

Research Article

## Correlation between Herpes Simplex Virus antibody titre and Duodenal Ulcer perforation

Authors

**Dr Nandagopal Vasudevan<sup>1</sup>, Dr Premlal A.P<sup>2\*</sup>, Dr Sareena Mathew<sup>3</sup>**

<sup>1</sup>Consultant Urologist, Sree Uthradom Thirunal Hospital, Pattom, Thiruvananthapuram

<sup>2</sup>Associate Professor, Department of Plastic Surgery, Govt. Medical College, Thiruvananthapuram, State Nodal Officer, Trauma & Burns, Kerala.

<sup>3</sup>Retd. Professor & Head of Department of Surgery, Govt. Medical College, Thiruvananthapuram

\*Corresponding Author

**Dr Premlal A.P**

Email: [premlalap717@gmail.com](mailto:premlalap717@gmail.com)

### Abstract

**Background:** *The high incidence of duodenal ulcer and perforated duodenal ulcers in this part of the country prompted us to test the hypothesis that, duodenal ulcer perforations may be associated with Herpes simplex virus infection. There is apparent absence of risk factors in DU perforation. Also it is associated with very short duration of pre-perforation dyspepsia in a significant number of perforated duodenal ulcers. It appeared that there is a high possibility that atleast a subset of these perforations could be due to Herpes simplex virus I or 2 infections.*

**Aim:** *To assess the role of Herpes Simplex Virus type I and II in duodenal- ulcer perforation.*

**Materials and Methods:** *This was a case-control study with three groups of 65 patients. Controls were formed by patients with no symptoms of upper gastrointestinal diseases who were admitted for non-gastrointestinal disorders. Case group again subdivided to a Second group of patients with acute duodenal ulcer perforation and a third group with chronic duodenal ulcer perforation.*

**Results:** *Significant statistical difference in seropositivity for HSV I & II was found in patients with both acute and chronic duodenal perforation vs controls. (P<0.05) For both HSV I and HSV II, there was a significant association between the seropositivity rates and size of acute DU perforation. P <0.05. Statistically significant difference in seropositivity to both HSV I and HSV II was seen in NSAID users. However, in the case of alcoholics and non- alcoholics, significant difference was noticed for seropositivity to HSV I only. In the case of smokers and non-smokers, there was no significant difference in the seropositivity to HSV I and II in all cases. There was a significant trend to higher seropositivity in patients with shorter pre-perforation duration and dyspepsia, for both HSV I and HSV II. There is a trend towards higher HSV titres in perforated DU compared to controls, which is statistically significant.*

**Keywords:** *herpes simplex virus (HSV), duodenal ulcer (DU).*

### Introduction

The question of infectious agents in the etiology of peptic ulcer has been raked in literature for

almost 100 years<sup>1</sup>. The evidence accumulated over the years resulted in implication of *Helicobacter pylori* as one of the causative factors in peptic

ulceration, in addition to other risk factors. Although, the role of other organisms has not been clearly defined, many authors have pointed to an association between peptic ulcer and Herpes simplex virus I and II<sup>2-5</sup>. That, both Herpetic infections and peptic ulcer exhibit recurrence, tends to remain localised, and demonstrate periodicity with remissions and exacerbations, point to the fact that Herpes simplex viruses could be associated with peptic ulcer<sup>6</sup>.

The high incidence of duodenal ulcer and perforated duodenal ulcers in this part of the country prompted us to test the hypothesis that, duodenal ulcer perforations may be associated with Herpes simplex virus infection. Another factor, which prompted us to proceed with this study, was the apparent absence of risk factors and the very short duration of pre-perforation dyspepsia in a significant number of perforated duodenal ulcers. It appeared that there is a high possibility that a subset of these perforations could be due to Herpes simplex virus I or II infections.

### **Aim of Study**

To assess the role of Herpes Simplex Virus type I and II in duodenal- ulcer perforation.

