INTRODUCTION

Pemphigus vulgaris (PV)–specific immunofluorescence pattern observed in the skin has been demonstrated in the outer root sheath (ORS) of plucked hair follicle.[1,2] Indirect immunofluorescence (IIF) is used to determine the circulating autoantibodies in cases of pemphigus. For IIF in pemphigus, several substrates had been used. This study was done to determine whether plucked hair can be utilized as a substrate for IIF among patients with pemphigus vulgaris. Aim & Objectives: To determine the efficacy of utilizing the plucked hair as a substrate for indirect immunofluorescence among patients with pemphigus vulgaris. Methodology: Thirty two consecutive patients with active and fresh diagnosed cases of pemphigus vulgaris (PV) who did not undergo any treatment and those patients who had positive DIF findings of characteristic fish net or chicken wire pattern of intercellular IgG deposits in the epidermis of perilesional skin were included in the study. A total of 32 control subjects without any auto immune disorders were selected and the blood samples were taken from these patients for IIF analysis. Anagen hair were collected from the healthy volunteers without pemphigus in the same way as for trichogram. Telogen hair were obtained by combing the hair and collecting the loose strands of hair on the comb and further confirmed under microscope. Three hairs each of anagen and telogen stage were collected from each subject to be utilized as a substrate for IIF. Results: Out of 32 samples 20 samples showed positive results for Ig G alone and 5 samples showed positivity for Ig G and C3. One sample showed positivity for C3alone. All these 26 samples were considered to have positive IIF test based on the intercellular pattern of deposit. Positive IIF results were observed in anagen hair samples and were negative in all the telogen hair samples. Six anagen hair samples did not show any positive findings in IIF for the study group. All the 32 control samples showed negative reports in IIF. Conclusion: In conclusion, IIF microscopy on plucked hair as a substrate is a more sensitive immunoassay for the detection of circulating intercellular autoantibodies in PV and the lower negative predictive value of this substrate is a limitation. Further large scale studies might provide better information regarding the practical utility.

Key words: Hair, indirect immunofluorescence, pemphigus
METHODOLOGY

Thirty-two consecutive patients with active and fresh diagnosed cases of PV who did not undergo any treatment and those patients who had a positive direct immunofluorescence (DIF) findings of characteristic fish net or chicken wire pattern of intercellular immunoglobulin (Ig) G deposits in the epidermis of perilesional skin were included in the study. Inactive cases and those cases undergoing treatment for PV were excluded from the study. A total of 32 controls without any auto immune disorders were selected and the blood samples were taken from these patients for IIF analysis. Anagen hair were collected from the healthy volunteers without pemphigus in the same way as for trichogram. Telogen hair were obtained by combing the hair and collecting the loose strands of hair on the comb and further confirmed under microscope. Three hairs each of anagen and telogen stage were collected from each subject to be utilized as a substrate for IIF hair sample were transported in Michelle’s medium and processed for IIF without sectioning. First, the hair samples were placed on a clean glass slide and then washed in phosphate-buffered saline (PBS) three times each lasting for 10 min. Then hairs were covered with sera of patients with pemphigus and those from the controls without pemphigus. Fluorescein-labeled anti-human antibodies was added to the processed samples and incubated for 1 h at 37°C. Hairs were washed again three times in PBS, each wash lasting for 10 min, air dried for 10 min, and finally mounted on slide with PBS-glycerol for examination. Hair samples showing intercellular IgG and or C3 deposits in the ORS were taken as a positive reading. For the IIF, a dilution of 1:20 was utilized for the study and the serum samples were considered to be positive if they stained ORS intercellular spaces at a titer of ≥20. Institutional ethical committee approval was obtained for the study.

RESULTS

Among the 32 individuals in the study group 18 individuals were male and 14 were female. Of 32 samples, 20 samples showed positive results for IgG alone and 5 samples showed positivity for IgG and C3. One sample showed positivity for C3 alone. All these 26 samples were considered to have positive IIF test based on the intercellular pattern of deposit. Positive IIF results were observed in anagen hair samples [Figures 1-3] and were negative in all the telogen hair samples [Figure 4]. Six anagen hair samples did not show any positive findings in IIF for the study group. All the 32 control samples showed negative reports in IIF. The sensitivity of using plucked hair as a substrate was 81.25%, specificity was 100%, positive predictive value was 100% and negative predictive value was 84%.

