶ HA Protein in *Klebsiella (K) pneumoniae* wild group have MW of 29,5 kDa.⁷ Sumarno (2000) has succeeded in isolating pili adhesin molecule of *V. cholerae* with MW of 38 kDa and Omp of 76 kDa.⁹ Up to now, however, no researcher as yet has reported the true molecular weight of HA of UPEC.

CONCLUSIONS

The pili HA protein subunit molecule of UPEC is adhesion molecule that play role in the early phase of the pathogenesis of UTI as adhesion process of the bacteria onto the epithelial cells of urinary bladder.

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