### **Materials and Methods**

**Study design and setting:** This is a prospective case-control study done in Department of Surgery, Medical College, Thiruvananthapuram, for one year. Sample size was calculated using standard statistical formula.

The study was conducted in three groups of patients with a total of 65 patients. Group 1: Controls = 30 patients. This group was formed by hospital based patients with no symptoms of upper gastrointestinal diseases, who were admitted for non-gastrointestinal disorders like hernia, hydrocele, etc. These patients were interviewed and examined in detail to exclude gastrointestinal disease. Upper gastrointestinal endoscopy was not performed in this group, due to ethical considerations. Group II: Acute duodenal ulcer perforation = 20 Patients with duodenal ulcer

perforation with symptoms of acid peptic disease, less than 3 months prior to perforation. Group III: Chronic duodenal ulcer perforation = 15 Patients with duodenal ulcer perforation with symptoms suggestive of peptic ulcer disease for more than 3 months prior to perforation.

Ethical clearance for the study was obtained. Informed consent was taken from each of the patients included in the study.

**Exclusion criteria:** Patients with known history of herpetic lesions were excluded from the study. A history of promiscuity was also elicited from the patients.

**Statistical analysis:** Statistical analysis was done by the Paired 't' test.

### **Methods**

Five ml of venous blood was collected from each of the patients in Group I to III. Serum was separated and stored at -20°C. The serum samples stored at -20°C was thawed and tested for IgG antibody by ELISA, as per the manufacturer's instructions. Each test kit contained micro titre strips coated with Herpes simplex antigen with 96 wells.

### **Test principle**

Micro titre strip wells as a solid phase are coated with HSV I and II antigens. Diluted patient specimens and ready for use controls are pipetted into these wells. During incubation (1 hr) HSV I and II specific antibodies of positive specimens and controls are bound to the immobilised antigens. After a washing step (3 washes) with ELISA washer to remove unbound sample and control material, horse radish peroxidase conjugated anti-human IgG antibodies are dispensed into the wells. During a second incubation (0 min) this anti-IgG conjugate binds specifically to IgG antibodies resulting in 111c formation of enzyme linked immune complexes.

After a second washing step (3 washes) to remove unbound conjugate, the immune complexes formed (in case of positive results) are detected by incubation for 15 minutes at room temperature with TMB substrate (tetra-methyl-benzidine) and

development of a blue colour. The blue colour is turned into yellow by stopping the enzymatic indicator reaction with sulphuric acid TMB stop reagent). The intensity of this colour is directly proportional to the amount of HSV I and II specific IgG antibody in the patient serum.

Absorbance at 450 nm is read using an ELISA micro titre plate reader. Total time taken for the test is 1 hour 45 minutes. The absorbance values are converted into arbitrary titres.

$\frac{\text{Mean Absorbance Value} \times 10}{\text{Cut off}} = \text{ARBITRARY VALUE}$

Cut off value is 10 arbitrary units. Values above 11 arbitrary units are considered positive by the manufacturer's criteria. The other values are considered negative.

## Results

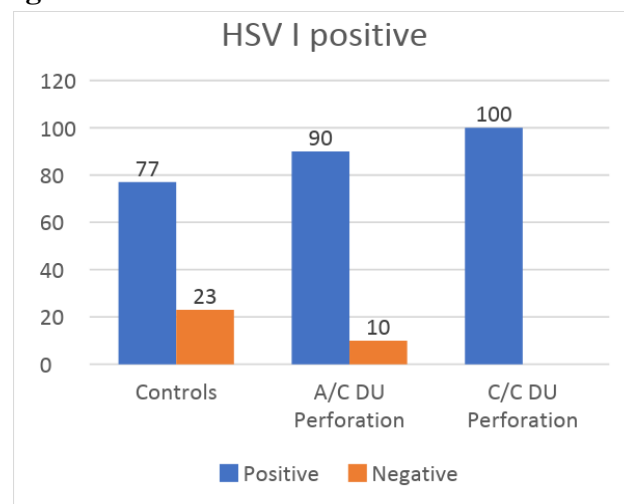
The study was carried out on three groups with 65 patients.

