

# PREVALENCE AND GENETIC DIVERSITY OF HERPES SIMPLEX VIRUS TYPE 2 AMONG MARRIED WOMEN OF SOUTH ANDAMAN ISLANDS

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## Abstract

The spread of HSV-2, a type of herpes simplex virus (HSV-2) a sexually transmitted infection (STI) which widely found all over the globe, which is considered as a significant public health problem and it is one of the main causes for increased risk of HIV infection. This research is based on the prevalence of HSV-2 among married women and the prevailing factors like risks in the Andaman and Nicobar Islands for the period 2020 to 2023. In the survey conducted on 675 women in 18-45 years, 4.8% of HSV-2 cases among female participants were recognized with the relative determinants such as age at first intercourse, reproductive history and living in rural areas. While traditional risk factors like tobacco and alcohol use were notably absent. Through genetic analysis it was revealed that there are resemblances with global strains, this may imply implications for transmission pattern and treatment procedure. As a result of these findings, the stakeholders can develop specific interventions and genetic characterization that can inform the preventative strategies to aid in reducing the prevalence and health outcomes associated to HSV-2 infection. By figuring out the HSV-2 epidemiology which is localized to remote island community and building pathways for layered and culturally appropriate targeted interventions, this research has played its part in shaping these public health efforts toward controlling STI and improving the overall population health.

**Keywords:** Herpes Simplex Virus type-2; Andaman and Nicobar Islands; Risk factor; Genetic analysis; Polymerase Chain Reaction (PCR).

## 1. INTRODUCTION

HSV-2 infection is a widespread sexually transmitted infection (STI) and persists throughout a person's lifetime [1,2]. Research findings point to a correlation between herpes simplex virus type 2 (HSV-2) infection and an increased susceptibility to acquiring HIV infection in subsequent instances [3]. The transmission of herpes simplex virus (HSV) infection occurs primarily through intimate personal contact, involving a susceptible individual without antibodies to the virus coming into contact with someone who is actively shedding HSV [4].

Initiation of infection requires the virus to encounter mucosal surfaces or damaged skin. Upon introduction to the skin or mucous membrane, there follows an incubation period lasting approximately four to six days before HSV commences replication within epithelial cells, mirroring the earlier discourse on transmission through close personal contact [5]. Herpes simplex virus 1 and 2 are capable of infecting both humans and other animals, including cats, mice, and dogs. However, clinical manifestations of the disease are observed solely in humans [6].

Genital herpes, predominantly caused by herpes simplex virus type 2 (HSV-2), impacts approximately 491 million individuals aged 15 to 49 globally. The majority of herpes infections is asymptomatic or goes undiagnosed, but painful blisters or ulcers that might eventually recur are signs of herpes. The risk of contracting and spreading HIV infection is elevated in individuals with HSV-2 infection. Blistering, lumps, or an ulcer around the genitalia or anus are common signs of genital herpes.

HSV-2 infection affects more than one in ten people of Asia. Three-quarters of cases of genital herpes were caused by HSV-2. Currently, there is no immunization against HSV-2 acquisition or reactivation. However, antiviral medications like acyclovir, valaciclovir, and famciclovir have been shown to effectively reduce HSV-2 shedding frequency and the length of genital ulcer outbreaks [7]. This strengthens the case for both universal access to sexual and reproductive health care and the development of HSV-2 vaccination [8].

A study from India revealed 11.3% of married women tested positive for HSV-2. The prevalence of HSV-2 rose with age: it was 5.4% in women between the ages of 15 and 20, 8.3% in those between the ages of 21 and 25, and 14.4% in those over 25. Women with a history of vaginal ulcers or sores, bacterial vaginosis, women who reported having intercourse with a partner while under the influence, and women who smoked were at risk for HSV-2 [9]. Past genital herpes infection may be a significant risk factor for Human Immunodeficiency virus (HIV) infection [10].

India's union territory includes the Andaman and Nicobar Islands which was situated closer to Indonesia and Thailand and it is situated in the southernmost part of the Bay of Bengal in the Indian Ocean [11]. South Andaman is a district of this union territory and it had a population of 2,38,142 including 1,10,859 females [12]. Viral Pathology of STI such as Human Papillomavirus (HPV) was previously was identified in this island. This study revealed various types of HPV among the women population [13]. Further, there was no information about HSV-2 and associated factors among married women of this Island. Modern preventive therapies could minimise the incidence of herpes genital ulcer disease (GUD) and enhance the well-being of countless individuals [3]. Therefore, we aimed to know the prevalence of HSV-2 and its related factors of married women.

