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Original Research Article

Epidermolysis bullosa in Greece: the patients' journey so far

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ABSTRACT

Background: Hereditary epidermolysis bullosa (EB) represents a group of rare, inherited disorders with different penetrance patterns characterized by skin fragility and easy inducibility of blisters. Mucosal involvement of internal organs may occur. As no published data on EB in Greece exist, this study aimed to record demographics and clinical characteristics of EB patients. Another objective was to identify the associations among clinical characteristics of different types in connection with immunofluorescence mapping (IMF) findings and molecular analysis (MA) used for the laboratory diagnosis of the disease.
Methods: This is a descriptive study conducted at the outpatient clinic of rare diseases of Andreas Sygros Hospital, Athens, Greece from March 2012 until February 2015. Adults and children presenting with EB were enrolled. Patients underwent a thorough clinical and laboratory assessment. Specific laboratory analyses were performed in Rome and two sets of data based on IFM and MA were collected.
Results: In total, 41 patients were enrolled. Prevalence rate of EB was 0.024%. The most frequent type was dystrophic EB, as it affected 20 patients (48.8%). Twelve patients (29.3%) were diagnosed with EB simplex, 6 patients (14.6%) with Kindler syndrome and 3 (7.3%) with junctional EB. IFM was performed in 26 patients and MA in 8 patients. The concordance among clinical and laboratory diagnosis was 88.5%.
Conclusions: This study is the first report on hereditary EB in Greece. Since there is a lack in diagnostic management of EB, we would strongly encourage an effort to perform the required laboratory tests in Greece.

Keywords: Hereditary epidermolysis bullosa, Immunofluorescence mapping, Molecular analysis, Mutations, Greece

INTRODUCTION

Epidermolysis bullosa (EB) encompasses a broad range of inheritable skin diseases. EB disorders are characterized by induced blistering of the skin and mucous membranes.

EB is classified into 4 major types: simplex (EBS), junctional (JEB), dystrophic (DEB) and Kindler syndrome (KS). Each main type, with the exception of KS, is further subdivided into several subtypes, covering the spectrum of the remarkable phenotypic variability of the disease.[1-3] In severe forms, the joints, gastrointestinal, genitourinary, and respiratory system can be affected resulting in a variable range of complications.[1-4]

Four international consensus meetings on diagnosis and classification of EB have taken place. The last one, held in London in 2013, concluded in a revised approach based on the type, mode of inheritance, phenotype,

immunofluorescence antigen mapping findings and the mutation(s) present in each EB patient.[1]

To our knowledge, there are no published data on EB regarding clinical characteristics of its different types and laboratory findings in Greece until present day. The diagnosis of the disease is clinical, since there are no facilities available in our country for performing immunofluorescence mapping (IFM) and molecular analysis (MA).

This study aimed to record demographics and clinical characteristics of EB patients. Moreover, since EB poses many diagnostic challenges, another objective of our study was to identify the associations among clinical characteristics of different types in connection with IMF findings and MA. For this purpose, our center was the first one in Greece that initiated a collaboration with 'Istituto Dermopatico dell' Immacolata' in Rome.

METHODS

The current study was conducted at 'Andreas Sygros' Hospital in Athens, Greece from March 2012 until February 2015. The study was approved by the Ethics Committee (Scientific Board) of 'Andreas Sygros' Hospital, on March 9, 2012. Patients who were above 18 years old granted their written informed consent, while in cases of patients younger than 18 years old the respective written informed consent was granted by their parents.

Patients underwent the following evaluation. Patients presenting with hereditary EB were recruited during their routine visit to the outpatient clinic of the hospital and they were subsequently referred to the outpatient clinic of rare diseases. A detailed history was recorded and data including sex, age, nationality, age at disease onset, positive family history of EB and consanguinity were collected. Then, a thorough clinical examination was carried out and photos were taken. Patients were categorized into different EB types according to clinical findings.

Patients were periodically re-evaluated. Duration of time intervals between re-evaluations depended on clinical and laboratory assessment by other specialties, i.e. pediatrician, dentist, ophthalmologist, gastroenterologist, urologist, cardiologist.

Patients underwent complete blood cell count and biochemical testing.

Perilesional skin punch biopsies were obtained from patients for IMF examination. IMF analysis using a three-step biotin-streptavidin-fluorescein procedure for dermal-epidermal junction antigens was performed on skin biopsy sections as previously described.[5]

Blood was sampled from probands and their parents for MA. MA of EB subtype genes was performed by polymerase chain reaction (PCR) of ethylenediaminetetraacetic acid (EDTA) blood-purified genomic DNA using appropriate primers, followed by denaturing high-performance liquid chromatography (dHPLC) pre-scanning and/or direct sequencing of amplified PCR products, as previously reported.[6,7]

Laboratory examinations were performed in 'Istituto Dermopatico dell' Immacolata' in Rome.

