Immunomodulating Effect of Phyto V7 in Preneoplastic Cervical Lesions

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Abstract

This study tested the hypothesis that an oral administration of a specific complex of phytochemicals and micronutrients (Phyto V7) induces an in situ cellular immune response and clearance of High Risk Human Papilloma Virus (HR-HPV) in preneoplastic cervical lesions. A randomized prospective 3 months study with 33 women with histopathological diagnosis of High Grade Squamous Intraepithelial Lesions (HGSIL) was performed. Patients were randomly divided into Control (CG, n=14) and Treatment (TG, n=19) Groups. Following an electro surgical excisional procedure, only the subjects who were included in the TG received Phyto V7 supplementation for 3 months. At Day 90 none of the groups showed colposcopically visible lesions and HR-HPV DNA was recovered from 50% and 26% of the CG and TG participants, respectively. While in the CG there was ~84% reduction in the percentage of CD8+ T-cells at Day 90, in the TG the reduction was of only ~5%. More remarkably, while there was a dramatic reduction in the percentage of CD56+ natural killer (NK) cells in the CG (>90%), there was a striking increase of >100% of these cells in the TG. This long-term prospective follow-up study reports a protective role of Phyto V7 supplementation in patients with HR-HPV.

INTRODUCTION

Human Papilloma virus (HPV) is one of the most common sexually transmitted viruses and women with HPV infections are at risk for developing squamous intraepithelial lesions (SIL) of the cervix and for developing cervical cancer, the second most common cancer in women worldwide [1,2]. There are over 100 types of HPVs, which are classified as high-risk (HR-HPV) or low-risk (LR-HPV) HPV. Infections with HR-HPV, especially HPV-16 followed by HPV-18, are strongly associated with anogenital cancer [2].

The immune deficiency that results from malnutrition increases the risk of HR-HPV infection and its permanence in the epithelial cells [3,4]. Studies have demonstrated weakness in a common chromosomal site of the host that is sensitive to micronutrient deficiencies and that matches an integration site of HPV-16 in the tissues of primary cervical carcinomas [5] and also with three of the four places in which HPV-18 integrates its DNA into the infected cell [6]. This finding suggests that suboptimal micronutrient concentrations may result in increased risk of cervical cancer [7]. A higher risk of persistent oncogenic HPV infection was observed for women with low dietary carotenoid uptake [8]. A negative correlation between frequent consumption of fruits high in antioxidant nutrients and reduced risk of SIL incidents was also found [9].

Plants contain a very large variety of phytochemicals and micronutrients. Phytochemicals (PHT) are a large group of biologically active plant chemicals that have positive effects on human health such as immune system stimulation, down regulation of inflammatory responses, radical scavenging activities, cell repair function, and antibacterial and antiviral activity [10-19]. Some of the most intriguing therapeutic properties include anti-hepatotoxic, anti-lithic, anti-hypertensive, and anti-hepatitis [20]. Some phytochemicals have been reported to possess potent anti-HIV in vitro activity, especially against the HIV-1 integrates [21-25]. Remarkably, administration of a specific mix of PHT that also contains micronutrients, called

ABBREVIATIONS

CIN: Cervical Intraepithelial Neoplasia; CG: Control Group; HGSIL: High Grade Squamous Intraepithelial Lesions; HPV: Human Papilloma Virus; HR-HPV: High Risk Human Papilloma Virus; NK: Natural Killer; PBS: Saline Buffered Solution; PHT: Phytochemicals; RVR: Relative Variations Rate; SIL: Squamous Intraepithelial Lesions; TG: Treatment Group

Thirty three women with histopathological diagnosis of High Grade Squamous Intraepithelial Lesion (HGSIL) attending the Panzeri Laser Surgical Center in Córdoba, Argentina were recruited over a period of one month. The inclusion criteria was the histopathological diagnosis of HR-HPV performed at least a year prior to enrollment of women that did not undergo surgical intervention. All participants read and signed the informed consent. All patients answered a questionnaire regarding potential risk factors. Pelvic exams with collection of cervical specimens for laboratory essays (flow Cytometry and HR-HPV DNA by HC-2) were performed by medical personnel. After the samples were obtained, all women were subjected to the loop electrosurgical excisional procedure standardized for HGSIL.