This study conducts a comprehensive exploration into HSV-2 infection and its associated factors among married women residing in the Andaman and Nicobar Islands. It delves into various dimensions, including socio-demographic characteristics, risk factors, menstrual history, symptoms, and the construction of a phylogenetic tree based on genetic analysis. Through this research endeavor, a detailed understanding of the prevalence and determinants of HSV-2 infection in this specific population, thus shedding light on crucial factors that contribute to its spread and persistence.

## 2. MATERIALS AND METHODS

### 2.1 Study Design and Population

A cross-sectional field study was conducted for three years from July of 2020 to August of 2023 in South Andaman district of India. In South Andaman district tertiary care hospitals, study participants were admitted as outpatients. Before being admitted to the study, all subjects were subjected to a mandatory comprehensive clinical examination that was conducted by trained health professionals. This study was aimed

at localizing sign and symptoms possibly associated with Lower genital tract infections. As well as, there were socio-demographic elements assessed and collected through a structured questionnaire to have a clear idea of the research population.

## **2.2 Exclusion Criteria**

Individuals who were already diagnosed with tuberculosis, those who had used antibiotics in the last two weeks before study commenced, and those who refused to take part in the study were not included in the study to preserve the quality and reliability of the results.

## **2.3 Sample Collection**

Qualified gynecologists and auxiliary midwives were carefully trained for the collection of vaginal swabs, to ensure that the results are accurate and consistent. Cotton-tipped sterile stick with a plastic handle was put into the vaginal canal and got firmly rotated for 15 to 20 seconds then carefully removed. Every swab collected is immediately transferred in a 15 ml screw-capped centrifuge tube containing phosphate buffered saline (PBS) to minimize the possibility of degradation. To avoid sample degeneration and maintain their stability, all samples were transported to the laboratory in a temperature-controlled container which kept the temperature range at 2-8°C. When arriving in labs, samples were placed in a freezer at -20°C until they were processed further.

## **2.4 Sample Processing and DNA Extraction**

Upon arrival at the laboratory, samples were then processed through the centrifugation step at 10,000 rpm for 10-20 minutes. This objective of this centrifugation step was to pellet the cellular molecules, thereby concentrating the DNA within the pellet. The process of DNA extraction was carried out with high accuracy, using a commercial DNA extraction kit. The DNA extraction protocol was precisely followed based on the instructions given by the manufacture to isolate most of the high-quality DNA from the cell pellets.

## **2.5 HSV-2 Specific PCR Assay**

The detection of HSV-2 was carried out by the specific Polymerase Chain Reaction (PCR) assay. PCR amplification was done with the help of PCR Master Mix (K0171, Invitrogen, ThermoScientific™, USA) and using the HSV-2 genome-specific primers: Pol F' forward primer, 5'-GTCCCACCT CAGCGATCTGCC T-3 and Pol R' reverse primer, 5'-CAGCAGCGAGTCCTGCACACAA-3'.

The primers were designed for high specificity and sensitivity. The PCR reactions were carried out using an ABI Veriti 96-well thermal cycler (Applied Biosystems Inc, USA) with optimized conditions, previously published in a scientific literature [14].

The formed PCR fragments were subsequently analysed using horizontal agarose gel electrophoresis stained with ethidium bromide (EtBr) to ensure the presence of HSV-2 DNA fragment and to evaluate the quality and completeness of the amplification. The amplification product consisted of approximately 490 bp from the UL 30 (DNA polymerase) primer, was visualized after electrophoresis through a 1.5% agarose gel containing ethidium bromide (EtBr).

## 2.6 Sequencing

Identification of HSV-2 strain dispersed over the South Andaman was done using DNA sequence analysis. All samples were submitted to DNA sequence analysis for the UL30 gene PCR products. For DNA sequencing, these amplicons were sent to the Centre for DNA Fingerprinting and Diagnostics (CDFD), Hyderabad, India. Using the illumina MiSeqFGx genomics device, DNA sequencing was done using the Sanger sequencing method.

