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Molecular Assessment Of Epstein–Barr Virus In Patients With Macular Amyloidosis

Dr. Chandan Kumar Reddy[1]*, Dr.Anusha Valati[2]

[1] Associate Professor, Department of Dermatology, Sri Lakshmi Narayana Institute of Medical Science, Pondicherry, India.
[2]Assistant Professor, Department of Dermatology, Sri Lakshmi Narayana Institute of Medical Science, Pondicherry, India.

***Corresponding Author**: Dr. Chandan Kumar Reddy
Associate Professor, Department of Dermatology, Sri Lakshmi Narayana Institute of Medical Science, Pondicherry, India.

ABSTRACT

An eosinophilic hyaline precipitate and special staining features are symptomatic of amyloidosis. Macular amyloidosis, which occurs only on the skin, is a result of several different factors. Considering that Epstein-Barr virus (EBV) was detected in this lesion, it seems likely that it could factor into the pathogenesis of this disease. The purpose. The EBV DNA was detected with PCR in 30 samples from patients with macular amyloidosis and 31 healthy samples taken from the margins of melanocytic nevi removed from patients. Results. Twenty-three patients tested positive for the BLLF1 gene of EBV in the control and eight in the case groups. A significant difference in EBV DNA levels was not observed between macular amyloidosis or control groups ($P<0.08$). Based on the findings of this study, EBV does not cause macular amyloidosis.

Keywords: Macular amyloidosis, Epstein-Barr virus, Eosinophilic Hyaline Precipitate, PCR.

INTRODUCTION

Amyloidosis is characterized by the extracellular deposit of fibrous proteins. Amyloidosis can be divided into systemic and localized types based on the type of organ affected. Both types involve skin. Acute cutaneous amyloidosis is classified as either keratinic or nodular, depending on its type: (i) primary or secondary. In contrast to the primary type, the secondary form can be caused by inflammatory skin disorders, skin tumors, or the effects of phototherapy. The more common form of keratotic amyloidosis is lichen amyloidosis, but there are also two types of macular amyloidosis. A majority of basal keratinocytes are positive for CK5 in keratotic amyloidosis. Asia, South American, and Chinese regions tend to suffer from keratotic types. Females are more likely to have the sporadic form (10%) than males [1]. There have also been cases reported on the face, trunk, and thighs [2]. Interscapular infections usually affect the upper back, arms and shins as well as the interscapular region. There are various types of macular amyloidosis lesions, including hyperpigmented patches with reticulated or rippled surfaces, which usually have indefinite margins [3]. In the early stages of amyloidosis, itchiness is common.
The pathogenesis of this disorder may be influenced by keratin deposits derived from keratinocytes . There are two theories as to how fibrillar or secretory mechanisms may cause disease [4]. As keratinocytes convert to amyloid, they degenerate their colloid bodies into amyloid, according to apoptotic theory. [5-6] Degenerating basal keratinocytes in the papillary dermis can cause amyloid deposits to spread into the lamina densa when the lamina densa is damaged. In addition to race, genetics, environment, hormonal changes, sunlight, friction, atopy, sarcoidosis, and IgA nephropathy, a number of etiological factors contribute to macular amyloidosis [7, 8], genetics, environment, hormonal changes, sunlight, friction, and atopicemia [9], autoimmunity, and infection with EBV are among the factors that contribute to it. An epidermal EBV infection is linked to macular amyloidosis [10].
Studying the role of EBV in cutaneous amyloidosis is limited to a case series with 27 patients from China [11]. Due to a lack of studies on this subject in Iran, we aimed to investigate the relationship between EBV and macular amyloidosis. EBV-related diseases may be treated with antiviral agents.