Data sets were extracted and analyzed using Microsoft Excel and SPSS statistical software.

RESULTS

Demographic data

A total of 41 patients aged from 3 days old to 55 years old were enrolled in the study. The mean age of the patient population was 18.65 years old. The majority of patients were children and adolescents <18 years old (63.4%). Age distribution at the time of consultation is presented in Figure 1.

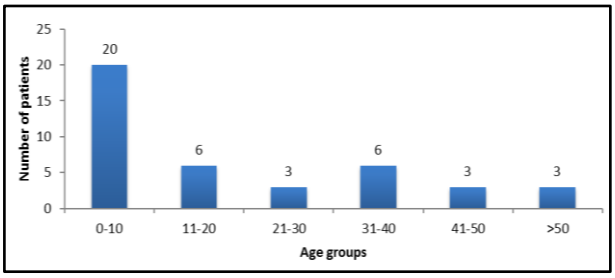

Figure 1: Number of EB patients for each age group.

Twenty-four patients (58.5%) were male, while 17 (41.5%) were female (M/F ratio 1.4:1).

Regarding nationality, 35 patients (85.4%) were Greek nationals, while only 6 patients (14.6%) were non-Greeks. Positive family history was reported in 55.9% of the study population. Consanguineous marriages were reported in three patients (7.3%).

In terms of geographical distribution, the majority of the patients (58.5%) lived in urban areas (U/R ratio 1.4:1).

During this 3-year period prospective study, 172,110 patients visited the Hospital's Outpatient Clinic for the first time. Thus, EB prevalence among patients with dermatological diseases examined during the aforementioned period was 0.024%.

Clinical manifestations

In more than half of the patients (53.6%), disease onset was reported at birth or neonatal period. The distribution of patients based on age at disease onset is shown in Figure 2.

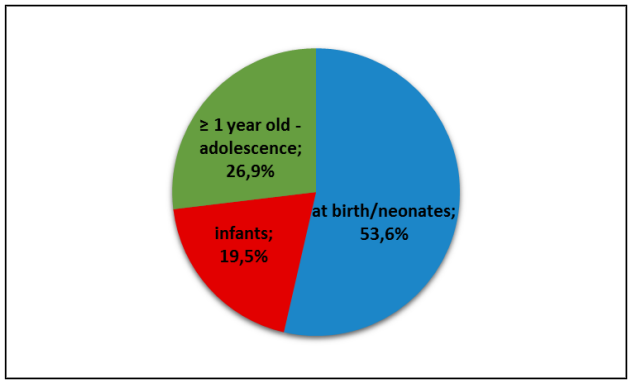

Figure 2: Distribution of patients based on disease onset.

Clinical findings regarding skin and its appendages are set out in Table 1. Figure 3 A-F corresponds to representative skin manifestations across various types and subtypes of EB.

Special referral is made to some of our patients who presented complications, necessary to be treated by implementing interventional procedures.

Due to painful erosions and severe stenosis that impaired food and liquid intake, three of our patients required endoscopic dilatation, after which symptoms were significantly improved and no additional intervention were necessary until present day.

Table 1: Clinical findings and associated features in 41 Greek EB patients.

Clinical characteristics of the skin and its appendages	Total no of patients	EBS	DEB	JEB (gen-i)	KS
Fragile skin	41	12	20	3	6
Blisters - Easy inducibility of blisters	36	12	20	3	1
Milia	7	-	2 (1 D, 1 R, gen-sev)	-	5
Hyperhidrosis	21	4 (1 loc, 3 gen-i)	12 (7 D, 3 R, gen-i, 1 gen-i/sev, 1 gen-sev)	1	4
Exacerbation during summer/heat	33	11 (All except 1 loc)	14 (All except 6 D)	2	6
Scarring	26	5 (1 loc, 3 gen-i, 1 gen-i/sev)	14 (9 D, 3 R, gen-i, 1 gen-i/sev, 1 gen-sev)	2	5
Hyperkeratosis of palms and soles	9	1 (gen-i)	2 (1 D, 1 R, gen-i)	1	5
Hyperpigmentation	18	4 (3 gen-i, 1 gen-i/sev)	10 (7 D, 1 R, gen-i, 1 gen-i/sev, 1 gen-sev)	1	3
Granulation tissue	7	-	6 (5 D, 1 R, gen-i)	1	
Poikiloderma	5	-	-	-	5
Photosensitivity	5	-	-	-	5
Skin atrophy	5	-	-	-	5
Flattening of palmar dermatoglyphic lines	2	-	-	-	2
Nail dystrophy	14	4 (1 loc, 2 gen-i, 1 gen-i/sev)	7 (5 D, 2 R, gen-i)	1	2
Absence of nails	13	2 (2 gen-i)	8 (4 D, 2 R, gen-i, 1 gen-i/sev, 1 gen-sev)	2	1
Scarring alopecia	2		1 (R, gen-sev)	1	
Associated features					
Pruritus	20	6 (2 loc, 2 gen-i, 2 gen-i/sev)	10 (5 D, 5 R, gen-i)	2	2
Pain	20	1 (gen-i/sev)	15 (7 D, 5 R, gen-i, 2 gen-i/sev, 1 gen-sev)	2	2