Statistical analysis consisted of univariate distributions study, Crosstabs, Proportions test Chi-square test and Contingency coefficient Q. Test Mann-Whitney rank test, analysis of Kruskal & Wallis nonparametric variance and Conover post-test. A significance level of 5% was used. To compare the fluctuations in the mean and in the 50th percentile values, variations relative rates (RVR) were calculated as follows:

$$RVR = \frac{(\text{cellvalue}_{\text{after}} - \text{cellvalue}_{\text{before}})}{\text{cellvalue}_{\text{before}}} \times 100$$

(Positive values indicate increases, negative values indicate decreases)

The analysis of viral load, as determined by HPV DNA, was conducted by the Proportion Test.

The statistical programs were Microsoft Excel 2010, Sigma Plot 12.0, SPSS 20.0 and InfoStat 2012.

RESULTS

Study of homogeneity at baseline

Table 1 shows the distribution of the age, number of sexual partners, type of cervical lesions, smoking habits and percentage of barrier method of contraception at baseline of the patients, respectively.

No significant differences were found at baseline between the proportion of patients in the TG and CG by age group (Pearson Chi-square= 1.442; Q=0.208, p=0.696); by smokers groups (Pearson Chi-square= 1.758; Q=0.228; p=0.185), number of sexual partners (Pearson Chi-square= 2.886; Q=0.288; p=0.236) and method of contraception as barrier (Pearson Chi-square= 2.81; Q=0.284; p=0.093).

Univariate analysis of risk factors

As it can be deducted from (Table 1), 85% of the patients were between 21 and 40 years of age, above 77% of the patients had 5 or more previous sexual partners, and 72.5% of the patients had cervical intraepithelial neoplasia (CIN) of type I. In addition, 28.1% of the patients were smokers and 44% of patients did not use a barrier method for contraception.
Analysis of risk factors

Having more than 5 sexual partners, smoking, age, type of cervical lesion and the high percentage of patients having no barrier method of contraception, were considered as risk factors for contracting the virus. By using the non-parametric variance Kruskal & Wallis analysis \[28\] we evaluated whether the above mentioned risk factors had a direct impact on the values of CD3+, CD4+, and CD8+ T-cells and on CD56+ natural killer (NK) cells.

No significant differences were found in the CD3+ (Kruskal & Wallis statistic \(H= 16.20; p=0.7520\)), CD4+ (\(H= 15.59; p=0.7878\)) and CD8+ (\(H= 19.25; p=0.5645\)) T-cells and CD56+ NK cells (\(H= 17.90; p=0.6337\)) before treatment. Similarly, no significant difference were found in the CD3+ (\(H= 17.52; p=0.6779\)), CD4+ (\(Pearson \text{ Chi-square}= 17.84; p=0.6583\)) and CD8+ (\(H= 16.71; p=0.4181\)) T-cells, and CD56+ NK cells (\(H= 17.28; p=0.3246\)) post treatment. Thus, the above risk factors did not affect the values of T- and NK- cells.

Once satisfied that the risk factors did not affect the values of T- and NK- cells, we analyzed the effect of Treatment (with and without Phyto V7) and time of sampling by endocervical brushing, before and after electrosurgical excision.

HR-HPV viral DNA Determinations

While at Day 0, HR-HPV viral DNA was recovered from all study participants, on Day 90, following the electrosurgical excisional procedure, HR-HPV viral DNA was recovered from 50% of the CG participants and only from 26% of the TG participants.

Table 1: Patients Description.

<table>
<thead>
<tr>
<th>Age Range</th>
<th>Sexual Partners</th>
<th>Cervical Lesion Type</th>
<th>Smoking</th>
<th>Method of Contraception</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 20</td>
<td>7.5 1-4</td>
<td>22.5 CIN I</td>
<td>Yes 72.5</td>
<td>Yes 28.1</td>
</tr>
<tr>
<td>21-30</td>
<td>50 5-9</td>
<td>67.5 CIN II</td>
<td>No 24</td>
<td>No 71.9</td>
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<td>31-40</td>
<td>35 ≥ 10</td>
<td>10 CIN III</td>
<td>No 2.5</td>
<td>No 44</td>
</tr>
<tr>
<td>≥ 40</td>
<td>7.5 7</td>
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CIN: Cervical intraepithelial neoplasia