MATERIALS AND METHODS

Using a nonrandom objective-oriented sampling strategy, 38 patients with melanocytic nevi excised from patients without macular amyloidosis were compared to 38 healthy skin samples. Inclusion criteria for the study were paraffin blocks from Imam Reza Hospital in Mashhad with sufficient tissue, according to the pathology report and clinical presentation of the patient. Blocks with imperfect data and insufficient samples were excluded from the PCR. Amyloid deposition in the case

group was confirmed by optical microscopy and Congo red staining. Following the preparation of sterile blades from each block, six sterile Eppendorf tubes were prepared in each case and control group. [12]
In the process of decomposing paraffin. A xylol/ethanol solution was used to deparaffinize the paraffinized tissues. For 30 minutes at room temperature, 1 mL xylol was added to microtubes containing tissue sections. A centrifuged microtube was discarded after being centrifuged for 10 minutes at 13,000 rpm. A second time was spent repeating this process. Inversions of the microtubes were performed several times before centrifugation at 13,000 rpm for 10 minutes. The precipitate was then dissolved in 500 liters of 100% ethanol. Several repetitions were needed. The precipitate itself, however, did not evaporate with ethanol at room temperature.
Extracting DNA from a sample. The DNA was extracted from the samples using kit 8401-140116. In order to extract the DNA, lysis buffer-T was used. To remove the proteinase from each microtube, 100 liters of extraction buffer was added to each microtube, followed by 10 liters of proteinase K. After incubation for 10 minutes, the mixture was collected. Proteinase K was inactivated in the samples after incubation for 3 minutes at 95°C. One hundred liters of Universal Buffer NST should be added to the tubes and re-inverted 10 times. The mixture obtained was analyzed by PCR. PCR. [13] The EBV genome was used for the detection of macular amyloidosis. Using beta-globin gene primers, DNA extracts from paraffin-embedded tissues were analyzed for quality after being extracted from paraffinized blocks. These primers amplified a 260 bp fragment of the beta-globin gene from the GH20 and PC04 genes. Following are the primer sequences: In samples that produced 260 bp fragments when the desired primers were used, EBV virus BLLF1 gene amplification was considered favorable. [14] Lot number 935701 of the Cinna Gen kit. was used to test the extracted DNA samples for the presence of EBV sequence. EBV DNA quality can be determined using this kit by using PCR. PCR buffers, MgCl2, dNTPs, and primers were mixed in an optimized 1x PCR method using recombinant Taq DNA polymerase, PCR buffer, and dNTPs. Primer sequences are used to amplify DNA from the BLLF1 gene encoding gp 350/220. Detection of EBV at least 30 copies is possible. SPSS 11.5 was used to analyze the data. The data were described using graphs and tables, and the samples of healthy people and patients were compared using Chi-square tests and independent t-tests. 0.05 was considered to be the significance level in all tests.

RESULTS

In the study (19/38), 50% of patients were males and 50% were females. Three patients were under 20 years (7.9%), five 20–30 year olds (13.2%), ten patients 30–40 years (26.3%), 13 patients in the 40–50 years age group (34.2%), four patients over the 50 years age bracket (10.5%), and three over 60 years aged (7.9%). Among the participants, there were 19 people under the age of 19 and 76 people over the age of 76. The trunk of the body was infected in 27 cases (71.1%), while the extremities were infected in 11 cases (28.1%). A 0.535 P value was obtained for the age of the study group and a 0.646 P value was obtained for the sex of the control group. We tested 76 samples using the PCR method for beta-globin gene expression (38 cases and 38 controls). There were 61 positive results (30 samples in the case group and 31 samples in the control group), while 15 samples showed negative results (8 samples in the case group and 7 samples in the control group). [15] In the study, 26.7% of participants were positive for the BLLF1 gene, whereas 48.4% of control participants were positive. EBV infection rate and macular amyloidosis did not correlate with one another (P = 0.08).

Table 1: Researchers conducted a study like this to find out the degree of differences between cases of macular amyloidosis and melanocytic nevi by analyzing 30 cases of macular amyloidosis and 31 cases of melanocytic nevi

Study groups					
EBV DNA PCR	Cases		Controls		Chi-square test results
	Number	Percent	Number	Percent	
Positive	8	26.7	15	48.4	P< 0.08
Negative	22	91.7	16	76.2	
Total	30		31		61

DISCUSSION

Hyaline deposits of self-origin characterized by eosinophilic staining are the result of amyloidosis. These disorders may be caused by systemic diseases or skin disorders. When there is macular amyloidosis, only the skin is affected.
[16]. As a result of EBV infection, keratinocytes secrete amyloid material or degenerate, resulting in amyloid being converted to filaments by degenerating filaments. Epithelial cells may contribute to the continued reproduction of EBV, according to recent studies. It is possible that epidermal keratinocytes are directly infected by EBV based on the presence of cell surface receptors on less differentiated squamous epithelium. A virus can replicate only after maturation and differentiation of cells, so it can be infected in germinal layers. As a result of infection with EBV, human keratinocytes become fibroblasts and express cytokeratin differently [17]. Amyloid can be produced by phagocytizing keratin aggregates by fibroblasts [18].

