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ORIGINAL ARTICLE

Abnormal Morphology of Blood Vessels in Erythematous Skin from Atopic Dermatitis Patients

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ABSTRACT

Background: Atopic dermatitis is a lesion of skin that affects the blood vessels originated from the vascular plexus. It is a pruritic inflammatory lesion with continuous relapses in between. It has an unknown etiology with no evidence of interaction between environmental and genetic factors. AD has many manifestations including; allergic skin reactions, rhinitis and asthma. This study will illustrate the morphological changes associated with the person suffering from erythematous skin in AD patients.

Methods: It is a case control study which has included 40 participants aged between 20-65 years including 20 AD and 20 healthy patients. Study was conducted between the time period of Dec 2021 to Nov, 2022. Cases included all those patients who were diagnosed with AD from a period of at least 3 months whereas controls were taken by matching the age and race with the cases population. Specimens of skin were collected from all the patients and then observed under a photon microscope.

Results: Results of the study states that the patients suffering from AD demonstrate thick, complex vessels of blood causing increased flow of blood within the erythematous as well as non-erythematous skin. It suggests that these morphological changes are not observed in all acute and subacute stages but only in chronic stages. In addition, bifurcation was absent in both the lesions either lichenification or erythematous.

Practical implication: This study will help the clinicians to understand the morphological changes that occur in atopic dermatitis patients in order to differentiate them from the normal population. Further, it will help the community to differentiate themselves on the basis of the signs and symptoms associated with Atopic dermatitis patients.

Conclusion: Study concluded that these results will be helpful in the development of clinical strategies for the treatment of eczema in AD patients.

Keywords: Atopic dermatitis, lichenification, flexuous blood vessels, pruritus, eczema, vascular endothelial growth factor.

INTRODUCTION

Vascular system comprises a network of capillaries that originates from the organization of superficial and deep horizontal plexus.[1] In acute inflammatory conditions, there are multiple mediators of inflammation that help in the activation of endothelial cells of the blood vascular system, most commonly vascular endothelial growth factor (VEGF).[2] These mediators display the inflammatory signs in the form of increased flow of blood due to dilation of vessels and formation of edema by increasing the permeability of vessels.[3] Increase in the size and number of blood vessels are dependent upon the type of inflammatory condition of skin.[2] Literature suggests that the quantity of VEGF in abrasions associated with AD shows 25 times more severity as compared to normal skin. Altered circulation of blood peripherally has a direct association with formation of erythema in patients of Atopic dermatitis (AD) on the basis of different phases.[4] Acute phase represents the formation of weeping eruption, crusting and vesicular development. Subacute phase demonstrates the formation of plaques, dry scales, and erythematous papules.[5] Chronic phase of AP represents the repeated scratching from Lichenification.[4]

Lesions associated with AD develop most commonly on upper trunk, face, knees, elbows, bends, and neck with discrete distribution however, sometimes erythematous delineation and scales.[5] The involved area may also display oozing, discoloration and formation of papular lesions most frequently in the popliteal region and antecubital fossa.[6] AD makes the skin itchy, weepy and cracked independent of age however, there are certain characteristics that may display differences between the presentation of children and adults.[2] In children, its presentation is different with the formation of red scaly plaques on the surface of cheeks rather than on the perinasal and perioral regions.[4] It displays a classical sign of Hertoghe causing the loss of eyebrow as a result of chronic rubbing on the area.[5] Another common symptom of AD is pruritis which may result in hyper and hypo pigmentation of the involved area.[2] Adults represent different expressions of AD with scaling and redness on the dorsum of hand along with crusting and oozing. AD patients may also suffer from secondary complications like herpes simplex and dermatophytosis.[6] Ribatti (7) carried a study for throwing light on the vascular system covering the smooth muscle cells and endothelial cells with their intimate involvement in the clinical presentation of AD such as edema, recruitment of leukocyte, erythema, and dermographism urticaria.[7]

On the basis of these reports, it has been hypothesized that in patients suffering from erythematous skin of AD, alterations in the capillaries and blood vessels were seen in the 3D structure.[8] For throwing light on this question, a thin section of the skin is usually observed under light microscope, however a simple light microscope can't differentiate between the increased vessel length and it's branching therefore, a 2-photon microscope with fluorescence imaging is required for exploring the depth [9]. This examination investigates the comparison of 3D blood vessel morphologies in the superficial layer of skin between the AD patients and healthy people.