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Figure 1. Percentage of immune cell types obtained from cervical smears. The percent of CD3+ T-cells, CD4+ T-cells, CD8+ T-cells and NK cells before and after 90 days following the electrosurgical excisional procedure are depicted for both the CG and TG. The boxes represent the middle 50% of the data values. The horizontal black line across the box marks the median value. The error bars show the 10th and 90th percentiles of the population. Individual data-points falling beyond these boundaries are shown as dots. The statistically significant differences \(p\) values between the groups are shown. NS- Non-statistically significant differences.
Lymphocytes statistical analysis from cervical smears

As it can be seen in (Figure 1A), at the commencement of the study there were no significant differences in all the analyzed cell subsets between the CG and TG. Ninety days following the electrosurgical excisional procedure, there were similar statistically significant reductions of ~60% in the percentages of CD3+ T-cells in both CG and TG groups (Figure 1A and Table 2). Similarly, there were reductions in the mean and median percentages of CD4+ T-cells in both groups, although these reductions were not statistically significant and the differences in the mean and median values between the two groups were not statistically significant at Day 90. In contrast, only in the CG there was a statistically significant reduction in the percentages of CD8 T-cells at Day 90 as compared to Day 0 (Figure 1A-1C). The mean and median decreases were of approximately 84% (Table 2). In contrast, in the TG the reductions of the mean and median were only of ~5 and 50% respectively, and there was a statistically significant difference in the CD8+ T-cells values between the groups at Day 90 (Figure 1D). Remarkably, while there was a dramatic reduction in the CD56+ NK cells at Day 90 as compared to Day 0 in the CG (Figure 1D and Table 2), there was a striking increase of ~136% and 100% in the mean and median percentage values in these cells in the TG, being the difference between the two groups statistically significant at Day 90 (Figure 1D).

**DISCUSSION**

Most HPV infections are cleared by the immune system and do not result in clinical complications [29]. However, when the immune system is not able to eliminate the HPV infection, the infection can progress to cancer, which can be fatal if treatment is delayed [30]. One to three percent of women have symptoms of HPV infection and ~250,000 women die worldwide from cervical cancer [31]. Current therapies for HPV infections include the excision or ablation of the affected tissue, and the stimulation of the immune system to generate clearance and prevent recurrence [32,33]. However, high frequency of recurrence occurs [29,34]. For the patient this may result in physical and psychological trauma. Whatever therapy will be chosen, HPV DNA can persist latently in surrounding tissue and may lead to recurrence of visible lesions [29].

One way to stimulate the immune system in order to help eradicate the HPV infection before or after the excision of the affected area is the local or systemic administration of interferon. However, administration of interferon is quite expensive [35], mild and transient side effects may occur and efficacy in preventing recurrence has not always been proven, when compared to placebo [36].

We have found that Phyto V7 has immune stimulatory properties [27] We hypothesized that, similarly to interferon treatment, administration of Phyto V7 to HPV affected women undergoing electrosurgical excision of cervical lesions, may reduce HPV recurrence. Indeed, in the group receiving Phyto V7 there was almost two-fold higher elimination of HPV, as demonstrated by the absence of HPV viral DNA in the cervical tissues. The increased clearance of the virus may be explained by the increased local cellular immune response in these women, exemplified by the much higher elevated presence of NK cells and cytotoxic T-cells (CD8+) in the cervical smears obtained in these women 90 days after the electrosurgical excisional procedure.

Phyto V7 is a complex of phytochemicals and micronutrients, registered as a nutritional supplement in several countries. Administration of Phyto V7 to chicks has been found to enhance their humoral immune responses against Newcastle Disease Virus following vaccination [27], indicating that Phyto V7 has immune-stimulatory properties. Other phytochemicals have been shown to stimulate nonspecific immunity, for example phytochemicals obtained from the coneflower [18]. Remarkably, administration of Phyto V7 to 9 terminally ill AIDS patients resulted in a dramatic improvement in their physical status [26]. Importantly, we have found an increase in CD4+ T-cells in HIV-1 infected individuals and AIDS patients taking Phyto V7 [unpublished results]. Taken together, this study indicates that administration of Phyto V7 to HPV infected women, before or after undergoing antiretroviral treatment, is beneficial by having an immune-stimulating effect allowing for a better clearance of the viral infection.

**CONFLICT OF INTERESTS**

Drs. Walter F. Goldman and Gadi Borkow are members of the Immune Nutrition Incorporated, the company that produces the PhytoV7 complex.

**REFERENCES**


Cite this article