

Comparison between high-frequency ultrasonography (Dermascan C, version 3) and histopathology in atopic dermatitis

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Background: The main purpose of this study was the exact comparison of B-scan images obtained from 16 patients suffering from AD with histological.

Methods: Sixteen patients diagnosed with AD aged between 14 and 57 years, were chosen for the study. In all patients before the introduction of proper treatment regimen, the high frequency skin ultrasonography (Derma Scan Cortex Technology, version 3, Hadsund, Germany) has been performed and then a 5 mm wide punch biopsies have been taken from the area of scanned lesions. Control group consisted of 15 healthy individuals without any signs of atopic and chronic diseases.

Results: There was $7.8 \pm 0.4\%$ mean skin echogenicity from lesional antecubital skin in AD patients (min value 7.2%, max value 8.5%). The skin hypoechoic band was detected in fifteen out of sixteen patients (93.7%). There were statistically significant correlations between the hypoechoic band thickness and

the following parameters: degree of epidermal hyperplasia, the degree of epidermal hyperkeratosis, the degree of parakeratosis and the degree of spongiosis as well as the intensity of inflammatory infiltrates. Skin echogenicity strongly correlated negatively with the intensity of inflammatory infiltrates.

Conclusion: On the basis of analysis of 16 specimens we can conclude that all pathological changes of AD influence ultrasound image and in addition, the USG picture depends on the phase of disease process.

Key words: atopic dermatitis – high frequency ultrasonography – skin sonography – histopathology

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Accepted for publication 23 February 2013

ATOPIC DERMATITIS (AD) belongs to the group of chronic, recurrent skin disorders, which diagnosis is based on well-known in daily routine practice clinical findings (1–3). Due to the high prevalence, considerable morbidity and chronicity of the disease as well as its high economic burden, there is a need for accurate evaluation of a treatment regimen being tested (4, 5). Unfortunately, a useful laboratory test to determine disease severity does not exist and skin biopsy, which is invasive, also presents no specific histological features (6). There are widely used scoring indexes, which allow to quantify skin lesions morphology and to measure the percentage of affected area. Until 2007 Schmitt et al. identified a total of 56 different objective measurements to assess clinical severity used in 94 trials and only three of them (EASI, POEM, SCORAD) have been validated adequately (7). The disadvantage of scoring

systems is that they require subjective judgment and have poor reproducibility, even though they are assessed by an experienced physician (8).

To provide objective evaluation of the disease severity, several noninvasive techniques have been proposed. Most of studies use instrumental measures to assess skin barrier function in AD, like transepidermal water loss (TEWL), and corneum hydration (9, 10). Both were stated as valuable in quantification of disease severity (11). Relatively new method in evaluation of AD, that is continuously developing, is high frequency ultrasonography (HF-USG). Characteristic sonographic feature of inflammatory dermatoses, such as eczema, is the presence of superficial hypoechoic band just beneath the echo entry, what may represent acute inflammatory process with edema or infiltration in the upper part of the dermis (12–14). However, these findings were not supported by a

histologic examination of biopsy specimen. In addition, within inflamed lesions the decrease of skin density (echogenicity) may be detectable. During therapy the changes of these two skin parameters: thickness of hypoechoic band and mean echogenicity may serve as useful and reliable indicators of its effectiveness (14).

It would seem that there is a need for broader evaluation of this method in inflammatory dermatoses, especially AD. The aim of this study was the exact comparison of B-scan images obtained from 16 patients suffering from AD with histological features in biopsies of scanned lesions. This article reports a pilot study on this subject and according to the authors' knowledge this is the first report dealing with this problem.

Materials and Methods

Sixteen patients diagnosed with AD, who have signed their informed consent, aged between 14 and 57 years, were chosen for the study. There were 5 men and 11 women. All patients recruited to the study were referred to the Department of Dermatology Poznan University of Medical Sciences due to the exacerbation of skin lesions. Before the introduction of proper treatment regimen, the skin ultrasonography has been performed and then a 5 mm wide punch biopsies have been taken from the area of scanned lesions. The clinical severity was evaluated on the basis of EASI score (15).

Control group consisted of 15 healthy individuals (aged between 18 and 39 years old, 4 men and 11 women) without any signs of atopic and chronic diseases.

Skin ultrasonography

USG examination produces a cross-sectional image of human skin with an axial resolution of 80 μ m and lateral resolution of 200 μ m with a sharp-focusing transducer. The ultrasonic wave is partially reflected at the boundary between adjacent structures and produces echoes of different amplitudes. The intensity of the reflection echoes is evaluated by the microprocessor and is visualized as a color-coded two-dimensional B-mode image (standardized code of 255 levels). The average amplitude of the echoes in a defined area of the image is known as echogenicity, which may be objectively measured with the computer-assisted image analysis.

