HPV TYPES OF 2, 3, 4, AND 5 AMONG PATIENTS WITH LICHEN-PLANUS VERSUS CONTROL GROUP

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ABSTRACT

Lichen planus is an idiopathic papulosquamous skin disease with purple itching papules mainly in extremities in leading to decreased quality of life and numerous problems in affected patients. Among the possible causes are viruses. Hence in this study the prevalence rate of HPV types of 2, 3, 4, and 5 was determined and compared among patients with lichen-planus and control group. In this case-control study, 28 consecutive patients with lichen-planus and 25 healthy subjects were enrolled. The prevalence rate of HPV types of 2, 3, 4, and 5 and existence of more than one type of HPV were determined by PCR method and compared across the groups. There were 50% and 84% HPV-2 positive samples in case and control groups, respectively showing significant difference (P=0.009). Also 42.9% in case and 8% HPV4 positive cases in case and control groups, respectively showing significant difference (P=0.004). Also the HPV types of 3 and 5 and existence of more than one HPV type were not significantly differed across the groups (P > 0.05). Totally it may be concluded that presence of some HPV types in lichen-planus patients is higher than control group ant lack of HPV type 2 and presence of type 4 may be risk factors for lichen-planus.

Key words: Lichen-planus, HPV, PCR

INTRODUCTION

Lichen planus is a papulosquamous disease with purple itching papules mainly in extremities in mid-age subjects (1). This is an autoimmune disease due to some problems in regulations by T cells affecting mucosal surfaces (2). The patients would have a decreased quality of life (3, 4). Hence recognition of possible etiological factors is important for prevention of disease and reduction in burden of disease.

One etiology is human Papilloma virus (HPV) which is seen in many skin diseases (5). Accordingly prompt diagnosis of infection would result in decreased burden of disease (6) and slower progression rate of Lichen (7, 8). It is important to reduce the burden of disease (9, 10). Accordingly in this study the prevalence rate of HPV types of 2, 3, 4, and 5 was determined and compared among patients with lichen-planus and control group.

MATERIALS AND METHODS

In this case-control study that was accepted by local ethical committee, 28 consecutive lichen-planus patients attending to a training hospital in 2014 and 2015 and 25 control subjects were enrolled. The prevalence rate of HPV types of 2, 3, 4, and 5 and existence of more than one type of HPV were determined by PCR method and compared across the groups.

The primers for PCR were as followings:

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BeG-F: 5'- ACACAACTGTGTTCACTAGC-3'

BeG-R: 5'- CAACTTCATCCACGTTCACC-3'

The PCR sequence was 102 bp which was run on agarose 2% gel and electrophoresis was done with 5 minutes in 95 centigrade degree, 30 seconds in 95 centigrade degree, 30 seconds in 72 centigrade degree, 35 cycles and then 7 minutes in 72 centigrade degree. For PCR, following materials were put under PCR hood on ice. Positive samples demonstrated correct DNA extraction and presence of DNA of virus; 12 lambdas Mastermix 2X, one lambda (10 picomol) Premier F, one lambda (10 picomol) Premier R, and 100 nanogram DNA and H2O with final volume of 25 lambda. In next step for each DNA sample four PCR with 4 programs and 4 primers were designed and four virus types of 2, 3, 4, and were done.

Sequence primers for type 2

CH2F: 5'-TCACTGTGGGTCATCCATATT-3'

CH2R: 5'-CCGAACCCTGTTTCAACCATATC-3'

Sequence primers for type 3

CH3F: 5'-TGACCGTGGGTCATCCTTATT-3'

CH3R: 5'-CCTGTGTCCACCATATCGCCATC-5'

Sequence primers for type 4

CH4F: 5'-TAACTGTAGGCCATCCTTATT-3'

CH4R: 5'-CCAATGTCACACATATCTCCATC-3'

Sequence primers for type 5

CH5F:5'-TAACTGTGTAGGTCATCCTTATT-3'

CH5R:5'=CCTATGTCTGCCATATCACCATC-5'

Program 3 was 5 minutes in 94 centigrade degree, 30 seconds in 94 centigrade degree, 30 seconds in 60 centigrade degree, 30 minutes in 60 centigrade degree, 45 minutes in 72 centigrade degree, and 10 minutes in 72 centigrade degree. The program for type 4 was 5 minutes in 94 centigrade degree, 30 seconds in 94 centigrade degree, 30 seconds in 56 centigrade degree, 45 minutes in 72 centigrade degree, and 10 minutes in 72 centigrade degree. The program for type 5 was 5 minutes in 94 centigrade degree, 30 seconds in 94 centigrade degree, 30 seconds in 55 centigrade degree, 45 seconds in 72 centigrade degree, and 10 minutes in 72 centigrade degree.

For electrophoresis, 0.6 gram of agarose gel 1% was mixed in 60 ml of TBE solution 0.5X and melted. Then it was left to be cool and 13 lambda of ethidium bromide was added and was inserted in Caset gel and then reformed in frames and the gel was put in TBE and a DNA ladder was run in plates from PCR samples. Then the electrophoresis devices were attaches to power source and run and the photography was developed.

