HUMAN VACCINES & IMMUNOTHERAPEUTICS
2017, VOL. 13, NO. 3, 676–679
http://dx.doi.org/10.1080/21645515.2016.1236880

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SHORT REPORT

The effect of perceived psychological stress on the immunogenicity of the quadrivalent human papillomavirus vaccine in males

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ABSTRACT

Background: The human papillomavirus (HPV) vaccine is recommended for male and female recipients aged 9–26 years, and is effective in preventing HPV infection and cancer precursors. However, there is variability in immunogenicity among recipients as measured by anti-HPV geometric mean titers. In this study, we explored the effect of stress level on the immunogenicity of the HPV vaccine among college age males. **Methods**: 220 males aged 18–25 y were randomly assigned to 6-month (0, 2, and 6) and 12-month (0, 2, and 12) dosing schedules. Antibody titers were measured before the first dose and 2–6 weeks following the final dose. We recorded participants' age and stress level, based on a 4-item Perceived Stress Scale (PSS-4) questionnaire. **Results**: The average age of participants was 21.3 y old. Inspection of titers by quartile on the stress scale generally showed highest titers with highest stress. Spearman correlation coefficients revealed significant correlation between stress and titers for HPV-6, 16, and 18 but not for HPV-16 in the group of 6-month dosing schedule; no associations were found for the 12-month dosing schedule. For most strains, linear regression revealed significant ($P > 0.05$) associations on antibody titer for categorical age and dosing schedule but not stress. **Conclusion**: The evidence is mixed for an association between stress and HPV vaccine response for the 6-month dosing schedule, but no association was found for stress for the 12-month dosing schedule. Further investigations with larger and more diverse population groups are needed to explore the association between stress level and vaccine immunogenicity.

ARTICLE HISTORY
Received 5 July 2016
Revised 26 August 2016
Accepted 11 September 2016

KEYWORDS
gardasil; HPV vaccine; human papillomavirus; immunogenicity; stress

Introduction

Human papillomavirus (HPV) is the most common sexually transmitted infection (STI) in the United States, newly infecting about 6.2 million people each year.[1] There exist a total of more than 100 different types of HPV. The majority of these HPV types may lead to asymptomatic infection that cannot be readily detected. However, HPV-6 and HPV-11 lead to genital warts, and persistent HPV-16 and HPV-18 infection are responsible for 70% of all cervical cancers.[1] Cervical cancer is the 2nd leading cause of cancer in women worldwide.[2] In the United States, 493,000 cases and 273,000 deaths are counted annually from cervical cancer. In less-developed countries, where 83% of all cases occur, cervical cancer is the leading cause of death from cancer. Consequently, HPV is a major public health concern.[2]

The quadrivalent HPV vaccination Gardasil, produced by Merck Company, has been shown to be highly effective in preventing persistent HPV infection, HPV-6/11 genital warts, as well as HPV-16/18 cervical cancer precursor lesions.[3] Gardasil vaccine is given to male and female recipients aged 9–26 y. Almost all (97%) of vaccinated males develop antibody response after a 3-dose regimen, and efficacy is 89%. Therefore, the HPV vaccination is an effective prophylactic solution for HPV.[3]

Although HPV vaccination is effective, there is some variation in immunogenicity as measured by anti-HPV geometric mean titers (GMT).[3] One factor which may affect vaccine immunogenicity is stress. Chronic stress is known to negatively affect cellular and humoral immunity.[4] Studies relating perceived psychological stress to immunogenicity exist, but the collection of research in this area is not extensive. Negative association between stress level and antibody response is evidenced in hepatitis B, influenza, rubella, and pneumococcus polysaccharide vaccinations.[5] In this study, we explored the relationship between perceived psychological stress and immunogenicity of quadrivalent HPV vaccination. We hypothesize that anti-HPV titers will decrease with increasing stress scores.

Materials and methods

Detailed methods on the parent randomized, open label clinical trial have been published in Lin, et al.[6]

Participants

Men ages 18–25 y were recruited during the period of October 2010 through May 2011. Participants were excluded if they had more than 4 lifetime sexual partners, health problems which would interfere with immune response or completion of the study, hospitalization within the past year, hypersensitivity to vaccine components, inability to complete scheduled appointments, had already received the HPV

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vaccine, or were taking immunosuppressive medications. 311 men were screened, 91 were excluded, and a total of 204 completed the study.

Interventions

Participants signed informed consent forms prior to study initiation and completed screening forms before each dose of the vaccine. Participants were randomized using a simple random number sequence into 6-month dosing schedule and 12-month dosing schedule groups. Participants were aware of their group assignment.