**A) Age:** There was no significant difference between the mean ages in the three groups. The mean age of controls was 42, acute duodenal perforation group – 47 and chronic duodenal perforation – 49.

**B) Gender:** Overall, males comprised over 90% of the patients in the different groups. There was no statistical difference in the proportion of males and females in the different groups.

**C) Seropositivity of HSV I** as per manufacturer's criteria in the 3 different groups is shown in figure 1. The positivity varied from 77% in controls to 100% in patients with chronic duodenal ulcer perforation (Fig.1), thus showing that there is a background of high seropositivity to HSV I in population study.

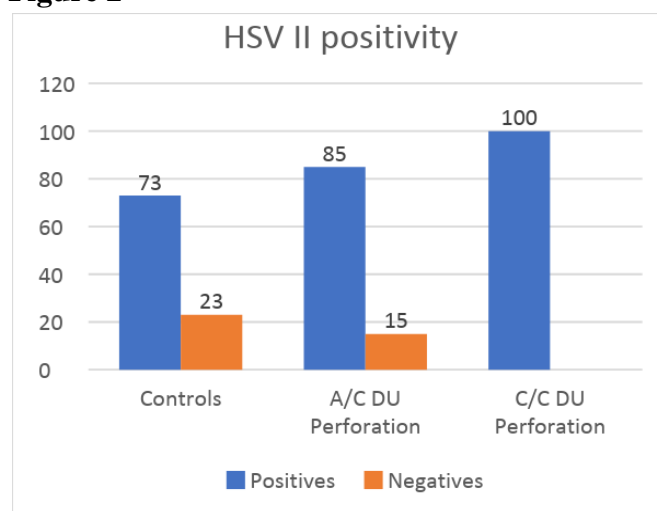
**Figure 1**



Acute DU Perforation Vs Controls  $P < 0.05$  95% CL = 2.21  
 Chronic DU perforation Vs Controls  $P < 0.05$  95% CL = 2.23  
 Significant statistical difference in seropositivity for HSV I was found in patients with both acute and chronic duodenal perforation vs controls.

**D) Seropositivity of HSV II** in the 3 groups is shown in Figure 2. Seropositivity rates varied from 73% in controls to 100% in patients with chronic DU perforation, again showing that there is high background of seropositivity rate to HSV II.

**Figure 2**



Acute DU Perforation VS Controls  $P < 0.05$  95% CL = 2.23  
 Chronic DU perforation VS Controls  $P < 0.05$  95% CL - undefined

Significant statistical difference in seropositivity for HSV II was found in patients with both acute and chronic duodenal perforation vs controls.

**E) Seropositivity for HSV I and II were compared with different patient parameters viz., alcoholism, smoking and NSAID intake in the three groups.**

Results for Group I (controls) are shown in Table

1. Statistically significant difference in

seropositivity to both HSV I and HSV II was observed in the case of alcoholics, smokers and NSAID users when compared with the non-alcoholics, non-smokers and non- NSAID users.

**Table 1:** Comparison of parameters like smoking, alcoholism, NSAID intake and seropositivity to HSV I and HSV II in group I (Controls)

Groups	N	HSV I Positive N (%)	HSV II positive N (%)	P Value HSV I	P Value HSV II
<b>Alcoholic</b>	10	10 (100%)	9 (90%)	<0.05	<0.10
<b>Non-alcoholic</b>	20	13 (92%)	13 (92%)	<0.10	<0.10
<b>Smokers</b>	13	12(77%)	12 (77%)	<0.05	<0.10
<b>Non-smokers</b>	17	11(65%)	10 (59%)	<0.05	<0.10
<b>NSAIDs (+)</b>	2	1 (50%)	1 (50%)	<0.05	<0.5
<b>NSAIDs (-)</b>	28	22 (79%)	21 (75%)	<0.05	<0.5

P <0.05 indicates significance at 5% level; p <0.10 is non significant.

