Vaccine xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Antibody response to human papillomavirus vaccination and natural exposure in individuals with Fanconi Anemia

Parinda A. Mehta [a,*], Sharon Sauter [a], Xue Zhang [a], Stella M. Davies [a], Suzanne I. Wells [a], Kasiani C. Myers [a], Gitika Panicker [b], Elizabeth R. Unger [b], Melinda Butsch Kovacic [a]

[a] Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States
[b] National Center for Emerging Zoonotic and Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, United States

ARTICLE INFO

Article history:
Received 6 April 2017
Received in revised form 5 October 2017
Accepted 6 October 2017
Available online xxxx

Keywords:
Human papillomavirus
Fanconi anemia
Serology
Vaccination

ABSTRACT

Fanconi anemia (FA) is a rare genetic disorder associated with predisposition to head and neck and gynecological squamous cell cancers. In the general population, these cancers are commonly linked to human papillomavirus (HPV) infection. Antibodies to natural HPV infection and HPV vaccination were evaluated in 63 individuals with FA while considering host immune factors. Approximately 30% of reportedly unvaccinated participants were seropositive (HPV6-38%, HPV11-25%, HPV16-26%, and HPV18-26%). Seropositivity was significantly associated with having had sex regardless of age (p = .007). Most participants showed seropositivity after HPV vaccination (HPV6-100%, HPV11-100%, HPV16-100% and HPV18-92%). Interestingly, titers for all 4 subtypes were significantly lower in the post-hematopoietic stem cell transplant (HSCT) participants compared to those who received the vaccine, but had not undergone HSCT (HPV6-p = .030, HPV11-p = .003, HPV16-p = .018, HPV18-p = <.001). It is unclear if these titers sufficiently protect from new infection since protective serologic cut offs have not yet been defined for the HPV vaccine. Individual immune functions were not associated with HPV seropositivity, however, underlying heterogeneous immune deficiency may explain higher rates of seropositivity in our younger unvaccinated participants (age 4–13 years). To better measure the efficacy of HPV vaccination in those with FA and other immune-compromised or cancer-prone disorders, future well-controlled vaccine studies are required.

1. Introduction

Fanconi anemia (FA) is a genetic disorder that is characterized by genome instability, progressive bone marrow failure and predisposition to gynecological and head and neck squamous cell carcinomas (SCC) [1]. Reports by the International Fanconi Anemia Registry and the German FA registry estimated that the risk of head and neck SCC is over 500-fold higher among individuals with FA compared to the general population. The risk remains high even after hematopoietic stem cell transplantation (HSCT) and the tumors occur at strikingly early ages and carry dismal prognosis [2–4]. Additionally, individuals with FA treated with HSCT who develop graft vs host disease (GVHD) have a higher incidence of head and neck cancers in the ten years following treatment (28% vs 0% in those without GVHD); this finding points to the importance of minimizing the risk of GVHD [5]. Increased risk for GVHD observed in earlier FA studies is now reduced significantly by T-cell depletion of the donor graft [6,7]. Chemotherapy and radiation are associated with high morbidity and mortality because of underlying DNA repair defects, making treatment of SCC difficult in this population [8–10].

Previous studies have found associations between human papillomavirus (HPV) infection, and cancers of anogenital and oropharyngeal regions in the general population [11–14]. Recent U.S. population-based studies conducted by the Centers for Disease Control and Prevention (CDC) show 62% of oropharyngeal cancers are attributable to HPV types 16 or 18 [15]. Studies of HPV in SCC tumors obtained from individuals with FA have shown contradictory findings [16–18]. The extent to which head and neck SCCs and anogenital cancers are associated with HPV in individuals with FA remains unanswered. However, these contradictory reports have increased interest in studies addressing the role of HPV infection and vaccination in individuals with FA.

In a study of individuals with FA living in Brazil, HPV positivity in oral samples was significantly higher than in non-FA controls

* Corresponding author at: Division of Bone Marrow Transplantation and Immune Deficiency, Cincinnati Children's Hospital Medical Center, 3333 Burnet Ave., Cincinnati, OH 45229, United States.
E-mail address: Parinda.Mehta@cchmc.org (P.A. Mehta).

https://doi.org/10.1016/j.vaccine.2017.10.015
0264-410X/

[19]. Our group has also reported similar findings of higher HPV positivity in oral rinses of individuals with FA compared to their first-degree relatives [20]. Typically, an intact immune system recognizes, eliminates, and protects the body from viral and bacterial infections, as well as from transformed cells (pre-cancer cells) [21,22]. Nevertheless, molecular and cellular mechanisms responsible for protection from and clearance of HPV infection are not completely understood [23]. Since our group and others have previously shown heterogeneous immune defects in those living with FA, a better understanding of the role of HPV and host immunity would allow for a more thoughtful approach towards prevention of HPV infection and risk reduction for SCC in this vulnerable population particularly as the HPV vaccine is available and recommended [24–27].

Data from clinical trials show that HPV vaccines, when given as a 3-dose series, have very high efficacy for prevention of vaccine type–associated cervical pre-cancers [28–30]. The prophylactic quadrivalent HPV vaccine has also been shown to prevent HPV16- and HPV18-associated vaginal, vulvar, and anal pre-cancers and HPV6- and HPV11-associated anogenital warts [24,31,32]. No clinical trial data are currently available to demonstrate efficacy for prevention of oropharyngeal cancers. However, because many of these are attributable to HPV16, the HPV vaccine is likely to offer protection against these cancers as well. The current study sought to measure HPV antibody titers in vaccinated and naturally exposed, unvaccinated individuals with FA while simultaneously evaluating humoral immunity to provide insights into the prevalence of exposure to HPV and response to vaccination.

