

# Haemolytic activity of *Trichomonas vaginalis* for human RBCs

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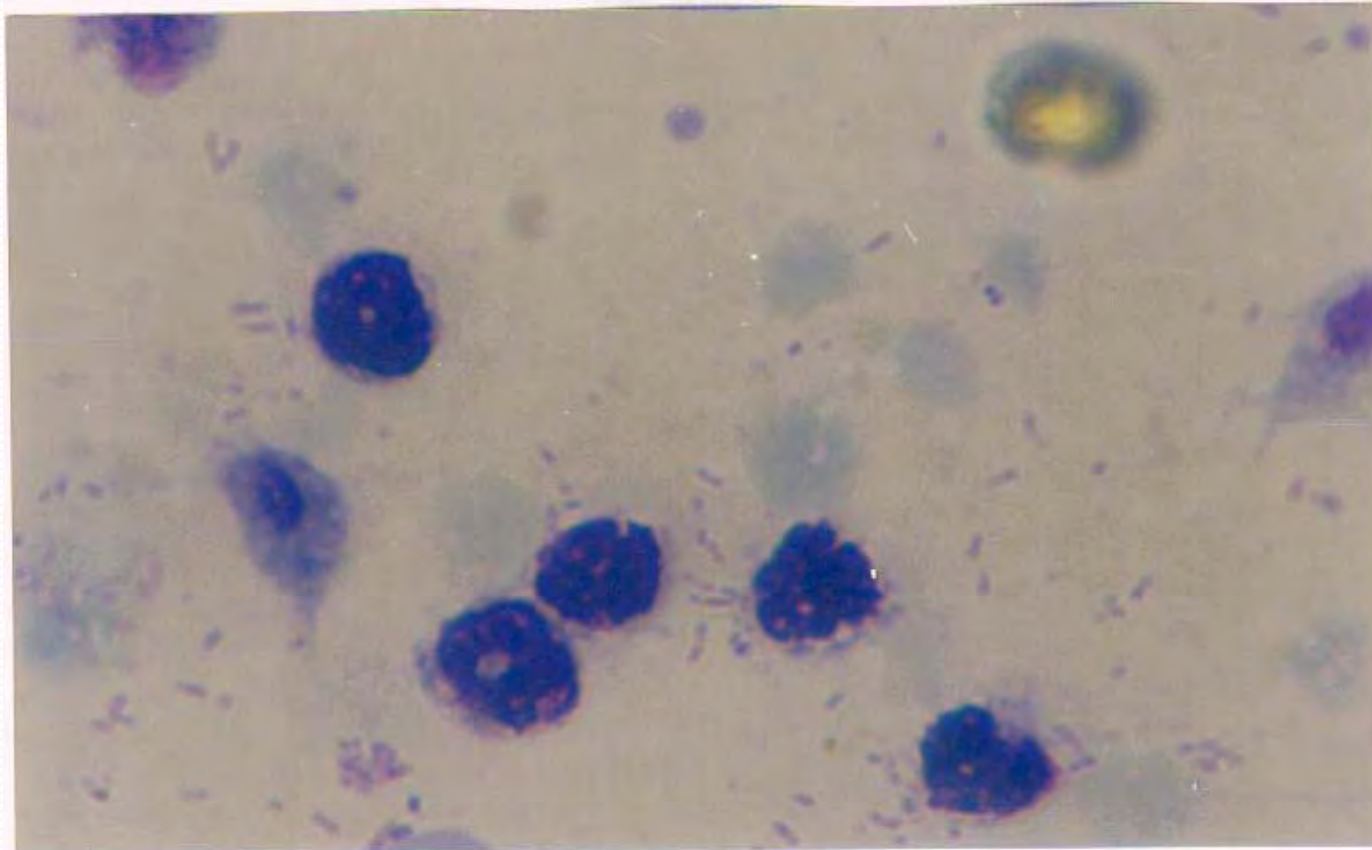
## ***Introduction:***

Human trichomoniasis is now widely recognised as prevalent STD, capable of causing considerable morbidity as well as significant emotional and economic burden on the community.



Cont.

The clinical spectrum of the disease in women, urethritis, vulvo-vaginitis, and cervicitis. Asymptomatic (carrier)





## Cont...

*T. vaginalis* haemolysin is considered as an important virulence factor mediated by pore-forming proteins. These molecules are capable of inserting into the lipid bilayer of the target cells, which play an important role in the establishment of the disease.



Cont...

Production of haemolysin results in  
RBCs lysis and utilization of  
haemoglobin released.



# Aims

- Comparative evaluation of haemolytic activity of *T. vaginalis* isolated from symptomatic and asymptomatic patients for human RBCs.



# Methods

*T. vaginalis* from symptomatic and asymptomatic women were isolated and maintained in axenic culture (TYI-S-33).

Blood group O, in equal volumes of a solution containing 10U of heparin per ml and 3% dextran in physiological saline





## Cont...

The mixture was allowed to sediment for 1 hr and supernatant and buffy coat layers were discarded and erythrocytes were washed twice with an equal volume of HBSS, containing 10U of heparin/ml.