**Table 2:** Acute duodenal ulcer perforation group II- comparison of parameters like smoking, alcoholism, NSAID Intake and seropositivity to HSV I and HSV II

Groups	N	HSV I Positive N (%)	HSV II Positive N (%)	P value HSV I	P value HSV II
<b>Alcoholic</b>	12	10 (83%)	10 (83%)	<0.05	<0.10
<b>Non-alcoholic</b>	8	8 (100%)	7 (88%)	<0.05	<0.10
<b>Smokers</b>	14	13(93%)	12 (86%)	<0.10	<0.10
<b>Non-smokers</b>	6	5(83%)	5 (83%)	<0.10	<0.10
<b>NSAIDS (+)</b>	5	5 (100%)	5 (100%)	<0.05	<0.5
<b>NSAIDS (-)</b>	15	13 (87%)	12 (80%)	<0.10	<0.10

P <0.05 indicates significance at 5% level; p <0.10 is non significant.

Results for Group II (Acute DU perforation) are shown in Table 2. Statistically significant difference in seropositivity to both HSV I and HSV II was seen in NSAID users. However, in the case of alcoholics and non- alcoholics, significant difference was noticed for seropositivity to HSV I

only. In the case of smokers and non-smokers, there was no significant difference in the seropositivity to HSV I and II in all cases. Results for Group III (Chronic DU Perforation) are shown in Table 3.

**Table 3:** Chronic DU perforation group III - comparison of parameters like smoking alcoholism, NSAID intake and seropositivity to HSV I and HSV II

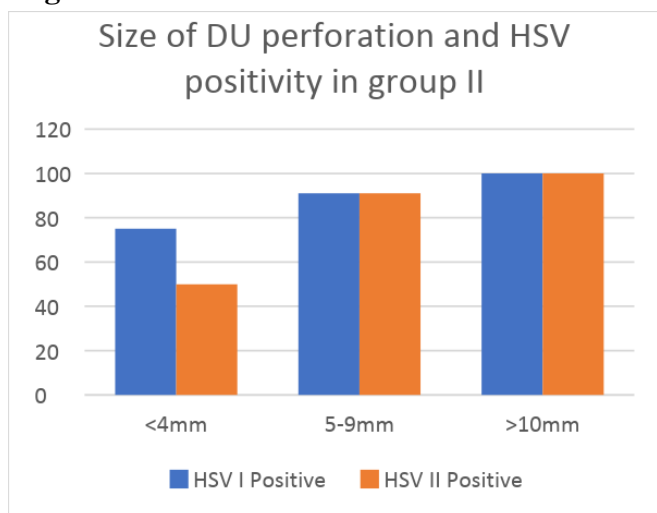
Groups	N	HSV I Positive N (%)	HSV II Positive N (%)
<b>Alcoholic</b>	5	5 (100%)	5 (100%)
<b>Non-alcoholic</b>	10	10 (100%)	10 (88%)
<b>Smokers</b>	5	5 (100%)	5 (100%)
<b>Non-smokers</b>	10	10 (100%)	10 (88%)
<b>NSAIDS (+)</b>	3	3 (100%)	3 (100%)
<b>NSAIDS (-)</b>	12	12 (100%)	12 (100%)

No statistics computed as the seropositivity was 100% in all groups.