Hypoechogenic is defined as an intensity <30 pixels. The A-mode interfaces are shown as well defined peaks. A typical gain curve was 25–70 dB. The velocity of ultrasound in the skin was set at 1.580 m/s (16, 17).

In all AD patients skin ultrasonography (Derma Scan Cortex Technology, version 3, Hadsund, Germany) was performed with the use of 20 MHz probe in B-mode scan within the representative skin region with the average intensity of the disease, within the flexural site of the forearm, 5 cm below antecubital fossa. Two skin parameters were assessed: skin echogenicity (%) and the thickness of hypoechoic band. The average hypoechoic band thickness was evaluated in millimeters by measuring the vertical distance between the lower edge of the entry echo and the posterior margin of the hypoechoic zone (A-mode scans). In control group ultrasound images were obtained from antecubital fossa. Scannings were performed by the same trained observer.

Histological examination

There were punch biopsies (measuring 5 mm) taken from the recorded skin area. After fixation in 10% buffered formalin all specimens were routinely processed, embedded in paraffin and 3 μ m thick sections were stained with Hematoxylin and Eosin. Microscopical slides were evaluated at $\times 4$, $\times 10$, $\times 20$, $\times 60$ magnification of microscope (Olympus CX41). Microscopic features such as hyperplasia, hyperkeratosis, parakeratosis, spongiosis and inflammatory infiltrates were scored semi-quantitatively on a 5 level scale (score from 0 to 4) (Table 1).

Statistical analysis

All data were elaborated by commercially available statistical software package (STATISTICA v 10.0). Statistical analysis was performed using Spearman correlation. Statistical significance was defined as $P < 0.05$.

Results

There was $7.8 \pm 0.4\%$ mean skin echogenicity from lesional antecubital skin in AD patients (min value 7.2%, max value 8.5%). The skin hypoechoic band was detected in fifteen out of sixteen patients (93.7%). Its mean thickness was

TABLE 1. Histological features (scored on a 5 level scale)

Histological feature	0 SCORE	1 SCORE	2 SCORE	3 SCORE	4 SCORE
Hyperplasia	Rete ridges length approx. equal to the thickness of epidermis	Rete ridges length approx. 2× thicker than epidermis	Rete ridges length approx. 3–4 times thicker than epidermis	Rete ridges length approx. 5–6 times thicker than epidermis	Rete ridges length more than 6× thicker than epidermis
Hyperkeratosis	Normal keratotic layer	Stratum corneum thicker <30% than the thickness of epidermis	Stratum corneum thicker 30–50% than the thickness of epidermis	Stratum corneum thicker 51–70% than the thickness of epidermis	Stratum corneum thicker above 70% than the thickness of epidermis
Parakeratosis	Not evident	<30% of the epidermis thickness	30–50% of the epidermis thickness	51–80% of the epidermis thickness	≥ 80% of the epidermis thickness
Spongiosis	Not evident	Minimal	Mild	Moderate	Marked
Inflammatory infiltrates	Not evident	Minimal	Mild	Moderate	Marked

0.22 ± 0.1 mm (min 0.12 mm, max 0.45 mm). Figures 1–3 present typical ultrasound image of AD skin in different severity of skin inflammation. Figure 1 characterizes with thin hypoechoic band, what reflects mild inflammation. On the other hand, Fig. 3 with wide hypoechoic band and low echogenicity represents severe skin condition. Clinical evaluation based on EASI score is presented in Table 2.

Mean skin echogenicity obtained from healthy volunteers was 8.8% ± 0.1% (min value 8.4%, max value 9.0%) and hypechogenic band underneath entrance echo was not observed in any image. Figure 4 presents USG image of healthy skin.

The histology revealed mainly reactive changes within epidermis in the form of hyperplasia with elongation of dermal papillae, hyperkeratosis, parakeratosis and slight intercellular edema. Lymphocytes were predominant cells in infiltrates, they were present in all patients. Six patients showed marked hyperplasia (score 3–4 points) and hyperkeratosis, which was associated clinically with chronic licheni-

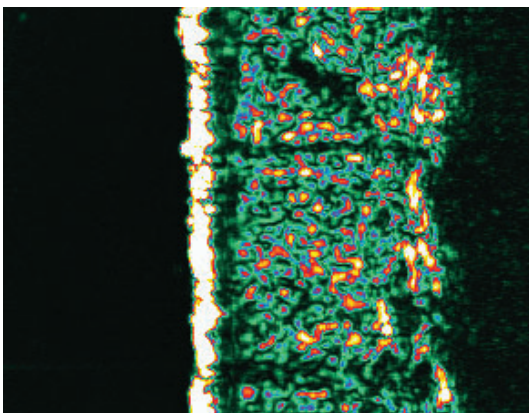


Fig. 1. Ultrasound image of mild severity of skin inflammation in atopic dermatitis.