Sample processing and immunogenicity testing

Vaccine storage and delivery followed standard procedures. Blood samples were drawn immediately prior to the first dose and 2 to 6 weeks after the third dose into serum separator tubes. Samples were spun at 3200 rpm for 10 to 15 min and serum was transferred to labeled nunc cryovials. Cryovials were stored at −70°C. Frozen nunc tubes were shipped on dry ice to the laboratory by an express carrier. Serology testing for each of the 4 HPV types was performed at PPD Vaccines and Biologics Laboratory (Wayne, PA) using a competitive Luminex immunoassay (cLIA) that measures type-specific antibodies to neutralizing epitopes on the virus-like particles (VLPs) as described in Dias et al.[7]

Stress measurement

A 4-item Perceived Stress Scale (PSS-4) questionnaire was used to measure each participant's degree of stress during each initial visit. The PSS-4 is a commonly accepted measure of stress that has proven to be a valid tool for assessing stress in mental and physical health research.[8] The PSS-4 consists of 4 questions designed to assess the perceived degree of unpredictability and uncontrollability in the subject's life.[9] Answers are converted into numerical values to produce a single final numerical stress score.[10]

Statistical analyses

The distribution of post-vaccination antibody titers was skewed; therefore, data were natural log-transformed and then used for calculation.

To examine the association between HPV type-specific titers and stress score within each dosing schedule, correlation coefficients were calculated for each study group using data from all participants as well as participants with stress scores in the lower and upper quartiles. We used the Shapiro-Wilk test to examine the normality of stress score and titers. Given non-normal distribution, we calculated non-parametric Spearman correlation coefficients.

To further explore association between HPV titers and stress score, as well as other variables, linear regressions were calculated for all subjects with natural log-transformed titers as the dependent variable and stress score at initial visit, age groups (under 22 vs. at least 22 y old), current smoking status (yes vs. no), and dosing schedule (6-month vs. 12-month) as the independent variables. Stress score was a continuous variable using participants' calculated stress scores at the initial dose. Age was incorporated as a categorical value. The age groups were participants aged 21 y and under, and participants aged at least 22 y. This age cutoff was chosen according to CDC recommendations for vaccination of men through age 21.[11] Smoking status was incorporated as a categorical value.

Statistical significance for the analyses was set at $P < 0.05$. SPSS Version 22 was used for statistical analyses.

Results

Baseline characteristics

The average age of participants was 21.3 y old. 121 (55.0%) of the participants were 21 y old or younger at the time of enrollment in the study. 99 (45.0%) of the participants were 22 y old or older at enrollment. 120 (93.0%) were non-smokers, and 9 (7.0%) were smokers. T-tests revealed no significant difference in initial stress score among age groups and smoking status. There was also no significant difference in baseline demographics of age and smoking status between the 2 study groups.

Antibody responses

Antibody titers

The post-vaccination geometric mean titers (standard error of the mean) was 926 (117) for HPV-6, 1417 (184) for HPV-11, 5228 (608) for HPV-16, and 852 (189) for HPV-18.

Comparison of antibody responses and stress

Although overall stress scores and titers were not significantly correlated, we further examined titers by stress quartile (Table 1). Anti-HPV titer levels appeared to follow a U-shaped pattern with higher titers in the lowest and highest stress quartiles for all HPV strains in the 6-month dosing schedule group, as well as for HPV-18 in the 12-month group. Spearman correlation coefficients for participants with lowest and highest quartiles of stress scores revealed significant correlation

Table 1. Geometric mean antibody titers post-vaccination by quartile of stress score by HPV vaccine type.

HPV titers (mM unit/mL) by HPV type and Stress Score Quartile Stress Score Quartile	HPV-6	HPV-11	HPV-16	HPV-18
6-Month Dosing Schedule (n=57)				
1st (Lowest)	860	1263	5261	941
2nd	640	712	3370	396
3rd	626	898	3563	506
4th (Highest)	**1498**	**1563**	**6924**	**1423**
12-Month Dosing Schedule (n=63)				
1st (Lowest)	**1223**	1916	6639	1154
2nd	1164	2216	6657	929
3rd	919	1760	4592	731
4th (Highest)	1006	**2223**	**7436**	**1408**

Highest titer values are bolded.
For both the 6-month and 12-month dosing schedule groups, the quartiles and their respective stress score ranges are: 1st quartile [0, 2], 2nd quartile [3, 3], 3rd quartile [4, 5], and 4th quartile [6, 12].

Table 2. Correlation between stress score and HPV titer post-vaccination by dosing schedule and HPV vaccine type.

	6-Month Dosing Schedule (n = 57)		12-Month Dosing Schedule (n = 63)	
HPV Type	Correlation Coefficient	*P*-value	Correlation Coefficient	*P*-value
HPV-6	0.35	**0.01**	−0.05	0.70
HPV-11	0.18	0.19	0.06	0.62
HPV-16	0.29	**0.03**	0.04	0.75
HPV-18	0.36	**0.01**	0.07	0.61

between stress and immunogenicity for HPV-6, 16, and 18 but not for HPV-11 in participants in the standard 6-month dosing schedule. No significant correlation was found in any of the HPV types in participants in the 12-month dosing schedule (Table 2).