## 3. RESULTS

### 3.1 Prevalence of HSV-2 Infection

A wide epidemiological survey of the South Andaman Islands was conducted by our research team during which samples from 675 women aged 18 to 45 years old were gathered and analysed. Through this process, the prevalence rate of HSV-2 infection among females of reproductive age was determined. The findings of our physiological tests were analysed and 32 samples were positive for HSV-2, this led to a prevalence rate of 4.8%. This study clearly demonstrates that, there is a need to develop prevention, screening and treatment strategies specifically meant for people with HSV-2 infection. The collected data are presented in Table 1 and Table 2 for a comprehensive overview of the study findings.

### 3.2 Demographic Characteristics

#### 3.2.1 Age Group Analysis

In the course of our study of age specificity, we discovered that the age group that was most affected by HSV-2 was 31 to 35 years old, with the number of cases reaching 11 (N=11) in this age group. The age group 36-40 had 10 cases (N=10), respectively. These trends highlight that women in their early to mid-thirties face a higher risk of HSV-2 infection, and therefore, the targeted interventions and educations ought to be implemented.

#### 3.2.2 Residence

Geographically, 59.3% of the HSV-2 positive cases were noted for females from the rural areas while 40.7% of them from the urban areas. This gap between rural and urban areas implies that there could be some differences in access to healthcare, awareness and preventive practices. Consequently, this would insist on the fact of health care delivery equity and the awareness campaigns that are equal.

#### 3.2.3 Educational and Employment Status

Among the infected women there were 12 graduates (N=12), which indicates a fairly high level of education achievements. But a substantial number of them have not managed to find a job. This unemployment rate may attribute to the socio-economic barriers to access healthcare testing and prevention strategies that now call for targeted interventions and community programs.

### 3.3 Sexual Orientation and Partners

#### 3.3.1 Sexual Orientation

Thirty (N=30) of them classified themselves as heterosexuals. A smaller fraction identified themselves as homosexual (N=1) or bisexual (N=1), which was an indication

of the diverse sexual orientations among this group. It reflects on the role of comprehensive sexual education and the importance of anti-discrimination campaigns in addition to healthcare facilities.

### **3.3.2 Number of Sexual Partners**

The data found that most of the participants (N=31) disclosed a rather monogamous life, with one lifetime sexual partner only. Single participant only had more than two sex partners. This binomial is evidence that searching for monogamy, using safe sexual practices, and regular testing of STIs are strategies to be implemented to reduce the risk of HSV-2 transmission and other STIs.

### **3.4 Risk Factors Associated with HSV-2 Infection**

#### **3.4.1 Age at First Intercourse**

56.2% of the infected individuals reported initiating sexual activity between the ages of 20 to 45 years. This age-group especially indicates a crucial period of susceptibility by saying that it is important sexual education that should be done early, preventions strategies, routine screening, and counselling services.

#### **3.4.2 Medical History**

25% women were found to have a past history of pelvic inflammatory disease (PID) which is well known as a potential source of HSV-2 infection. HSV-2 infected women never had the history of STD. Additionally, 34.4% of patients who take antidepressants have comorbid conditions like diabetes and hypertension. These results demonstrate the possible role of these conditions as predisposing factors. Also, these results give prominence to integrated healthcare models, specialist multi-disciplinary services, and chronic disease management.

### **3.5 Marriage and Pregnancy**

Majority of women were married between 19 and 25 years, of which 50% were confirmed to be infected with the disease. Majority of the women had their first pregnancy after 20 and 69% of them already had one or two pregnancies whereas only five women conceived more than three times. However, five of them never had a conception. These reproductive patterns could end up bringing about the risk transmission of HSV-2, back home or within the families, influencing the services on family planning, prenatal care and maternal care.

#### **3.5.1 Delivery Method**

The most prevalent delivery method among the cases was a natural delivery through the vagina, 15 cases (N=15) were reported in this group. This was followed by cesarean section in 12 cases (N=12). Particularly, five out of women do not have prior delivery, so the signs of fertility, as well as the need of perinatal care tied with HSV-2 infection, are highly likely.

### **3.6 Reproductive Health**

#### **3.6.1 Menarche Age**

The age of menarche has been detected at 13 years old for the majority of the infected women. Adolescent females with early menstruation may be considered at greater risk of acquiring HSV-2 and therefore suggests that hormonal, developmental, and



reproductive factors should be explored such as menstrual hygiene practices and regularity of menstrual cycle.