DISCUSSION

Pemphigus is a group of autoimmune blistering disease of the skin and mucosa characterized by antibodies against desmoglein (Dsg) 3 and Dsg 1.[1] The ORS of hair follicle is structurally analogous to epidermal keratinocytes.[2] Pemphigus antigens are distributed throughout the ORS.
and in the dermal bulb matrix cells. Pemphigus-specific immunofluorescence pattern seen in the skin has been demonstrated in the ORS of plucked hair follicle. Dsg 3 is responsible for anchoring the telogen hair to the follicle. The increase in amount of target antigen (Dsg 3 and Dsg 1) in the follicular epithelium could be a factor determining scalp involvement in pemphigus. Pemphigus-specific immunofluorescence pattern in the ORS of plucked telogen and anagen hair and their correlation with perilesional skin has been reported.

There are a number of sensitive and specific assays for the detection the circulating autoantibodies, including western blotting of cell-derived and recombinant forms of the target antigens, immunoprecipitation, and enzyme-linked immunosorbent assay for the diagnosis of pemphigus. IIF is still the most widely employed technique to detect the circulating and antibodies against the pemphigus antigens. The value of IIF titers in disease monitoring has been a subject of debate. Although early studies reported that intercellular antibodies levels measured by IIF were a useful marker of disease activity, later studies concluded that IIF titers did not always correlate with the disease severity, and are not consistent enough to serve as a guide for therapy or for monitoring the disease activity.

The widely used substrate for IIF in pemphigus is monkey esophagus, skin biopsy samples from individuals without pemphigus, guinea pig esophagus, and bovine tongue. Although a universally sensitive substrate has not been established, IIF on monkey esophagus has been elucidated as the most sensitive screening test. In general, IIF positivity is observed in 70%–90% of pemphigus patients and lacks the ability to differentiate definitively between PV and pemphigus foliaceus since both have IgG antibodies directed against keratinocyte cell surface.

In our study, we utilized plucked hair as a substrate for IIF due to the following reasons, simple, noninvasive method to obtain the samples, cost-effective, less time consuming while processing the substrate, was found to be a good substrate in DIF in earlier publications and was not studied as a substrate for IIF till now.

The sensitivity of IIF varies according to the epithelial substrate the sera are incubated with, monkey esophagus when used as a substrate, the sensitivity reported ranged from 81% to 100% and with guinea pig esophagus the sensitivity ranges from 80% to 87%. In our study, the sensitivity of the IIF with plucked hair as a substrate was 81.25%. The sensitivity was lower in our study when compared with the earlier publications; this could be attributed to the smaller sample size or to the inherent deficiency of the substrate used for IIF.

Our study demonstrated a specificity of 100%, this could be attributed to the fact that the control population did not have any autoimmune disorders. The negative predictive value in our study is only 84%, this could be attributed to the drawback of using plucked hair as a substrate for IIF. The positive predictive value in our study was 100% and this could be due to the criteria of including only DIF-positive patients in the study group.

All the telogen hair samples gave negative IIF findings. This could be due to the insufficient ORS component in the telogen follicles. There are no reports on the effectiveness of utilizing telogen hair as a substrate in IIF till now and hence we could not validate the negative findings in the telogen hair substrate.

Studies on IIF generally utilized IgG antibodies for IIF, we studied the antibodies against c3 in our substrate and found that a total of 6 (18.75%) samples showed reactivity and among them one sample showed intercellular deposit of c3 alone without IgG. Significance of these findings could not be interpreted due to the smaller sample size of the study group.

The drawback of this study is a smaller study group, comparison with the skin as a substrate for IIF in the study group was not done and characterization of the specific subclass of IgG antibodies could not be performed.
CONCLUSION

IIF microscopy on plucked hair as a substrate is a more sensitive immunoassay for the detection of circulating intercellular autoantibodies in PV and the lower negative predictive value of this substrate is a limitation. Further large scale studies might provide better information regarding the practical utility.

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Conflicts of interest

There are no conflicts of interest.

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