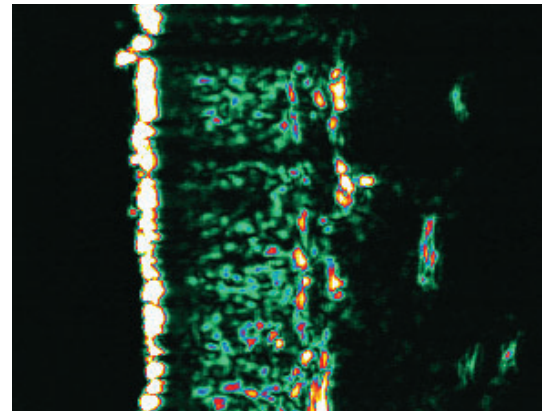


Fig. 2. Ultrasound image of moderate severity of skin inflammation in atopic dermatitis.

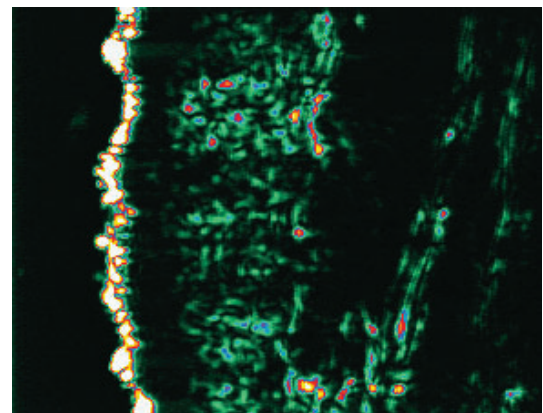


Fig. 3. Ultrasound image of advanced severity of skin inflammation in atopic dermatitis.

fied plaques. The spongiosis (scored 1–2 points) was observed in 6 patients. The detailed summary of histological features in analyzed patients is presented in the Table 2.

There was a statistically significant correlation observed between the degree of epidermal hyperplasia and hypoechoic band thickness ($R = 0.58$, $P = 0.02$), whereas in relation to the skin echogenicity such correlation was not

TABLE 2. The histological features (scored on a 5 level scale) and EASI (range 0–72)

Number of patient	EASI	Hyperplasia	Hyperkeratosis	Parakeratosis	Spongiosis	Inflammatory infiltrates
1	47.2	3	4	2	1	3
2	34.5	4	4	4	1	4
3	35.2	2	1	1	1	4
4	41	2	1	1	1	1
5	4	1	1	0	0	1
6	1.6	1	1	1	0	2
7	1	3	4	1	2	4
8	32	3	4	4	0	2
9	41	1	2	1	0	1
10	4.4	2	1	0	0	2
11	45	3	4	4	0	4
12	4	2	1	1	1	2
13	34	2	4	0	0	3
14	0.4	2	2	4	0	3
15	20.8	1	1	0	0	1
16	40	3	4	3	0	1

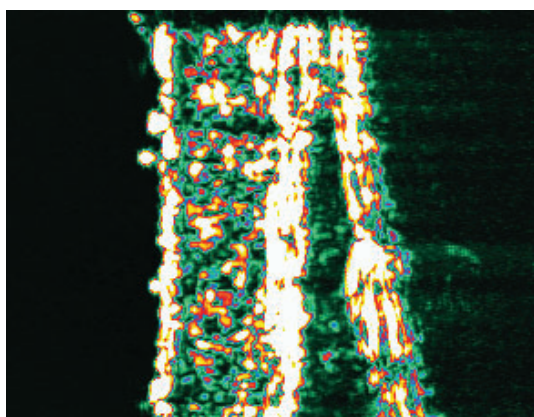


Fig. 4. Ultrasound image of healthy skin.

detected. In addition, there were also revealed statistically significant correlations between the hypoechoic band thickness and the following parameters: the degree of epidermal hyperkeratosis ($R = 0.57$, $P = 0.04$), the degree of parakeratosis ($R = 0.58$, $P = 0.02$) and the degree of spongiosis ($R = 0.54$, $P = 0.03$). There was also found a statistically significant correlation between the intensity of inflammatory infiltrates and hypoechoic band thickness ($R = 0.63$, $P = 0.008$). Skin echogenicity strongly correlated negatively with the intensity of inflammatory infiltrates ($R = -0.68$, $P = 0.003$).