### **3.6.2 Contraception Methods**

The fact that most of the infection was among women who didn't use any forms of contraception, stands out. On the other hand, two female participants revealed that they had used condoms, hormonal birth control pill and intrauterine device (IUD) while eight got a tubectomy as a contraceptive measure. This data underscores the requirement of multidimensional sexual education, coitus apprehension, family planning service and reproductive rights.

### **3.6.3 Sanitary Pad Usage**

Sanitary pads were the most mentioned among the infected women, with 22 women (N=22) changing their pads three to four times per day. Most of the participants expressed a preference for pads made from synthetic materials (N=27) compared to cotton pads or cloth, which could be a stepping stone towards educating them on proper menstrual hygiene, availing them with quality products, good sanitation and promoting environmental sustainability considerations.

### **3.7 Clinical Symptoms**

Four of the HSV-2 infected participants revealed uncommon symptoms attributed to coitus or menstruation. Majority of the women with the infection reported lower abdominal pain, chronic pelvic pain, abnormal vaginal discharge, itching or irritation, as well as painful urination as the most common symptoms in South Andaman.

These symptoms reveal the clinical picture of HSV-2 infections and the importance of early diagnosing, treating, managing, and providing support. In addition, few of them reported with Menorrhagia. Apart from this, only two were infected with inguinal swelling, weight loss without diet, and anemia (Fig.1). These mixed symptoms testify to the different clinical display of the HSV-2 infection, possible consequences, and the significance of a full scrutiny of symptoms, diagnoses and management.

### **3.8 DNA Sequencing Results**

#### **3.8.1 Strain Analysis**

The DNA sequencing analysis unveiled a close genetic relationship between the HSV-2 strains we found on the South Islands of Andaman and global strains from United States and Europe. This genetic similarity shows chances of the world interconnectedness through transmission dynamics, viral evolution and spread patterns of HSV-2 strains among different populations occupying various regions in the globe.

#### **3.8.2 Gene Mutations**

No mutations have been observed in the UL 30 gene in the investigated strains (Fig. 2), which symbolizes stability, conservativeness and consistency. Generally, this may have short-term and long-term implications for antiviral therapy resistance patterns, therapeutic strategies, vaccine development, and future research in molecular epidemiology, pathogenesis, and virology of HSV-2.

#### 4. DISCUSSION

The study revealed a 4.8% HSV-2 prevalence among married women in South Andaman. Unconventional risk factors were noted, while age at first intercourse and reproductive history emerged as potential determinants. Diverse symptoms were reported, and genetic analysis showed similarities with global strains. This highlights the need for targeted interventions in the region.

A study from India revealed 10.1% prevalence of HSV-2 among the general population in India [15]. One in five women aged 14 to 49 years had a risk of genital herpes infection [16]. Positive PCR results for HSV were strongly associated with specific clinical diagnosis and HSV-2 specific assay had highest diagnostic value [17].

Research found that the HSV-2 prevalence rising with the increasing age. Moreover, global analysis revealed that HSV-2 prevalence was high in women aged less than 30 years old [18]. Furthermore, a study from India revealed that women aged over 25 years had high prevalence of HSV-2 [9]. Age-wise analysis of the current study revealed that women aged over 30 years were more prone to HSV-2 infection.

There was a significant correlation between marital status and HSV-2 prevalence, in line with the findings of Kalu et al [19], who also reported a significant correlation between marital status and IgG prevalence, with a majority of married women with HSV-2 infection identified as unemployed or housewives [9]. Moreover, our study found a high prevalence of HSV-2 among unemployed women, particularly those residing in rural areas, indicating the importance of not overlooking prevention efforts in rural and remote communities [20]. The study revealed a notably elevated prevalence of HSV-2 infection, reaching 100% among respondents with low educational qualifications and 59.6% among those with high educational qualifications, suggesting a potential correlation between educational level and HSV-2 susceptibility [21].