After centrifugation at  $250\times g$  for 10 min, RBCs were used for haemolysis assay.

## Haemolytic activity assay:

Washed RBCs (7.0 $\mu$ l) were mixed with 2.5 ml of TYI-S-33 medium without serum containing a total of  $10^6$  trophozoites.

After 24 hrs of incubation at 37°C in 5% CO<sub>2</sub>, the culture was centrifuged at 250 $\times$ g for 10 min. Absorbance value of the supernatant was measured at 470 nm with a spectrophotometer.



## standard curve

Standard curve made by plotting absorbance versus haemoglobin concentration (0.25-3 mg/ml) in distilled water.

Control tubes containing erythrocytes without trichomonads, were also incubated along with tubes containing trichomonads.



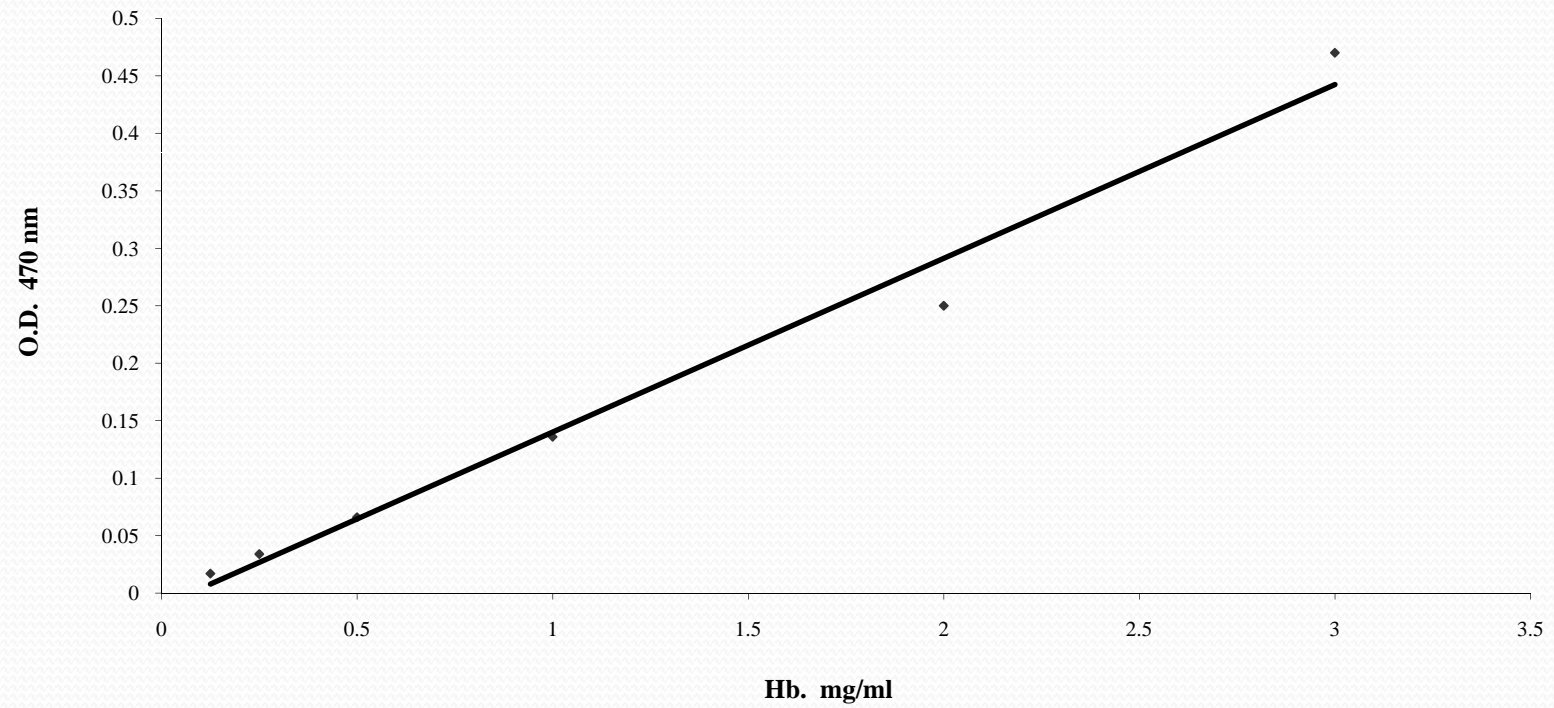
# Advantages of using RBC:

- I. Susceptible target cell for cytolytic effectors
- II. Lysis is easily measurable
- III. Serves as best model for membrane damage



# **Results and conclusion:**

## Standard curve for measurement of haemoglobin





## Cont...

No haemolytic activity was detected in concentrated culture supernatants after overnight growth of *Trichomonas*.