EBS: epidermolysis bullosa simplex; loc: localized; gen-i: generalized intermediate; gen-i/sev: generalized intermediate/severe; DEB: dystrophic epidermolysis bullosa; D: dominant; R: recessive; gen-sev: generalized severe; gen-i: generalized intermediate; gen-i/sev: intermediate/severe; JEB: junctional epidermolysis bullosa; gen-i: generalized-intermediate; KS: Kindler syndrome.

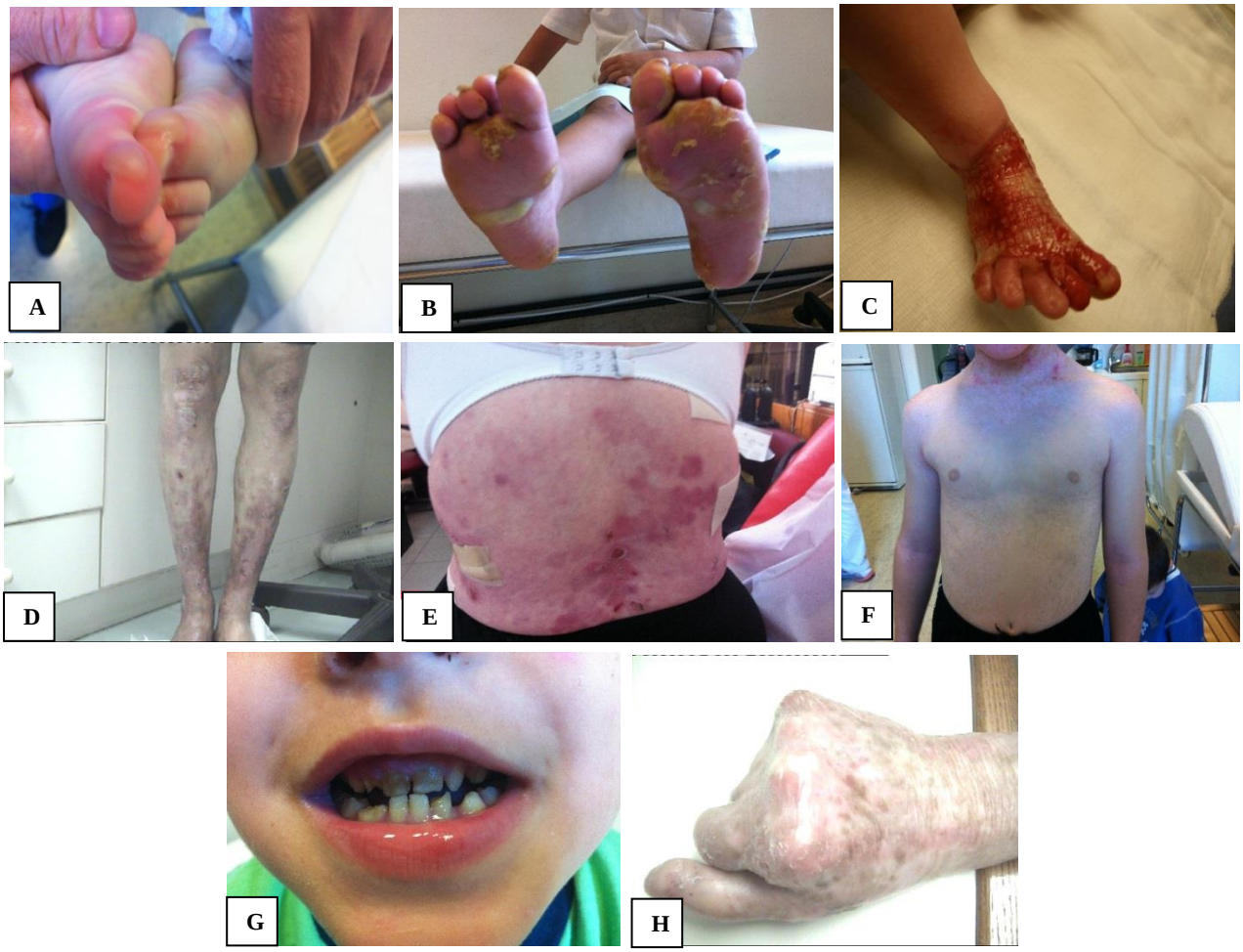

Figure 3: Cutaneous features of: (A) Localized blistering in epidermolysis bullosa simplex, localized; (B) Generalized blistering without scarring in epidermolysis bullosa simplex, generalized intermediate; (C) Large erosions in recessive dystrophic epidermolysis bullosa, generalized intermediate; (D) Scarring and hyperpigmentation in dominant dystrophic epidermolysis bullosa, generalized; (E) Erosions and scarring in junctional epidermolysis bullosa, generalized intermediate; (F) Poikiloderma in Kindler syndrome. (G) Dental enamel hypoplasia with caries in epidermolysis bullosa simplex, generalized intermediate; (H) Flexural contracture and pseudosyndactyly with severe loss of function of the right hand in recessive dystrophic epidermolysis bullosa

Table 2: Extracutaneous involvement of all Greek EB patients.

Extracutaneous features/manifestations		Total No of patients	EBS	DEB	JEB (gen-i)	KS
Physical development	Growth retardation	1	-	1 (R, gen-sev)	-	-
Musculoskeletal system	Pseudosyndactyly	1	-	1 (R, gen-sev)	-	-
	Flexural contracture/Stiffness	5	-	2 (1 D, 1 R, gen-sev)	1	2
Oral cavity	Erosions	6	1 (gen-i/sev)	3 (1 D, 1 R, gen-i, 1 R, gen-i/sev)	1	1
	Enamel hypoplasia	12	3 (1 loc, 1 gen-i, 1 gen-i/sev)	6 (4 D, 2 R, gen-i)	-	3
	Caries	8	3 (1 loc, 2 gen-i)	1 (D)	-	4
	Artificial denture	5	-	2 (1 D, 1 R, gen-sev)	2	1
	Microstomy	2	-	-	-	2
	Hoarseness	5	1 (gen-i)	1 (D)	-	3

Continued.