**Perforations were classified based on the size as seen at laparotomy into three groups.** This is shown in figure 3 for Group II (Acute DU perforation) Statistical comparison was made

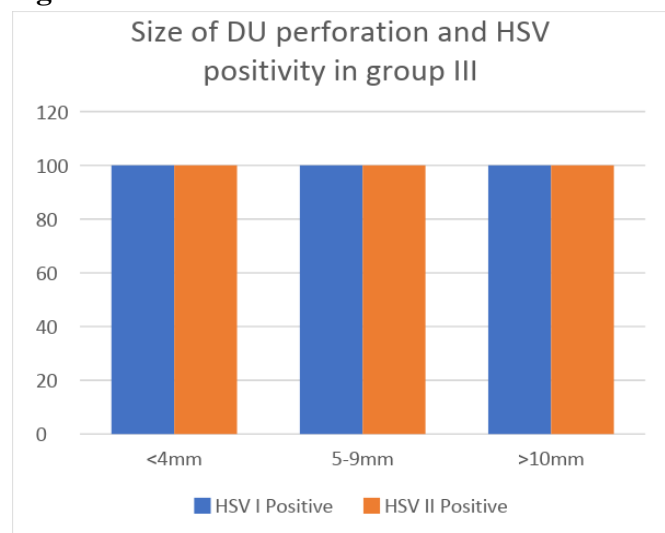
between 5-9 mm perforation and <4 mm perforation as well as between >10 mm perforation and <4 mm perforation

**Figure 3:**



For both HSV I and HSV II, there was a significant association between the seropositivity rates and size of acute DU perforation. P <0.05 for both HSV I and HSV II

**Figure 4**



P' value not computed since all values were 100%.

**F) Duration of Symptoms prior to perforation and seropositivity:** Analysis was done to see whether the duration of symptoms prior to perforation was related to seropositivity. Duration was classified into < 1 week, 1-3 weeks and > 3 weeks for acute DU perforation group. When thus classified, there was a significant trend to higher seropositivity in patients with shorter pre-perforation duration and dyspepsia, for both HSV I and HSV II (refer table 4)

**Table 4:** Duration of Symptoms prior to perforation and seropositivity in acute duodenal ulcer perforation (group II)

Duration of symptoms	N (%)	HSV I Positive N %	HSV II Positive N %
<7 days	9 (45%)	9 (100%)	8(89%)
8-21 days	7(35%)	6 (86%)	6 (86%)
>22 days	4 (20%)	3 (75%)	3 (75%)

Statistical comparison was made between 8-21 days duration and <7 days duration as well as between >22 days duration and, 7 days duration. For both HSV I and HSV II, P. <0.05

In patients with chronic DU perforation, the duration of pre-perforation symptoms were classified into three groups viz., 3 months to 1 year, 1-2 years and > 2 years. All the patients were seropositive and therefore the association between the seropositivity and pre-perforation duration could not be defined. (refer table 5)

**Table 5:** Duration of Symptoms prior to perforation and seropositivity in chronic duodenal ulcer perforation (group III)

Duration of symptoms	N (%)	HSV I Positive N (%)	HSV II Positive N (%)
3 months – 1 year	7 (47%)	7 (100%)	7 (100%)
1-2 years	5 (33%)	5 (100%)	5 (100%)
>2 years	3(20%)	3 (100%)	3 (100%)

P value is not computed since all values were 100%.

**G) Mean titres in various groups:** An attempt was made to see whether actual titre levels for HSV I and HSV II were different in the three groups. The arithmetic mean titres were used for comparison of titre values between the various groups (Table 6). Once again, a trend towards higher titres is seen in perforated DU compared to control group, which is statistically significant.



**Table 6:** Mean titres in various groups

	NOMENCLATURE	GROUPS	N	MEAN	SD
HSV I	Controls	I	30	40.69	22.92
	Acute DU perforation	II	20	60.78	25.61
	Chronic DU perforation	III	15	58.29	15.64
HSV II	Controls	I	30	21.42	16.37
	Acute DU perforation	II	20	50.80	24.38
	Chronic DU perforation	III	15	45.69	20.15

## Discussion

It was in 1967 when the possibility of a viral etiology in peptic ulcer was first raised by Neumann<sup>2</sup>. Since then many studies have appeared in the literature on this topic. Although, a few of them had reported that Herpes virus could be related to peptic ulcer<sup>7,8,9</sup> others did not support such an association<sup>10,11</sup>.

