Original Article

Preparation and standardization of Human papillomavirus (HPV) (Genotype 6) Nosode

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Abstract

Introduction The Homeopathic nosodes are the preparations sourced from organisms or diseased materials such as warty growths or cancerous tissues. The nosodes such as Psorinum (1826-30), Tuberculinum (1879), Medorrhinum (1880-85), Syphillinum (1880) and others have been in use in homeopathic practice since the early 19th century. In the recent years, certain new nosodes such as HIV nosode, Hepatitis C nosode, Mycobacterium Tuberculosis nosode, Plasmodia Falciparum Nosode, Swine Flu and more have been systematically introduced. Materials and methods The scrapping and the tissues from the genital warts clusters were collected carefully and were tested for the evaluation of STD (Sexually Transmitted Diseases) panel and HPV-DNA-PCR for screening high risk type followed by genotype determination. The collected tissue sample was processed as per the procedure defined developing the Nosodes, in the Homeopathy Pharmacopoeia of India. Sterility testing and PCR tests were carried out for safety purposes. Result Human papillomavirus (HPV Genotype 6) Nosode sourced from the genital wart tissues; detected positive for Human papillomavirus genotype 6 was prepared and standardized for future research and clinical exploration. Discussion and conclusion The HPV (Genotype 6) nosode contained the whole organisms in pure form. The therapeutic potential of HPV (Genotype 6) Nosode may be explored against viral warts affecting the genitals, anus, mouth, and respiratory tract as well as other relevant clinical conditions.

Keywords: Human papillomavirus, HPV, HPV nosode (genotype 6), nosode, potentization, homeopathy

Introduction

Human papillomavirus (HPV) causes Human papillomavirus infection. This sexually transmitted infection may resolve spontaneously, may remain latent and can cause warts on and cancer of cervix, vulva, vagina, penis, anus mouth and throat [1,2]. HPV is a DNA virus having over 170 types [3]. Based on their association with cervical cancer and precursor lesions, HPV can also be grouped to high-risk and low-risk HPV types. Low-risk HPV types include types 6, 11, 42, 43, and 44. High-risk HPV types include types 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 70 [4].

Genital warts due to HPV usually appear as a small bump or group of bumps in the genital area around the penis, scrotum, rectum, anus, vulva, vagina or cervix.

These warts might be small, large, raised, flat, single or in clusters, shaped like a cauliflower. Genital warts can return even after treatment [5]. Warts have been reported in 2–25% of sexually
transmitted disease clinic attendees in India; however, there are no data on the burden of anogenital warts in the general community [6]. HPV types 6 and 11 are common causes of genital warts (90%) and respiratory papillomatosis (nose, mouth, and lungs) [1]. Although these conditions very rarely result in death, they may cause a significant occurrence of the disease. Genital warts are very common and highly infectious [7].

The Homeopathy nosodes are the preparations sourced from organisms and diseased material such as warts and other cancerous tissues. The nosodes such as Psorinum [8] (1826-30), Tuberculinum [9] (1879), Medorrhinum [10] (1880-85), Syphillinum [11] (1880) and others have been in use in homeopathy practice since early 19th century. Most of these nosodes were originally prepared directly from the diseased materials. However, European guidelines suggest sterilization or inactivation of organisms before processing for potentization [12]. The author disagrees with the process of inactivation as it may lead to denaturation of proteins and loss of antigenicity. In the recent years, certain new nosodes such as HIV nosode [13], Hepatitis C nosode [14], Mycobacterium Tuberculosis nosode [15], Plasmodia Falciparum Nosode [16], Swine Flu [17] and more have been systematically introduced.

The nosodes prepared from biological materials have shown anti-disease efficacies [12,15,18] in cell-line and human models, opening avenues for new drug research. Human papillomavirus is one such new drug development opportunity. The HPV nosode can be prepared using different genotypes and tested for therapeutic action. The Author has prepared the nosode from the genital wart of a male patient. The limitation that the HPV virus cannot be cultured easily and presently the culture is not available, the nosode has to be sourced from the infected tissues or vaccines. Currently, the nosode development from the wart or vaccine strain is the only option. There are few preparations available in the market from Remedia (prepared from Gardasil and Cervarix vaccine) [19], Homeoforce (prepared from Gardasil Vaccine) [20] and professional complementary health formulas which are prepared from Adenovirus and Papillomavirus [21].

**Objectives**

Preparation and standardization of homeopathic nosode prepared from Human papillomavirus, genotypes 6.

**Materials and methods**

A male patient who presented with clusters of genital warts was identified for evaluation. The patient was informed about the new nosode development and the procedure was explained to him in detail. The patient had voluntarily signed the Patient Informed Consent Form. A clearance from the Institutional Ethics Committee was obtained.