Extracutaneous features/manifestations		Total No of patients	EBS	DEB	JEB (gen-i)	KS
Gastrointestinal tract	Erosions/Stenosis	7	-	1 (R, gen-sev)	1	5
	Constipation	7	1 (loc)	3 (2 D, 1 R, gen-i)	-	3
Genitourinary tract	Urethral stenosis	1	-	-	-	1
	Phimosis	2	-	1 (R, gen-i)	-	1
	Adrenal insufficiency	1	-	-	1	-
Ocular	Corneal/conjunctival erosions	6	-	1 (R, gen-i)	2	3
	Ectropion	-	-	-	-	1
	Endothelial apoptosis	-	-	-	-	1
	Vitreous body detachment	-	-	-	-	1
Neurological system	Multiple sclerosis	1	-	-	1	-
Cardiovascular system	Cardiomyopathy	1	-	1 (R, gen-sev)	-	-

Table 3: Immunofluorescence antigen mapping results in 26 Greek EB patients.

EB type	EB subtype	Patient No	Intensity of staining pattern	Antibody location in the blister
EBS	EBS, loc	4	plectin: slightly reduced linear labelling	laminin-332, type IV, VII and XVII collagen and a6 integrin subunit: blister's floor b4 integrin subunit and plectin: blister's floor or to both blister's floor and roof keratin 5: blister's roof or both floor and roof keratin 14: both floor and roof
	EBS, gen	4	plectin: reduced linear labelling keratin 5 or 14: normal labeling, focally cytoplasmic labelling of basal keratinocytes	
DEB	DDEB	5	collagen type XVII: slightly reduced or reduced labeling	laminin-332, type IV, VII and XVII collagen and a6 integrin subunit: blister's roof
	RDEB	6	collagen type XVII: significantly reduced or absent	
JEB	JEB, gen-i	1	laminin-332: reduced	laminin-332, type IV and VII collagen: blister's floor a6 integrin subunit and keratin 5: blister's roof type VII collagen: blister's floor
		1	laminin-332: absent	
KS		5	laminin-332, collagen type IV and VII: thickened labeling	

EBS: epidermolysis bullosa simplex; loc: localized; gen: generalized; DEB: dystrophic epidermolysis bullosa; D: dominant; R: recessive; JEB: junctional epidermolysis bullosa; gen-i: generalized-intermediate; KS: Kindler syndrome.