All the studies which had been done on the herpetic etiology of peptic ulcers had been on either non-perforated duodenal ulcers or gastric ulcers. The clinical picture and course of herpetic infections closely resembles peptic ulcer. Both peptic ulcers and herpetic infections recur at roughly the same site, often two or three times a year and both tend to arise especially in the spring and autumn<sup>4,5</sup>. The incidence of duodenal ulcer perforation in this part of the country is high. Many of these patients have very short or no antecedent history of ulcer like dyspepsia and no other associated risk factors. Hence this study was planned to assess the correlation if any between herpetic infection and perforated duodenal ulcer.

### A) Age Distribution

In the present study the mean age distribution of the patients in the different groups ranged from 42 years to 49 years, suggesting a prevalence of these conditions in the young adults and others in their early forties. In patients with DU perforation, the presentation was predominantly around 45 — 50 years of age, which was comparable to other reported studies<sup>12,13</sup>. Khoursheed et al in a study on duodenal ulcer perforations reported a mean age of 36.4 + 11.8 years which was comparable to the present study<sup>14</sup>. On the other hand, reports from western literature give a later age of presentation lying in the late fifties for patients with DU perforation (59 + 10 years)<sup>15</sup>. This may

probably be due to differing etiologies for the disease state. NSAID abuse related perforated duodenal ulcer is more common in the west.

### B) Gender Distribution

In the present study there was a very high male preponderance in all the groups. Hence 90% in patients with acute duodenal ulcer perforation and 93% in patients with chronic duodenal ulcer perforation were males. This predominance amongst males was also reported in the previous by Khoursheed et al<sup>14</sup>.

### C) HSV Seropositivity Status

In the present study, the seropositivity to HSV I varied from 77% in controls to 100% in chronic duodenal ulcer perforation. The high HSV seropositivity in patients with acute duodenal ulcer perforation (90%) and chronic duodenal ulcer perforation (100%) was significantly higher than that of controls. A high seropositivity in controls suggests the possibility of a higher prevalence of HSV I in the study population due to prior exposure.

Many other studies have also reported a higher seropositivity to HSV I in the normal population. Westergaard et al<sup>16</sup> reported 80% seropositivity in healthy controls, similar to another study by Kottardis et al<sup>11</sup>, which reported a seropositivity of 94% in normal healthy controls. Archimandritis et al<sup>17</sup> reported 81% seropositivity to HSV I in patients with Non-ulcer Dyspepsia. A high seropositivity of 92% for HSV I was reported from the same study in patients with non-perforated duodenal ulcers. The significantly higher seropositivity in chronic duodenal ulcer perforation group could be due to flaring up of a latent herpes infection which is responsible for the perforation of the ulcer.

The seropositivity of HSV II has been seen to vary in different populations and geographic areas. It varied from 12% in adolescents to 78% in sex workers in the United States<sup>18</sup>.

In the present study, the seropositivity for HSV II varied from 73% in controls to 100% in patients with chronic duodenal ulcer perforations. The seropositivity to HSV II in the various groups was similar to the trend seen with HSV I. We found that HSV II seropositivity in acute duodenal ulcer perforation was 85% and 100% in chronic duodenal ulcer perforation which was significantly higher than the control group. The higher seropositivity to HSV II found in our study probably represents a geographical variation. The seropositivity to HSV II in perforated duodenal ulcer's has not been reported previously in the literature.

#### **D) Comparison of seropositivity and alcoholism, smoking and NSAID intake**

We did not come across any study which compared these parameters to HSV seropositivity. In the control group, statistically significant difference in seropositivity to both HSV I and HSV II was observed in the case of alcoholics, smokers and NSAID users when compared with the non-alcoholics, non-smokers and non-NSAID users. In the acute duodenal perforation group, statistically significant difference in seropositivity to both HSV I and HSV II was observed in the case of alcoholics, smokers and NSAID users when compared with the non-alcoholics, non-smokers and non- NSAID users.