The sample preparation for sequences was with selection of few positive samples for each type and then again put on PCR samples with final volume of 10 to 100 microliter and run on pure agarose gel and the band was seen with portable UV device and was cut with scalpel and the section was put in microtube and DNA was extracted for sequencing. The extraction was done with 3 to 8 cuts of 5 to 10 micron from paraffin tissue and one milliliter of xylene was added to cut tissues in microtubes of 1, 2, and 5 milliliter and was fixed for 10 seconds. Then with maximal speed was centrifuged for three minutes and supernatant was removed and one milliliter of ethanol 96 to 100 percent was added to vertex. Again was centrifuged for two

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minutes and supernatant was removed completely by sampler. Then the microtubes were opened and incubated in 37 centigrade degree for 10 minutes. Thereafter 180 microliter of ATL and 20 microliter of K proteinase was added to the precipitated portion to be mixed and vertex. Then was incubated in 56 centigrade degree for one hour and 200 microliter of AL buffer was added and vertex was done. Again 200 microliter of 96 to 100 percent ethanol was added and vertex. This was done several times to attain homogenous mixture.

Then mixture was transported to kit and centrifuged in 6000 g (18000 pm) for one minute. The collection tube was removed and new tubes were pot and 500 microliter of AW2 buffer was added and was centrifuged for one minute with 8000 pm (600 g). Again new collection tube was put and with maximal speed of 14000 pm (20000 g) was centrifuged for three minutes and finally these filters were put in 1.5 to 2 milliliter microtubes and again 10 to 100 microliter of ATG buffer was added and incubated in room temperature for 5 minutes and was centrifuged with 14000 pm maximal speed for one minute and the precipitation was kept in -20 centigrade degree.

Data analysis was performed among 53 subjects including 25 subjects in control group and 28 patients in case group. Data analysis was performed by SPSS (version 13.0) software [Statistical Procedures for Social Sciences; Chicago, Illinois, USA]. Chi-Square, Fisher, and Independent-Sample-T tests were used and were considered statistically significant at P values less than 0.05.

RESULTS

The mean (± standard deviation) age was 45.4 ± 13.6 and 49.9 ± 13.2 years in case and control groups, respectively (P > 0.05). Eight patients (28.6%) and one subject (4%) were male in case and control groups, respectively (P > 0.05). the frequency rates of different HPV types are shown in Table 1. The frequency of HPV types according to age and gender were not differed in two groups (P > 0.05).

Table 1. Frequency rates of different HPV types in two groups

HPV Type	Case Group	Control Group	P Value
2	14 (50%)	21 (84%)	0.009
3	6 (21.4%)	2 (8%)	0.05 <
4	12 (42.9%)	2 (8%)	0.004
5	12 (42.9%)	12 (48%)	0.05 <
More than one	17 (60%)	13 (52%)	0.05 <

DISCUSSION

Lichen planus is an idiopathic papulosquamous skin disease with purple itching papules mainly in extremities in mid-age subjects leading to decreased quality of life and numerous problems in affected patients. One of the possible causes may be viruses. Accordingly in this study the prevalence rate of HPV types of 2, 3, 4, and 5 was determined and compared among patients with lichen-planus and control group. In this study HPV type 2 was significantly less common and HPV 4 was significantly more common among patients with lichen-planus. The other type was alike across the groups and presence of more than one HPV was same in

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groups. In the study by Gorouhi et al (11) it was reported that HPV type 6 and 11 are involved in pathogenesis of lichen-planus. However in our study type 2 and 4 were related to disease.

Boyd and colleagues (12) reported that 84.6 percent of subjects had HPV infection in PCR which is similar to our finings. Matilla et al (13) reported positive HPV rate of 16 percent generally including types of 6 and 11 which the general frequency rate was higher in our study.Also Jontell and colleagues (14) reported that HPV type 6 was present in 55% but type 16 and 18 were not seen in any patients.

The study by Razavi et al (15) showed that 31 percent and 7 percent had HPV type of 11 showing no significant difference. This matter demonstrates that only some HPV types may be involved in pathogenesis of lichen-planus and assessment for all types may be beneficial to recognize those types with etiological role. Vesper and colleagues (16) reported the prevalence rate of some HPV types among patients with lichen-planus to be between zero and 87 percent according to the subtypes. Difference in various subtypes was also seen in our study.

The study by Sahebjamiee et al (17) showed 27.5 versus 7.5 percent rate for HPV prevalence in case and control groups, respectively as well as our study. Pierangeli and colleagues (18) reported 75 percent for HPV prevalence in lichen-planus patients which was more than control group in congruence with our study. Also Cao et al (19) reported 12.1% versus 2.6% rate for HPV infection in case and control groups, respectively showing the risk factor status of HPV for lichen-planus as well as our study.

Totally, according to the obtained results, it may be concluded that presence of some HPV types in lichen-planus patients is higher than control group ant lack of HPV type 2 and presence of type 4 may be risk factors for lichen-planus. This matter may be considered in preventive approaches to lichen-planus. However further studies with larger sample size would result in more definite results.

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