One patient had a history of pyloric stenosis and underwent surgery during neonatal period. Urethral stenosis was recorded in one patient with KS, who underwent surgery.

Phimosis was identified in two patients, one with recessive generalized intermediate DEB (RDEB, gen-i) and one with KS. The last one underwent surgical restoration.

Due to severe pain suffering, 5 patients were treated with oral analgesics.

Extracutaneous manifestations of all EB types are summarized in Table 2. Figure 3 G-H corresponds to characteristic extracutaneous features/complications of different types and subtypes of EB.

Laboratory results

The final diagnosis was based on a combination of clinical observation and IMF staining and MA.

Biopsies for IMF were taken from 26 patients.

Eight cases out of the nine clinically diagnosed with EBS had consistent IMF results. IMF findings in the ninth patient were compatible with KS. Eleven out of thirteen clinically assessed DEB patients had also consistent IMF findings. IMF results for the other two patients were

compatible with JEB, gen-i, caused by laminin-332 deficiency in the one patient and by laminin-5 defective expression in the other patient. In all four clinically suspected KS cases and in one clinically typed EBS, IMF results were compatible with KS and suggestive, although not diagnostic, of KS. IFM results are shown on Table 3.

Table 4: Results of molecular diagnosis in 8 Greek EB patients.

EB type	Gene	Patient	Exon	Mutation 1	Mutation 2
EBS (generalized intermediate or severe)	KRT5	1.	7	p.IIe467Phe	-
DEB (recessive generalized)	COL7A1	2.	19/108	c.2503G>T	c.8026delG
		3.	7/74	c.857_870del14	c.6205C>T
		4.	104/105	c.7759G>A	c.7864C>T
KS	FERMT1/ KIND1	5,6.	5/10	c.676dupC	c.1209C>G
		7,8.	5/7	c.676dupC	c.877T>G

EBS: epidermolysis bullosa simplex; DEB: dystrophic epidermolysis bullosa; JEB: junctional epidermolysis bullosa; KS: Kindler syndrome.

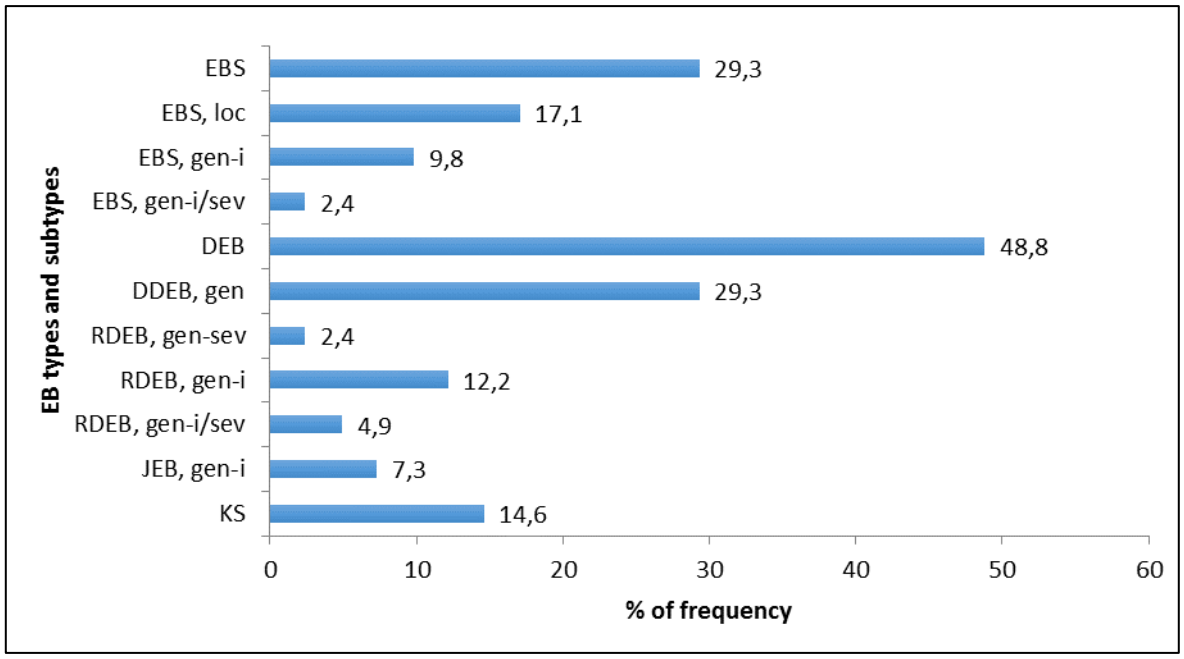

Figure 4: Frequencies of types and subtypes, based on clinical diagnosis, immunofluorescence antigen mapping and molecular analysis in Greek EB patients.