#### **E) Seropositivity and size of DU perforation:**

In the present study, when the size of DU perforation was compared as found at laparotomy to seropositivity to HSV, it was found that for both HSV I and HSV II, there was a significant association between the seropositivity rates and size of acute DU perforation. As regards the correlation between the size of chronic DU perforation and seropositivity, it was seen that seropositivity was 100% in all patients, irrespective of perforation size. The reason for association of higher seropositivity to increasing

size of ulcer remains unclear. It may suggest that a larger ulcer is more prone for reinfection with HSV with consequent risk of perforation.

#### **F) Seropositivity and duration of pre-perforation symptoms**

Regarding the duration of symptoms and seropositivity in acute DU perforation, there was a significant trend to higher seropositivity in patients with shorter pre-perforation duration of dyspepsia for both HSV I and HSV II. This finding might suggest a link between herpetic infections and acute perforations with shorter history of pre-perforation dyspepsia. In patients with chronic DU perforations, all the patients were positive for HSV I and II infections suggesting even stronger relationship between herpetic infection and chronic DU perforation.

#### **G) Antibody Titres**

When the mean titres of HSV I and HSV II antibody in the different groups were compared, it was found to be higher in the perforated duodenal ulcer groups (Groups II and III), which was statistically significant. The higher titres or antibodies could be due to the recrudescence of the latent infection in patients chronically infected with Herpes simplex virus. This could be a causative factor for perforated duodenal ulcer.

The titre of HSV II was also significantly lower compared to the HSV I titre in the different groups. This points to the fact that the predominant infection may be due to HSV I. The high titres of HSV I and HSV II antibodies in perforated duodenal ulcers could be taken as indirect evidence of an association between Herpetic infection and duodenal ulcer perforation. The higher titres of antibodies could be due to the recrudescence of the latent infection in patients chronically infected with Herpes simplex virus. This could be a causative factor for perforated duodenal ulcer. The relationship of HSV II infection and duodenal ulcer perforation remains less clear compared to HSV I.

### Limitations of Study

The IgA titre in the gastric aspirate could not be studied in the different groups due to technical problems. If the seropositivity of HSV I and II to IgA antibody and IgA antibody titres in the various groups could have been studied, it would have given further information on the association between Herpes simplex virus and duodenal ulcer perforation.

Patients with non-ulcer dyspepsia, acute duodenal ulcer and chronic duodenal ulcer was not included in the study and the presence of these groups would have made the interpretation of the results easier.

Only serology was used to determine the presence of Herpetic infection in the different groups. Viral culture is suggested to be the gold standard in diagnosis of Herpetic infection<sup>[20]</sup>. Use of modalities like viral culture, immunofluorescence, complement fixation tests and histopathological examination could have been more appropriate in defining herpetic infection, especially as the prevalence of the infection in normal population is high.

The control group in this study were hospital based patients with no apparent history or evidence of gastrointestinal disease. This control group may not be similar to a field based control group.

### Conclusion

This study provides some evidence that Herpes simplex virus may be associated with perforated duodenal ulcers. Significant statistical difference in seropositivity for HSV I & II was found in patients with both acute and chronic duodenal perforation vs controls. ( $P < 0.05$ ) For both HSV I and HSV II, there was a significant association between the seropositivity rates and size of acute DU perforation.  $P < 0.05$ . Statistically significant difference in seropositivity to both HSV I and HSV II was seen in NSAID users. However, in the case of alcoholics and non- alcoholics, significant difference was noticed for seropositivity to HSV I only. In the case of smokers and non-smokers,

there was no significant difference in the seropositivity to HSV I and II in all cases. There was a significant trend to higher seropositivity in patients with shorter pre-perforation duration and dyspepsia, for both HSV I and HSV II. There is a trend towards higher HSV titres in perforated DU compared to controls, which is statistically significant.

The exact nature of the association and the probable role of other agents need to be ascertained by further studies using better diagnostic methods and by a larger sample size of subjects in the study.

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