In 1996, Drago et al. demonstrated this correlation. They saw a 30-year-old woman who had been experiencing itchy brown papules and macules on her chest and back for ten years. Through in situ hybridization, they were able to demonstrate the EBV genome in epidermal lesions. Cells from the basal epidermis and upper layers were found to contain EBV genomes, especially in the cytoplasm of these cells. [19] The patient's serology for EBV also revealed a positive result. The patient's skin lesions and general symptoms improved with acyclovir and interferon alpha therapy [20]. It was reported that 27 patients with lichen erythematosis and macular amyloidosis had been subjected to a skin biopsy. The investigation demonstrated no evidence of EBV DNA positivity in the DNA of five patients with chronic simplex lichen, three in the case of secondary cutaneous amyloidosis, and two in the case of primary systemic amyloidosis.
EBV was not associated with macular amyloidosis in this study ($P = 0.08$). Eight patients with macular amyloidosis and 15 controls tested positive for EBV DNA. According to this study, EBV DNA was detected at different levels in patients with macular amyloidosis compared with controls. Several studies have found insufficient evidence to conclude that EBV infection and macular amyloidosis are related, so we could draw this conclusion from the results of this study. In situ hybridization versus PCR for DNA detection (Methodology): The detection of EBV DNA was conducted with a sensitive method that included positive and negative controls. In situ hybridization is more sensitive than PCR based on previous studies [21]. Our controls, however, did not undergo in situ hybridization, so it was not possible to identify the exact infected cells, which may have been circulatory B cells instead of keratinocytes. As we used positive and negative controls in our EBV PCR kit, the positive cases in our control group could not be false positives. [22] Chang's study used controls comprised of skin with other cutaneous disorders, while our study used controls surrounding melanocytic nevi. Our macular amyloidosis was found to be more widespread than lichen amyloidosis reported in a study.

CONCLUSION

EBV was not associated with macular amyloidosis, according to this study. A biopsy punch made from fresh tissue or quickly frozen should be used for EBV DNA comparative analysis in macular amyloidosis, as well as serological testing to detect anti-EBV antibodies. As well as in situ PCR, samples can be examined for EBV DNA positive cells. This study can detect EBV infection by using other genes besides BLLF1, since some samples contain mutations in this gene. As a further recommendation, we recommend comparing EBV detection in macular amyloidosis patients with and without involved skin.