Discussion

Typical ultrasound image obtained from healthy skin is composed of three layers of different echogenicity (Fig. 4). The outermost layer is known as an epidermal entrance echo and is highly echogenic. Underneath there is a dermal layer, which is less echogenic than entrance echo and contains many different echoes of various intensities. The

last layer is related to the non-echogenic subcutaneous tissue (12, 17). The pathological processes located within the epidermis, dermis and subcutaneous tissue may change echogenicity of these layers. According to the literature, reduced echogenicity in the form of low reflective band (hypoechoic band) underneath the entrance echo can be caused by edema or by infiltrates of inflammatory cells (e.g. atopic dermatitis) or of carcinomatous cells (e.g. mycosis fungoides) (12, 17, 18). The decreased echogenicity can be detected as a typical ultrasound aspect of positive patch test (19). Similar features may be observed in ultrasound images obtained from psoriatic skin. It is stated that both elongation of the rete ridges and inflammatory infiltrate in the papillary dermis can be responsible for that USG picture (20). In relation to AD, that is perceived as inflammatory disorder of epidermis and papillary dermis and which clinical picture may be different in acute and chronic state, we may also observe lower skin echogenicity and the presence of hypoechoic band (9, 16). But until now this problem was not clearly investigated and supported by the histological assessment. Hoffmann et al. took the biopsy from lesional skin of 5 AD patients and compared the depth of infiltrate and edema with the width of the subepidermal echolucent area and showed the corresponding results (21).

In this study, we have investigated skin echographic features in patients suffering from AD and compared them with histological examination. Ultrasound images were obtained and 2 parameters were assessed: skin echogenicity and width of hypoechoic band. We found lower skin echogenicity among AD patients in comparison to healthy volunteers (7.2% vs. 8.8%, respectively).

Almost all USG images of AD patients (15 out of 16) revealed the presence of echo-poor superficial band of the mean value 0.22 ± 0.1 mm, while none was present in the control group.

Five histological parameters were evaluated in skin biopsies of AD patients: hyperplasia, hyperkeratosis, parakeratosis, spongiosis and inflammatory infiltrates. In acute lesions there is slight hyperplasia of the epidermis, slight hyperkeratosis and some parakeratosis. The spongiosis may be observed, which reflects intercellular edema. Subadjacent dermis shows perivascular inflammatory infiltrates which are composed of lymphocytes and histiocytes. Chronic lichenified plaques present as moderate or marked hyperplasia of the epidermis with elongation of rete ridges and prominent hyperkeratosis with areas of parakeratosis. Dermal infiltrate consists of mononuclear cells (lymphocytes, histocytes, macrophages), which form perivenular aggregates. Also fibrosis of the papillary and upper reticular dermis can be seen (6).

There was an agreement in our study between hypoechoic band thickness and all evaluated histological features. In relation to skin echogenicity we have observed negative relationship with only one parameter – the degree of inflammatory infiltrates. On the basis of analysis of 16 specimens we can conclude that all pathological changes of AD influence ultrasound image and in addition, the USG picture depends on the phase of disease process. The presence of hypo-

echoic band in chronic lesions is associated with reactive changes of epidermis, like its hyperplasia, hyperkeratosis and parakeratosis as well as presence of subepidermal inflammatory infiltrates. While in acute stage of the disease the thickness of subepidermal low-echogenic band may be dominated by the intercellular edema. The general lower skin echogenicity within lesional AD skin reflects the presence of inflammatory infiltrates, which may differ in composition and severity according to the disease phase. Therefore, only one analyzed histological feature – level of inflammatory infiltrates is related to both ultrasound changes observed in AD (hypoechoic band, lower echogenicity).

Conclusion

We have confirmed the previous reports that echo-poor band in AD is related to the edema and inflammatory infiltrates (9, 14, 15, 17, 19). But we have additionally found that this phenomenon is strongly associated with the phase of the disease process and that in the case of chronic lesions also depends on the presence of hyperplasia, hyperkeratosis, and parakeratosis. Moreover, we have revealed that another ultrasound parameter, that is skin echogenicity in AD shows difference in relation to healthy skin and mostly is associated with inflammatory infiltrates. To conclude, there is a strong agreement between HF-USG and histological features in evaluation of AD.

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