EBS: epidermolysis bullosa simplex; EBS, loc: epidermolysis bullosa simplex localized; EBS, gen-i: epidermolysis bullosa simplex generalized intermediate; EBS, gen-i/sev: epidermolysis bullosa simplex generalized intermediate/severe; DEB: dystrophic epidermolysis bullosa; DDEB, gen: dominant dystrophic epidermolysis bullosa generalized; RDEB, gen-sev: recessive dystrophic epidermolysis bullosa generalized severe; RDEB, gen-i: recessive dystrophic epidermolysis bullosa generalized intermediate; RDEB, gen-i/sev: recessive dystrophic epidermolysis bullosa generalized intermediate/severe; JEB, gen-i: junctional epidermolysis bullosa generalized-intermediate; KS: Kindler syndrome.

Eight patients underwent a formal genetic analysis. DNA analysis was performed in one EBS patient, which detected a heterozygous A>T transversion at nucleotide 1399, in exon 7 of KRT5. In one patient with DEB, 2 heterozygous mutations designated c.2503G>T (exon 19) and c.8026delG (exon 108) were identified in the COL7A1 gene. In another DEB patient, compound heterozygosity for the c.857_870del14 (exon 7) and c.6205C>T (exon 74) in the COL7A1 gene was revealed. The third patient with DEB (with IFM result of possible bullous dermolysis of the newborn variant) presented 2 heterozygous mutations, c.7759G>A (exon 104) and c.7864C>T (exon 105) in the COL7A1 gene. The results of these three DEB patients confirmed the diagnosis of RDEB. Regarding the four patients with clinical and probable IMF diagnosis of KS, MA identified in the first two patients, a compound heterozygosity for the c.676dupC (exon 5) and c.1209C>G (exon 10) mutations and in the other two patients, 2 heterozygous mutations designated c.676dupC (exon 5) and c.877T>G (exon 7) in the FERMT1/KIND1 gene. The first two patients were sisters and shared a similar clinical picture and same

findings in IMF analysis. The same applied for the other two affected patients, who were brothers. MA results confirmed the diagnosis of KS in two families. MA results are shown on Table 4.

In all patients the relevant mutations identified, were compatible with IMF results, except from the patient with possible diagnosis of bullous dermolysis of the newborn variant.

Table 5: Concordance between clinical and laboratory diagnosis of Greek EB patients.

EB type/subtype	Clinical diagnosis	Laboratory investigation	Concordance of clinical and laboratory diagnosis	
	No. of patients	No. of patients	No. of patients	%
EBS	9	8	8	88.9
EBS, localized	5	5	4	80.0
EBS, generalized (intermediate/severe)	4	4	4	100.0
DEB	13	11	11	84.6
DEB, dominant	5	5	5	100.0
DEB, recessive (intermediate/severe)	8	6	6	75.0
JEB				
JEB, generalized intermediate	0	2	0	0
KS	4	5	4	80.0
Total	**26**	**26**	**23**	**88.5**

EBS: epidermolysis bullosa simplex; DEB: dystrophic epidermolysis bullosa; JEB: junctional epidermolysis bullosa; KS: Kindler syndrome.

Based on clinical diagnosis, IMF and MA results, DEB was the most frequent type, since it affected 20 patients (48.8%). Sub-classification of DEB included 12 patients with DDEB, 5 with RDEB, gen-i and 1 with RDEB, gen-sev. Two patients with RDEB presented clinical features that overlapped between severe and intermediate subtypes. Twelve patients (29.3%) were diagnosed with EBS. To further sub-classify EBS, 7 patients presented localized form and 4 gen-i subtype. One patient had overlapping clinical features between intermediate and severe subtype of generalized EBS. Six patients had KS (14.6%) and 3 JEB; all presented gen-i subtype (7.3%). The frequency of each EB type and subtype is shown in Figure 4.

Laboratory investigation was performed in 26 patients (63.4%) out of the 41 presenting with EB. In 23 of them (88.5%), clinical diagnosis matched the laboratory one, based on IMF and MA, that posed the final diagnosis (Table 5). The highest rate of concordance between clinical and laboratory diagnosis was recorded for EBS, followed by the dystrophic type of the disease, and subsequently by KS.

DISCUSSION

This is the first descriptive study, which aimed to present clinical and laboratory data of EB patients in Greece.