To further explore the relationship between HPV titers and stress scores, while considering other variables including age, smoking status and dosing schedule, linear regression models with HPV-type specific, log-transformed titers as the dependent variable were performed (Table 3). Significant negative relationship between age groups and antibody titer response was found for HPV-11, 16, and 18, but not for HPV-6. Significant negative relationship between smoking status and antibody titer response was found for HPV-18, but not for HPV-6, 11, and 16. Significant positive relationship was also found between dosing schedule and antibody titer response for all HPV types (Table 3). After adjustment for age, smoking status, and dosing schedule, stress scores were not associated with antibody titers.

Discussion

Examination of the effect of perceived stress level on the immunogenicity of the quadrivalent HPV vaccination produced mixed results. We found statistically significant Spearman correlation for HPV-6, 16, and 18 in the 6-month dosing schedule subgroup analysis among participants with stress scores in the lower and higher quartiles. However, analyses of associations for the 12-month dosing schedule proved statistically insignificant. Linear regression revealed no significant association with stress scores; however there was significant relationship between age groups and dosing schedule on antibody titer response.

Stress affects the immune system through indirect and direct mechanisms. Indirect effects may stem from poor lifestyle habits that often develop in response to stress, such as alcohol consumption, smoking, and poor diet. These factors are linked to decreased vaccine immunogenicity for pneumococcal, influenza, and hepatitis B vaccinations.[5] Stress may directly influence vaccine efficacy via the hypothalamic-pituitary-adrenal axis. Stress increases cortisol, which may be related with decreased antibody response. Direct effects may also be mediated through sympathetic nervous system activation, perhaps due to release of catecholamines or direct innervation of lymphoid organs.[5]

There are several possible explanations of the insignificant correlation between stress and antibody responses in the 12-month dosing schedule group. Immune response is known to be strongest during the adolescent and early adulthood years.[12] Ages of all study participants fell within this range. It has been previously published that the 12-month boost is substantially larger than the 6-month boost.[6] Therefore, the booster effect in the 12-month group may have overwhelmed the effect of stress that was seen in the 6-month group.

In addition, the stress level of each participant may have been variable throughout the study period. Perhaps participants' stress levels were higher at the beginning of the study period and decreased in intensity during the course of the academic year, particularly because many participants were recruited earlier in the academic year. This might lead to a significant correlation in the 6-month group but not in the 12-month group.

This study is significant because it is one of the first studies examining the relationship between perceived stress level and quadrivalent HPV vaccine immunogenicity. However, this was an exploratory study with several limitations including modest sample size, inclusion of only males between 18–25 y of age, measurement of stress at one time point, and evaluation of stress with a single subjective PSS-4 score. This study deserves to be replicated in other age groups, with a larger sample size, inclusion of both genders, measurement of stress scores throughout the study, and evaluation of stress levels using other assessment tools.

In conclusion, there is mixed evidence relating stress to quadrivalent HPV vaccine response at 6 months but not at 12 months. Further investigations and larger sample size are needed to explore the effect of stress on vaccine immunogenicity.

Table 3. Regression results: Association between stress score, age, smoking status, dosing schedule and HPV post-vaccination titer by linear regression by HPV vaccine type.

Standardized Coefficients (B) and P values by HPV Type HPV Type	Initial Stress Score	Age Group (Reference: 21 y and under)	Smoking Status (Reference: Non-smoker)	Dosing Schedule (Reference: 6-month schedule)
HPV-6	0.02 (P = 0.78)	−0.13 (P = 0.07)	−0.13 (P = 0.07)	**0.14 (P = 0.04)**
HPV-11	0.02 (P = 0.75)	**−0.16 (P = 0.02)**	−0.10 (P = 0.14)	**0.30 (P = 0.00)**
HPV-16	0.01 (P = 0.86)	**−0.16 (P = 0.03)**	−0.12 (P = 0.08)	**0.14 (P = 0.05)**
HPV-18	0.02 (P = 0.79)	**−0.14 (P = 0.05)**	**−0.15 (P = 0.03)**	**0.16 (P = 0.03)**

$Y = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + B_4X_4 + e$

Where Y = antibody titer, X_1 = initial stress score, X_2 = age (< 21 vs. > 22), X_3 = smoking status (yes vs. no), X_4 = dosing schedule (6-month vs. 12-month), and e = error.

Disclosure of Potential Conflicts of Interest

Drs. Zimmerman and Lin have research funding from Merck & Co., Sanofi Pasteur and Pfizer Inc.

Funding

The parent trial was funded by Merck & Co., Inc. This current paper was funded by the University of Pittsburgh.

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