REFERENCE

1. Fernandez-Flores, “Cutaneous amyloidosis: a concept review,” *The American Journal of Dermatopathology*, 34(1), 2012, 1–14,.
2. J. T. Al-Ratrout and M. B. Satti, *et al*. “Primary localized cutaneous amyloidosis: a clinicopathologic study from Saudi Arabia,” *International Journal of Dermatology*, 36(6), 1997, 428–434,
3. Bandhlish, A. Aggarwal, and R. Koranne, *et al.,* “A clinico- epidemiological study of macular amyloidosis from North India,” *Indian Journal of Dermatology*, 57(4), 2012, 269– 274.
4. Y. Horiguchi, J.-D. Fine, I. M. Leigh, T. Yoshiki, M. Ueda, and S. Imamura, *et al*., “Lamina densa malformation involved in histoge- nesis of primary localized cutaneous amyloidosis,” *Journal of Investigative Dermatology*, 99(1), 1992, 12–18,.
5. H. Kobayashi and K. Hashimoto, “Amyloidogenesis in organ- limited cutaneous amyloidosis: an antigenic identity between epidermal keratin and skin amyloid,” *Journal of Investigative Dermatology,* 80(1), 1983, 66–72.
6. D. M. Touart and P. Sau, *et al.,* “Cutaneous deposition diseases: part I,” Journal of the American Academy of Dermatology, vol. 39(2), 1998, 149–171,
7. W.-J. Wang, C.-Y. Huang, Y.-T. Chang, and C.-K. Wong, *et al*., “Anosacral cutaneous amyloidosis: a study of 10 Chinese cases,” *British Journal of Dermatology*, 143(6), 2000, 1266–1269,
8. V. Eswaramoorthy, I. Kaur, A. Das, and B. Kumar, *et al*., “Macular amyloidosis: etiological factors,” *The Journal of Dermatology*, 26(5), 1999, 305–310,
9. A.-G. Kibbi, N. G. Rubeiz, S. T. Zaynoun, and A. K. Kurban, *et al*., “Primary localized cutaneous amyloidosis,” International *Journal of Dermatology*, 31(2), 1992, 95–98,
10. Y. T. Chang, C. K. Wong, K. C. Chow, and C. H. Tsai, *et al*., “Apoptosis in primary cutaneous amyloidosis,” *British Journal of Dermatology*, 140(2), 1999, 210–215,
11. Rasi, A. Khatami, and S. M. Javaheri, *et al*., “Macular amyloidosis: an assessment of prevalence, sex, and age,” International Journal of Dermatology, 43(12), 2004, 898–899,
12. V. K. Somani, H. Shailaja, V. N. V. L. Sita, and F. Razvi, *et al*., “Nylon friction dermatitis: a distinct subset of macular amyloidosis,” *Indian Journal of Dermatology, Venereology and Leprology,* 61(3), 1995, 145–147,
13. L. Onuma, M. Vega, R. Arenas, and L. Dominguez, *et al*.,“Friction amyloidosis,” *International Journal of Dermatology*, 33(1), 1994, 74,

14. M. Siragusa, R. Ferri, V. Cavallari, and C. Schepis, *et al.,* "Friction melanosis, friction amyloidosis, macular amyloidosis, towel melanosis: many names for the same clinical entity," *European Journal of Dermatology,* 11(6), 2001, 545–548,
15. D. D. Lee, C. K. Huang, P. C. Ko, Y. T. Chang, W. Z. Sun, and Y. J. *et al*., Oyang, "Association of primary cutaneous amyloidosis with atopic dermatitis: a nationwide population-based study in Taiwan," *British Journal of Dermatology*, 164(1), 2011, 148– 153,
16. Tanaka, K. Arita, J. E. Lai-Cheong, F. Palisson, M. Hide, and J. A. McGrath, *et al.,* "New insight into mechanisms of pruritus from molecular studies on familial primary localized cutaneous amyloidosis," *British Journal of Dermatology*, 161(6), 2009, 1217–1224,
17. M. J. Dahdah, M. Kurban, A.-G. Kibbi, and S. Ghosn, *et al*., "Primary localized cutaneous amyloidosis: a sign of immune dysregula- tion?" *International Journal of Dermatology*, 48(4), 2009, 419–421,
18. F. Drago, E. Ranieri, A. Pasturino, S. Casazza, F. Crovato, and A. Rebora, (1996). "Epstein-Barr virus-related primary cutaneous amy- loidosis. Successful treatment with acyclovir and interferon- alpha," *British Journal of Dermatology*, 134(1), 170– 174.
19. Y. T. Chang, H. N. Liu, C. K. Wong, K. C. Chow, and K. Y. Chen, *et al*., "Detection of Epstein-Barr virus in primary cutaneous amyloidosis," *British Journal of Dermatology,* 136(6), 1997, 823–826,.
20. N. Suh, H. Liapis, J. Misdraji, E. M. Brunt, and H. L. Wang, *et al*., "Epstein-Barr virus hepatitis: diagnostic value of in situ hybridization, polymerase chain reaction, and immunohisto- chemistry on liver biopsy from immunocompetent patients," *American Journal of Surgical Pathology,* 31(9), 2007, 1403– 1409.
21. Z.-L. Qi, X.-Q. Han, J. Hu *et al.,* "Comparison of three methods for the detection of Epstein-Barr virus in Hodgkin's lymphoma in paraffin-embedded tissues," *Molecular Medicine Reports,* 7, 189–92,
22. Janz, M. Oezel, C. Kurzeder, *et al.,* "Infectious Epstein-Barr virus lacking major glycoprotein BLLF1 (gp350/220) demon- strates the existence of additional viral ligands," *Journal of Virology*, 74(21), 2000, 10142–10152,