2. Methods

2.1. Informed consent

Child and adult participants with FA followed at the Fanconi Anemia Comprehensive Care Center (FACCC) at Cincinnati Children's Hospital Medical Center (CCHMC) were invited to participate in the study. Individuals with FA participating in the Fanconi Anemia Research Fund (FARF) sponsored Adult or Family Meetings at Camp Sunshine were also offered the opportunity to participate. Informed consent was obtained from participants at least 18 years of age. Parental permission was obtained for children less than 18 years of age. The study was approved by CCHMC's Institutional Review Board.

2.2. Risk factor survey

Health related surveys were administered by either paper or using the REDCap (Research Electronic Data Capture) system [33]. The survey collected demographic, socioeconomic, clinical, sexual history, lifestyle and environmental exposure information. Participants ages 15 years and older were asked to complete surveys for themselves unless they were physically or mentally unable to do so, in which case a parent or guardian was asked to complete the survey on their behalf. For children less than 12 years old, a parent/guardian was asked to complete the survey for their participating children. For children between the ages of 12 and 15 years old, a parent/guardian was asked either to complete the survey or assist their child in completing the survey. However, the sexual questions were ascertained by study staff interview whenever possible for this age group provided a parent/guardian gave permission to do so. When available, biological mothers of FA participants were also asked whether they had genital or anal warts, a dysplastic Pap smear, or had a positive HPV test within a 3-year period before their child's birth.

2.3. Blood collection, processing and HPV antibody testing

Venous blood was collected using appropriate universal precautions and aseptic techniques by trained personnel. Approximately 50% of the samples were collected at the FACCC, centrifuged at 2400*g* and aliquoted, and immediately placed into storage at −80 °C (N = 33). Samples collected at the FARF-sponsored Family Meeting at Camp Sunshine or the Adult Meeting in Austin Texas were kept at room temperature and centrifuged at 2400*g* within 8 h of collection, aliquoted and then stored on dry ice until they were shipped via Same-Day FedEx (N = 20). Alternatively, they were shipped by FEDEX priority overnight to CCHMC, centrifuged within 24 h, aliquoted and stored at −80 °C (N = 12). An aliquot of each serum (0.5–1.0 mL typically) was sent via Next-Day FedEx to CDC where HPV antibody testing was performed using a multiplex ELISA (M4ELISA) to simultaneously measure antibody responses to HPV 6, 11, 16 and 18, as well as the pseudovirion-based neutralization assay (PBNA) for HPV16 and 18 as previously described [34,35]. The RLU (Relative Light Units) at 99th percentile of Johnson-Su probability distribution of children's sera run at 1:100 (N = 49, gift from Dr. Joakim Dillner, Lund University, Sweden) was set as the assay threshold for seropositivity for each HPV type evaluated as previously described [35]. The RLU (signal) threshold calculated using Johnson-Su distribution for these specific assays were 7998, 3225, 18,372, and 7426 for HPV 6, 11, 16, 18 respectively. Inter-assay variation between batches was less than or equal to 25%. Among the 63 total participants, 8 (12.3%) had samples collected at two different time points. Four of these participants were vaccinated and the most recent time point was used in order to allow for the most complete immune profiles.

2.4. Immune assays

Participant sera were tested for lymphocyte subsets, B cell panel, immunoglobulin levels (IgG, IgM and IgD) along with tetanus and diphtheria titers. The B cell panel was not performed on the first 9 study participants. Immunoglobulin levels were determined by standard methods in the CCHMC Clinical Laboratory. Tetanus and diphtheria titers were determined by Quantitative Multiplex Bead Assay in CCHMC's Clinical Diagnostic Laboratory. Remaining assays were performed at CCHMC's Diagnostic Immunology Laboratories. Results were interpreted with respect to age-appropriate reference ranges established in the laboratories. Evaluation of participant lymphocyte subsets was performed via routine four-color flow cytometric analysis of EDTA preserved whole blood using fluorochrome-labeled monoclonal antibodies to lineage-specific cell surface markers for T cells (CD3, CD4, CD8), B cells (CD19), and natural killer (NK) cells (CD16, CD56). All antibodies were obtained from BD Biosciences (San Jose, CA, USA). Briefly, erythrocytes were lysed by incubation in FACSLyse (BD Biosciences) and then stained with antibody and analyzed on a FACSCalibur flow cytometer (BD Biosciences) using multiset software (BD Biosciences). A similar flow-based approach was applied for assessment of the B-cell panel [23].

2.5. Statistical analysis

Statistical analyses were performed using SAS, version 9.3. Fisher's exact tests were used to (1) compare the demographics and disease characteristics between vaccinated and unvaccinated participants; (2) assess the association between HPV seropositivity and potential risk factors for HPV infection; and (3) compare the measurements of HPV titers in vaccinated participants stratified by immune function. To assess the agreement in seropositivity assayed by different methods, we used Cohen's Kappa. Since many tests have been performed; a p-value correction is usually needed.

Please cite this article in press as: Mehta PA et al. Antibody response to human papillomavirus vaccination and natural exposure in individuals with Fanconi Anemia. Vaccine (2017), https://doi.org/10.1016/j.vaccine.2017.10.015