METHOD

Study procedures and participants: This case control study was conducted on 40 participants (24 males and 16 females) aged between 20-65 years of age (mean age 45 years) during Dec 2021 to Nov 2022 in Watim Medical college, Rawat after the approval of ethical research committee. Samples of skin were collected from healthy as well as from chronic AD patients having lichenification.[10] Informed consent was taken from each participant before the study and everyone was being informed about the procedure. For including the patients, it was made sure to rule out other comorbidities like asthma, allergic rhinitis, and other skin infections. Patients were screened with scoring atopic dermatitis (SCORAD) scale to rule out the eligibility criteria for enrolling the patients. A score of > 25 was considered as the eligibility criteria

for enrollment [11]. The study was conducted according to the suggested guidelines of Watim medical college during Dec 2021 to Nov 2022 after the approval from the ethical research committee of the Institute.[12] Patients were given local anesthesia with lidocaine for performing the tape stripping of 10 – 30 minutes. Biopsy of each patient was then performed through the back skin by a 3 mm punch.[13] In patients suffering from AD, samples were obtained from the involved as well as uninvolved areas. After obtaining all the samples, they were then immediately soaked in the 4 % solution of formaldehyde throughout the night period at 4 ° C.[10]

Inclusion and exclusion criteria: Inclusion criteria include patients aged between 20-65 years of age. Patients diagnosed with AD for a period of at least 12 weeks. Peak pruritus numerical rating scale (PPNRS) of 2 or more. A rating score of 3-21 on Eczema Area Severity index (EASI). Score of less than 2 on both PPNRS and EASI should be excluded from study. [11]

Controls: Control group included participants that matched the age and distribution of the population selected for the study. Participants having no history of eczema, dermatitis or any other skin infections were included for the study [2]. Control group had gone through the same procedure for the diagnosis as for the patients with AD.

Outcome measures: Pain associated with AD was measured by using the Numerical pain rating scale (NPRS) or Visual analogue scale (VAS) [14]. Eczema was measured by using the EASI and pruritus was measured by using PPNRS [11].

Visualization and identification of tissue antigens: After soaking the samples of 40 participants in formaldehyde overnight, the samples were then immersed in the solution of sucrose (30 %) till descending at the base of the container. Samples were then submerged in a thermocouple compound and were sectioned at 100 micrograms through cryostat for the purpose of immunostaining [10]. The cross-sectioned parts were then managed by H_2O_2 (10 %), and phosphate buffered saline of Triton X (0.3 %) for a total time period of 10 minutes at ambient temperature [16]. These samples were then treated with blocking solution at room temperature for a time period of 2 hours before contacting them with first antibodies. During the whole procedure, it was observed that the samples were free floating [10]. Antibodies (type IV) were diluted in a blocking solution with a ratio of 1:500. After that, intubation of sections was done at temperature of 4 ° C for a period of 2-3 days with the solution of antibodies [16]. These sections were washed with the buffer phosphate solution of 2.5 %, 3 times and for 30 minutes [10].

2-photon Microscopy: The sections of the samples were mounted on the slides of glass with a mounting medium of Fluoromount plus. Laser scanning microscope having 2 photon power with Ti-sapphire and objective lens was used for observing the sections [9].

Statistical analysis: Statistical analysis of descriptive and demographic data was done by using t test and chi-square test in case of normal data distribution whereas Wilcoxon signed rank test in case of non-normal data distribution [16]. For testing correlations, spearman correlation coefficient was used. All the analysis was done by using SPSS version 23 [17]. In statistical analysis, a P value of < 0.05 is considered as statistically significant [18].