The scrapping and the tissues from the genital warts clusters were collected carefully by a practicing surgeon in an operation theatre in a private hospital set-up. The tissue samples were sent for the detection of C. trachomatis (CT), N. gonorrhoeae (NG), M. genitalium (MG), M. hominis (MH), U. urealyticum (UU), U. parvum (UP), T. vaginalis (TV), Haemophilus ducreyi (HD) by real-time multiplex PCR and HPV DNA by hybrid capture technology and genotype determination. For PCR test the samples were collected in specific SP medium (Sample transport medium), as the kit was provided by the molecular biology laboratory.

The collected tissue sample was processed as per the procedure defined developing the Nosodes, in the Homeopathy Pharmacopoeia of India [22]. The 50 mg (1 part) of the tissue sample was triturated...
with nine parts of Saccharum Lactis powder to achieve 1X potency. The vehicle Saccharum Lactis powder was procured from a commercially available source.

One part of 1X potency and nine parts of Saccharum Lactis powder were taken to make subsequent 2X potency. Likewise, potencies till 6X were prepared by trituration method, prescribed in Homeopathy Pharmacopeia of India (HPI) [10]. Further, 8X potency (equivalent to 4c) was prepared using 50:50 proportion of water for injection and dispensing alcohol (92% sourced from Merck). Potencies 5c, 6c and further up to 30c were prepared by using electromechanical potentizer developed by the author with well-defined force parameters 12,099 Nm in 30c potency [23]. All the potencies were labelled and stored appropriately in controlled access. Bio-safety laboratory guidance was followed during the sample handling and preparation.

**Results**

*Human papillomavirus (HPV genotype 6) Nosode* sourced from the genital wart tissues; detected positive for Human papillomavirus genotype 6 was prepared and standardized for future research and clinical exploration. The STD screening panel at InfeXn Laboratory (specialized for diagnosis of infectious diseases) was carried out using Multiplex PCR. The wart tissue sample was found negative for bacterial agents such as Chlamydia trachomatis, Neisseria gonorrhoeae, Mycoplasma genitalium, Mycoplasma hominis, Ureaplasma urealyticum, Ureaplasma parvum, Treponema pallidum, Haemophilus ducreyi; parasitic agent Trichomonas Vaginalis and the viral agents such as Herpes Simplex 1, Herpes Simplex 2, Varicella Zoster and Cytomegalovirus. The nosode was tested negative for sterility as per HPI guidelines for nosodes. The sterility testing of HPV Nosode (genotype 6) in 30c potency was prepared as per the Indian Pharmacopoeia (IP) monograph [24].

Polymerase chain reaction (PCR test) for HPV (genotype 6) Nosode 6c and 30c was performed by CFX-96 real-time PCR detection system (BIO-RAD). The safety testing was performed with an objective to trace whether any nucleic material is present in two dilutions. The test report was reported as negative (Cut-off Ratio is less than 1.0 Unit). Agarose gel electrophoresis (Figure 1: PCR Image) was performed for HPV nosode 30c at the molecular laboratory to reconfirm the outcome. Human papillomavirus DNA was not detected in hybrid capture assay (make: Qiagen).

The Human papillomavirus HPV (genotype 6) Nosode was prepared, standardized and tested free from any infective material in 30c potency.

**Figure 1**: PCR Image
Discussion and conclusion

The homeopathic materia medica includes literature on the source materials used in over 45 nosodes, developed before the year 1920 [25]. In the last seventy years, there are many advancements in the understanding of microbiology, calling for redevelopment of the old nosodes as well as introducing certain new nosodes. In this direction, some research work is being carried out by the author by developing a series of nosodes. Being the most common sexually transmitted infection globally [26] a nosode developed from HPV is called for. The preparation of HPV (Genotype 6) nosode is an addition.

Some additional nosodes from other major genotypes of the HPV could be developed in future, which may be prepared and used as a single strain or as bivalent or polyvalent, a combination of two or more genotypes. For all practical reasons, the Author recommends developing polyvalent nosodes using multiple types of the organism.

The comparable HPV nosodes available in the market are those prepared from the attenuated vaccines, (19,20), while the HPV (Genotype 6) nosode contained the whole organisms in pure form.

The therapeutic potential of HPV (genotype 6) nosode may be explored against viral warts affecting the genitals, anus, mouth, and respiratory tract as well as other relevant clinical conditions. Since the HPV are known carcinogens and tumorogens, the nosode calls for evaluating their anti-cancer and anti-tumor properties in cell-line, animal, and human trials. Homeopathic Pathogenetic Trials (drug proving) may be conducted for determining the therapeutic indications of the nosode.

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References


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