Regarding the frequency of EB types, our results are in agreement with the results of studies carried out in Italy, Romania, Croatia, Mexico, Japan, Saudi Arabia and Iran, according to which DEB prevalence is preceded against EBS, followed by JEB.[8-15] However, there are seven other studies, which demonstrate that the most common type is EBS, pursued by DEB. In these studies, JEB follows next, while KS is recorded as the least common type of EB.[16-22]

A possible explanation for the low incidence of EBS could be that patients with this type of disease usually have milder symptoms and they do not often seek for dermatologic consultation. Thus, EBS is often underdiagnosed, leading to a relative lack of cases reported. Recording high rate of KS in contradiction to all other studies is due to EB updated classification, since KS was not included in most of the above-mentioned studies. Furthermore, our patients were of age that would manifest characteristic clinical findings of KS.

As far as the frequencies of EB subtypes are concerned, the results of the present study are similar to those from U.S.A, where the most frequent subtypes were localized EBS (EBS, loc), dominant DEB, generalized (DDEB, gen) and JEB, gen-i. A difference regarding the frequency of generalized intermediate EBS (EBS, gen-i) was found. This was higher than generalized severe EBS in Greece, while the opposite was observed in the study conducted in U.S.A.[14] Our results were also in agreement with the Netherlands' study, where the most common subtypes were EBS, loc, DDEB and JEB, gen. Concerning the second-frequency subtypes, these were EBS, gen-sev and DDEB, gen-sev or RDEB in the Netherlands' study, while in the present study were EBS, gen-i and RDEB, gen.[15] Similarly to our results, a study in Scotland demonstrated that the rarest subtype of EBS was generalized severe and the most common one of DEB was DDEB, gen followed by RDEB subtypes.[17] On the contrary, in one study in Italy and in another study in Croatia, the most frequent subtype of EBS was

generalized following intermediate followed by the localized form and the most common ones of DEB were either DDEB, following gen-sev or following RDEB pursued by DDEB, gen. In agreement with our data on JEB, generalized following intermediate subtype was recorded as the most frequent subtype.[8,11]

Pertaining to EB onset, EBS patients have reported disease onset in childhood. This is justified by the fact that the disease may have appeared with very mild clinical symptoms, to which no adequate attention was given earlier. Patients in the group of DEB have stated childhood period as disease onset. This result does not match with international literature. However, it can be explained since it derived from adult patients, who roughly remembered their disease history and they were mainly based on their parents' narrations. The reference of childhood as disease onset by KS patients, although not consistent with literature, may be attributed to the fact that they had missed parental attention, due to a non-specific appearance and less severe symptoms, not properly evaluated.

Regarding age distribution of patients, our results indicated that the presence of several adult patients in this study is attributed to the inability to diagnose EB in the past, leading to a delay in recognizing such patients. Additional impediments were imposed by the subsequent stigmatization, which inhibited a search for specialized medical approach, and the consequent lack of information related to the disease by both patients and physicians.

The results of clinical cutaneous and extracutaneous summary, as well as of IMF findings in this study are in accordance with literature.[1-4,8,10,12,14,15,20] To our knowledge, patient with JEB, gen-i, who presented demyelinating disease (multiple sclerosis) has not been described in literature until present day.

Unraveling the genotype – phenotype correlation in EB, there is a known moderate correlation among EBS phenotypes and the functional fragment of either KRT5 or KRT14, where the mutation is detected. Mutations in the ligands (L1 and L2) and in section 1A correlate with localized EBS. Mutations in fragments 1A or 2B of KRT5 and KRT14 are often identified in the intermediate EBS. Mutations at the beginning of section 1A, or at the end of 2B in KRT5 and at the beginning of sections 1A or 2B in KRT5 and KRT14 are typical for severe EBS.[23,24]

MA performed in the EBS patient identified a nucleotide change that results in an isoleucine to phenylalanine amino acid substitution at residue 467 (located at the conserved heterologous terminator peptide segment: HTM) of the keratin 5. This genotype was compatible with the clinical picture of EBS, gen-i/sev; nevertheless other mutations in K5 p.IIe467 have also been reported in association with different forms of EBS.[25-27]

In RDEB, most severe forms are caused by biallelic mutations, resulting in null or non-framework mutations from insertions/deletions, one-base changes and ligation. Severity may be related to the position of the termination codon, although the presence of partially functional protein appears to be the most important factor in improving the severity of the disease. Moderate forms are typically due to replacement of glycine in an allele and to an early termination codon in another allele, whereby only a small amount of partially functional protein is synthesized. Less severe forms generally result from other (non-glycine) amino acid substitution and binding mutations, leading to a variety of phenotypes (>700 mutations in the literature).[28-34]