RESULTS

Demographic data and severity of disease: Characteristics of patients are presented in a tabulated form below (see table 1). Among 40 patients, 20 were having chronic atopic dermatitis scored through SCORAD whereas other 20 participants were normal. It has been observed from the table below that there are statistically significant differences between the NPRS, SCORAD and EASI values of both the groups with a p value of < 0.001 [11].

Table 1: Characteristics of study population.

Characteristics	Atopic dermatitis	Control group	P value.
Age (mean ± S.D)	10.0 ± 3.2	10.5 ± 3.3	0.68
Gender male	12 ± 65	12 ± 65	0.79
Gender female	7 ± 35	7 ± 35	0.65
Race/ethnicity	5 ± 25	4 ± 21	0.89
Comorbidities			
Skin allergies.	14 ± 65	3 ± 45	< 0.001
Allergic reactions.	12 ± 55	0 ± 0	< 0.001
Asthma	10 ± 20	0 ± 0	< 0.001
Allergic rhinitis	13 ± 17	0 ± 0	< 0.001
Severity of AD (mean ± S.D)	20 ± 57	0 ± 0	< 0.001
SCORAD (mean ± S.D)	46 ± 16	0 ± 0	< 0.001
EASI (mean ± S.D)	24 ± 14	0 ± 0	< 0.001
NPRS (mean ± S.D).	6 ± 2.8	0 ± 0	< 0.001

Table 2: SCORAD and EASI scoring for Atopic dermatitis patients.

Patients	SCORAD scoring		P value	EASI scoring		P value
Experimental group.						
	Assessment 1	Assessment 2		Assessment 1	Assessment 2	
Patient 1	10 ± 1.1	11 ± 5.5	0.3	37 ± 5.4	41 ± 2.2	0.3
Patient 2	13 ± 2.1	10 ± 4.5	0.4	32 ± 3.1	36 ± 2.5	0.23
Patient 3	12 ± 2.2	10 ± 3.4	0.33	31 ± 3.6	36 ± 2.7	0.33
Patient 4	14 ± 3.2	12 ± 4.5	0.23	30 ± 7.3	42 ± 1.9	0.04
Patient 5	18 ± 1.4	13 ± 6.5	0.21	29 ± 6.6	48 ± 2.9	0.03
Patient 6	21 ± 1.5	16 ± 2.1	0.04	26 ± 4.3	31 ± 3.9	0.4
Patient 7	13 ± 1.0	15 ± 3.5	0.2	25 ± 3.5	29 ± 4.2	0.2
Patient 8	24 ± 1.1	19 ± 5.2	0.01	31 ± 3.3	29 ± 3.1	0.5
Patient 9	31 ± 5.4	28 ± 6.4	0.02	36 ± 2.8	31 ± 2.9	0.1
Patient 10	13 ± 6.2	11 ± 1.2	0.4	39 ± 3.7	32 ± 3.0	0.2
Patient 11	15 ± 8.2	10 ± 9.2	0.23	24 ± 3.3	33 ± 3.1	0.4
Patient 12	12 ± 4.2	11 ± 3.2	0.2	21 ± 3.5	31 ± 3.3	0.23
Patient 13	17 ± 4.4	11 ± 4.6	0.04	19 ± 5.4	25 ± 3.9	0.35
Patient 14	17 ± 3.2	13 ± 6.7	0.1	30 ± 5.5	35 ± 4.1	0.2
Patient 15	16 ± 3.9	12 ± 5.7	0.12	32 ± 4.7	39 ± 3.9	0.11
Patient 16	13 ± 4.2	10 ± 5.1	0.33	33 ± 4.4	30 ± 5.4	0.32
Patient 17	12 ± 4.4	9 ± 4.9	0.23	38 ± 3.2	29 ± 3.3	0.05
Patient 18	21 ± 3.9	12 ± 7.1	0.21	37 ± 3.3	31 ± 4.3	0.4
Patient 19	12 ± 6.2	10 ± 6.8	0.2	33 ± 2.9	32 ± 3.8	0.5
Patient 20	13 ± 3.8	8 ± 5.0	0.05	28 ± 3.7	36 ± 2.9	0.34