The observed inverse relationship between HSV-2 infection and education level suggests that higher education may lead to greater awareness of the infection, its transmission routes, and prevention methods. This underscores the positive impact of education and public awareness on reducing the carrier rate of HSV-2 infection, while highlighting that inadequate education may contribute to ignorance regarding HSV acquisition and prevention [22]. Educational reforms aimed at enhancing access and community education levels can elevate future aspirations, and consequently lower the risk of HSV-2 and other STIs in young women [23]. Additionally, married women from rural areas demonstrated a similarly high prevalence of HSV-2 infection in our study.

The age of first intercourse has been identified as a significant factor contributing to the risk of herpes infection, with studies indicating a higher prevalence among individuals who initiated sexual activity at a younger age [19]. This association is further underscored by research revealing that a considerable proportion of married women with HSV-2 infection reported having their first sexual experience between the ages of 20 to 45 years. Additionally, a study highlighted that a majority of women diagnosed with HSV-2 had engaged in sexual activity with more than four partners, with multiple sexual partners being significantly linked to HSV-2 infection [9,24]. Notably, our study observed a different trend, as most married women included reported having only one sexual partner.

Women who have sex under the influence of alcohol and smoking were more linked with HSV-2 infection, based on previous study [9]. Meanwhile, tobacco usage, illicit drug usage, and alcoholic consumption were not reported among the infected population in this study. Women who had the history of Pelvic Inflammatory Disease were more prone to HSV-2 infection [25]. However, the current study revealed that history of PID was low among HSV-2 infected population.

Herpes infection may increase the risk of hypertension and Diabetes, according to previous studies. The present study found that more than 30 percent of HSV-2 infected women had other illness like diabetes and hypertension [26]. A study from Africa found that earlier menarche at age less than 14 years and earlier marriage had high risk of HSV-2 infection among married women in rural population [27]. Similarly, the findings of the present research revealed most of the women infected with HSV-2 had their menarche at the age of 13. In addition, married women at earlier age were also identified with HSV-2. Women those not using the contraceptives were more prone to the HSV-2 infection. This is similar to findings of our study. In addition, another study found that women those using tubal ligation, and intra-uterine devices were at high risk of HSV-2 infection [9]. Sanitary pad usage was lower the risk of sexually transmitted infection among women [28]. In this study, women those used sanitary pads more than four times in a day were predominantly infected with HSV-2.

Most common symptoms associated with genital herpes were pain in lower abdomen, chronic pelvic pain, itching, painful urination, and vaginal discharge [29]. In our study, very few infected women had complained of bleeding that was not related with coitus and menstruation. Most common symptom was abnormal vaginal discharge. Moreover, HSV-2 infected women present with lower abdominal pain, chronic pelvic pain, itching or irritation, and painful micturition. Polymorphisms of DNA polymerase gene for HSV-2 were aid in the analysis of genotypic antiviral resistance [30]. Moreover, phylogenetic analysis revealed that the strains identified in this island were closely associated with the global strains found in USA and Europe. There were no polymorphisms were identified in DNA polymerase gene.

## 5. CONCLUSION

Hence the study furnishes a deep understanding about the epidemiology of HSV-2 amongst married women in the remote island population of India. As the study reveals a higher prevalence rate at (4.8%) the community is urged to understand the local burden of HSV-2 and this will shed light on new risk factors which reproductive history and age at first intercourse. The different clinical presentations seen reinforces the complexity of both making HSV-2 diagnosis, as well as its management in remote areas. Genetic analysis of local strains showed similarities to others from around the world. It provided with information about transmission and antiviral resistance. These findings are the explosive approach to the planning of preventive intermediate actions and treatment strategies, which take into account the specific needs of remote island communities. Obtaining data in regards to HSV-2 epidemiology in such communities is a crucial element to successful longitudinal efforts of controlling the spread of the virus and improving health outcomes of affected individuals. The role of genotyping in public health in remote settings is important. Hence, additional studies into antiviral resistance and pathogenic characteristics are pertinent to counter the ongoing challenges posed by HSV-2.



### Data Availability

The analysed data used to support the findings of this study are included within the article.

### Ethical Approval

Ethical clearance was obtained from the Institutional Human Ethical Committee (IHEC) of Indian Council of Medical Research, Regional Medical Research Centre, Port Blair, Department of Health Research, Ministry of Health and Family Welfare, Government of India.

### Consent

Informed consent was obtained from study participants after a necessary explanation about the purpose, also their right on decision of whether or not participating in the study was made.

### Conflicts of Interest

The authors declare that they have no conflicts of interest.

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