## Optical density (OD) values and Haemoglobin (Hb) concentration of *T. vaginalis* strains isolated from asymptomatic patients (n=10)

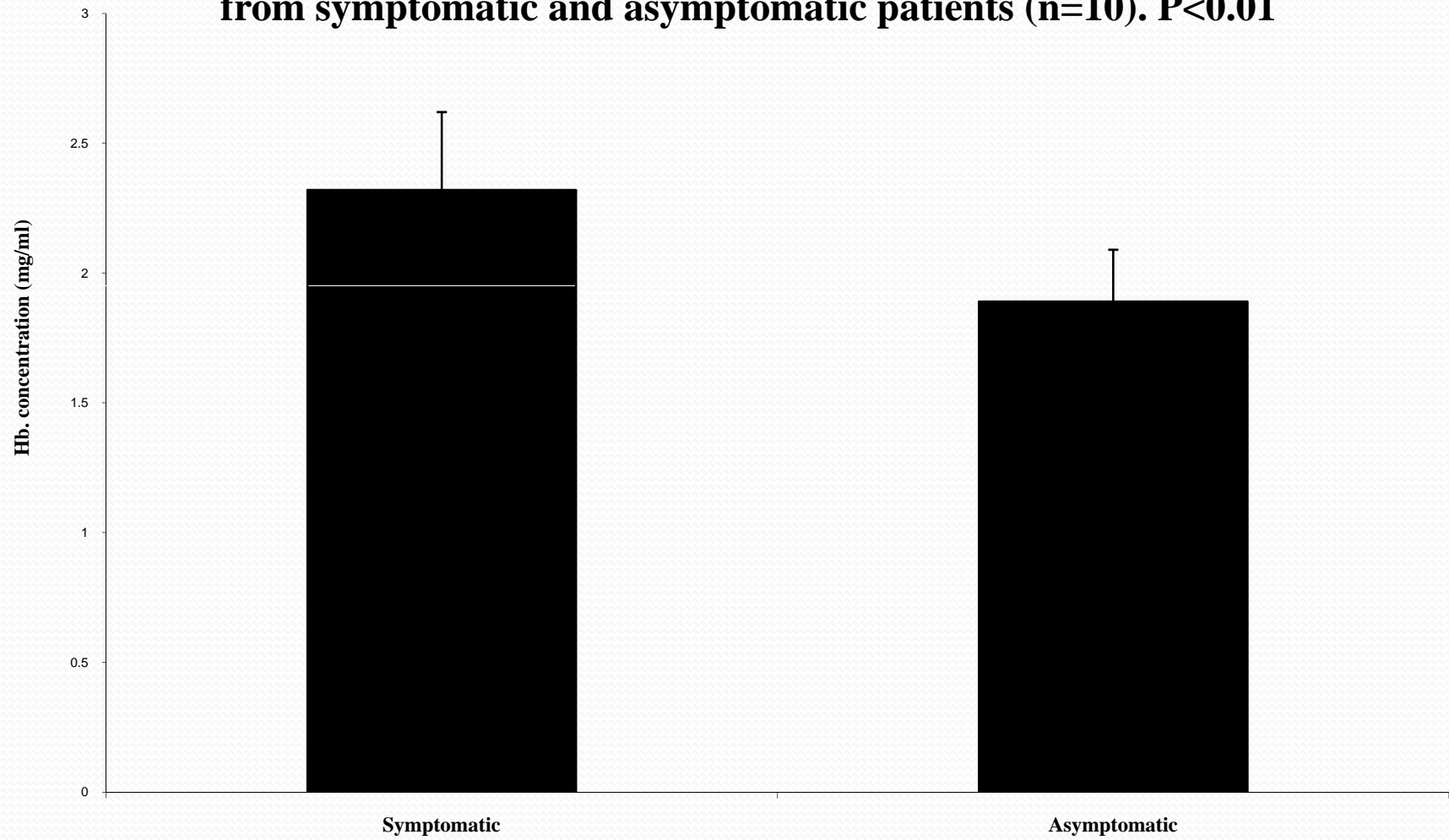
Hb concentration	OD values	T. vaginalis isolates
2.00	0.282	1
2.16	0.305	2
1.41	0.198	3
2.08	0.293	4
1.44	0.202	5
1.77	0.250	6
2.07	0.211	7
1.86	0.262	8
1.97	0.287	9
2.12	0.290	10
1.89 ± 0.2	0.258 ± 0.04	Mean ± SD



## Optical density (OD) values and Haemoglobin (Hb) concentration of *T. vaginalis* strains isolated from symptomatic patients (n=10)

Hb concentration	OD values	<i>T. vaginalis</i> isolates
1.95	0.275	1
2.21	0.312	2
2.19	0.309	3
2.42	0.342	4
1.52	0.213	5
2.71	0.384	6
2.69	0.381	7
2.65	0.375	8
2.57	0.307	9
2.28	0.352	10
2.32 ± 0.3	0.325 ± 0.05	Mean ± SD

**Comparison of average haemolytic activity of *T. vaginalis* isolated from symptomatic and asymptomatic patients (n=10). P<0.01**





Cont...

The vaginal mucosa is a poor nutritional milieu for microbes. The ability to synthesize lipids and to acquire iron is lacking in *T. vaginalis*.



## Cont...

Erythrocytes provide both lipid and iron for the growth and multiplication of parasite, which may be responsible for the exacerbation of symptoms observed during and following menstruation.