Representation of skin sections: In healthy participants, samples displayed normal vascular plexus superficially and normal loops of capillary originated from the vascular system [5]. In participants suffering from AD, representation is different with erythema along with twisted complex vessels in lesional and nonlesional skin [7]. In the region of papillary, this complex network of vessels is thick and packed tightly [6]. In chronic cases of AD, patients were having lichenification which can be observed easily under a photon microscope. No bifurcated blood vessels observed in any of the area [7].

DISCUSSION

To the best of the author's knowledge, it is the first largest case control study for assessing the abnormal morphology of the vessels associated with erythema in AD patients. In this study patients with AD were not suffering from any other comorbidities in order to clearly visualize the abnormalities of vessels associated with disease. Previously, it had been observed from studies that there were associations between the development of AD with other clinical conditions like urticaria, alopecia, and vitiligo but the current study didn't highlight any such evidence. Generally, the findings of this study are in line with some previous studies however, there remain some differences in terms of population size and study type. Furthermore, it had included a broad perspective to highlight the targeted variables.

After analyzing the samples of 40 participants under photon microscope, it has been revealed that the specimens that contain lesions were showing looped and curled vessels without any bifurcated vessel within the capillary loops under 3-dimensional (3D) analysis of structure. Previously, it was found that an enormous number of blood vessels were observed in the affected skin seen by thin sections under light microscope. It has also been observed in some sections that the cutting of a single complex vessel can result in increasing the number of vessels in the sections that were immune-stained. Previous study has been carried by Tsutsumi (19) in which he explored the abnormal morphology but the study was unable to conclude whether the formation of these flexuous and complex structures was from the alteration of superficial plexus or loops of capillaries therefore, they recommended to carry a more precise study on the abnormal morphology of blood vessels during AD [19]. The author has worked on this gap of the study and carried out a more precise and level III evidence study by recruiting a large number of the population (40 participants).

Author of the study concluded that after visualizing the skin sections of healthy and AD patients, it has been confirmed that the vessels of all the patients demonstrate similar characteristics under photon microscope. All the patients appear to have the formation of looped vessels regardless of the age, gender, ethnicity, and other comorbid conditions. There were no genetic associations found in patients suffering from AD. However, it was observed that the individuals having dry skin and those utilizing too much cosmetic products were more prone towards the development of AD as compared to normal skin individuals. All the healthy individuals in the study were observed to have moisturized and fresh skin. The strength of the study is that it has included a large population belonging to a geographic area where participants were belonging to different cultural backgrounds. However, it has a limitation that it had only included the patients suffering from chronic AD suffering from lichenification and excluded patients in acute and sub-acute stages of AD. Future researchers can also carry this study to a broader perspective by working on a cross-sectional study involving a large number of populations from different geographic regions of the world. Future researchers can also highlight the use of cosmetic products in the development of AD by focusing on the components of the products causing skin diseases.

CONCLUSION

Patients suffering from AD may always demonstrate changes within their vascular system owing to the formation of erythematous areas over the skin resulting in complex and twisted vessels. Patients may always complain of pain, pruritus, eczema however, its severity differs from person to person. AD can occur in any individual without any known genetic and environmental cause and may even affect the children aged between 1-3 years onward. Further, microscopic evaluation revealed that bifurcated vessels were not observed in either of the cases of AD patients or healthy individuals.

Conflicts of interest: There are no conflicts of interest in this study.

Abbreviations:

AD = Atopic dermatitis.
VEGF = Vascular endothelial growth factor.
3D = 3 dimensional.
SCORAD = Scoring Atopic dermatitis.
NIH = National Institute of health.
PPNPRS = Peak pruritus numerical pain rating scale.
EASI = Eczema Area severity index.
SD = Standard Deviation.

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