In the three patients with RDEB, the results of MA were in line with their clinical picture. In the first patient, mutation c.2503G>T results in a stop codon at residue 835 of the protein type VII collagen (p.Glu835X). The c.8026delG mutation causes a frame shift, which is predicted to result in a premature termination codon. These findings were compatible with the clinical picture of RDEB, gen-sev. In the second patient, mutation c.857_870del14 is predicted to result in a frame shift with formation of a premature termination codon. The c.6205C>T mutation is predicted to cause the amino acid substitution p.Arg2069Cys in the triple helix domain of collagen VII. Both findings were consistent with the clinical picture of RDEB, gen-i. The p.Arg2069Cys mutation of this patient was also detected in 14 patients with the inverse form of the disease; this was taken into account, as the patient was an infant when MA was performed and had not yet fully developed all of disease's features. In the third patient, mutations c.7759G>A and c.7864C>T are predicted to cause amino acid substitutions p.Gly2587Ser and p.Arg2622Trp, respectively, in the triple helix domain of collagen VII. These findings were compatible with the clinical picture of RDEB, gen-i.

Most mutations associated with KS are null mutations. It has been suggested, that pathogenic missense mutations and in-frame deletions were associated to milder manifestations of the disease and late onset of complications.[35-41]

The four patients with KS in the study presented a common mutation of c.676dupC (exon 5) in the FERMT1/KIND1 gene that results in a frame shift and is predicted to cause a premature termination codon. In the first two patients, the second mutation c.1209C>G mutation causes the formation of a nonsense codon at amino acid residue 403 (p.Tyr403stop); this finding was consistent with the clinical picture of the patients, since they presented a serious disease phenotype. In the other two patients, the second mutation c.877T>G mutation causes the formation of a missense codon at amino acid residue 293 (p.Tyr293Asp), although these patients had a severe phenotype.

Mutation c.676dupC, common in all four patients, has been reported in several other studies recording patients originated from Albania and Pakistan.[37,39,41] Mutation c.676dupC has been recognized as a hotspot and a founder mutation for KS; this was also confirmed by our findings.[37,38,41]

The mutations present in two of these cases were reported in literature for the first time. The above-mentioned mutations c.1209C> G and c.877T> G have not been recorded in literature.[42]

In the present study, overall concordance of clinical and laboratory diagnosis was 88.5%, while the respective percentage of the study in India was 57%.[20] The high rate of concordance is attributed to the small number of infants in the present study and to the fact that all IFM results were conclusive.

Certain limitations to our study should be noted. The study population did not represent the entire EB population in Greece. In addition, the design of the study was observational. Moreover, some recall bias should be taken into account, as patients were asked to provide information on their EB history.

CONCLUSION

Overall, patients with EB in Greece need to struggle against a complex disease under difficult circumstances. The current study is the first report on EB in Greece and constitutes an initial effort for optimizing disease diagnosis and management.

The study highlights the necessity to perform MA in a larger number of patients, in order to draw conclusions about the existing mutations in Greek population and enhance the link between genotype and phenotype characteristics.

To conclude, we would strongly encourage an effort to perform the required laboratory tests in Greece, in order EB families to meet their needs under difficult economic conditions, by saving the cost of performing the aforementioned examinations abroad.

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REFERENCES

1. Fine JD, Bruckner-Tuderman L, Eady RA, Bauer EA, Bauer JW, Has C, et al. Inherited epidermolysis bullosa: updated recommendations on diagnosis and classification. J Am Acad Dermatol. 2014;70(6):1103-26.
2. Intong LR, Murrell DF. Inherited epidermolysis bullosa: new diagnostic criteria and classification. Clin Dermatol. 2012;30(1):70-7.
3. Laimer M, Prodinger C, Bauer JW. Hereditary epidermolysis bullosa. J Dtsch Dermatol Ges. 2015;13(11):1125-33.
4. Murat-Sušić S, Husar K, Skerlev M, Marinović B, Babić I. Inherited epidermolysis bullosa - the spectrum of complications. Acta Dermatovenerol Croat. 2011;19(4):255-63.
5. Castiglia D, Posteraro P, Spirito F, Pinola M, Angelo C, Puddu P, et al. Novel mutations in the LAMC2 gene in non-Herlitz junctional epidermolysis bullosa: effects on laminin-5 assembly, secretion, and deposition. J Invest Dermatol. 2001;117:731–9.
6. Posteraro P, Pascucci M, Colombi M, Barlati S, Giannetti A, Paradisi M, et al. Denaturing HPLC-based approach for detection of COL7A1 gene mutations causing dystrophic epidermolysis bullosa. Biochem Biophys Res Commun. 2005;338(3):1391-401.
7. Castiglia D, Zambruno G. Molecular testing in epidermolysis bullosa. Dermatol Clin. 2010;28(2):223-9.
8. Tadini G, Gualandri L,Colombi M, Paradisi M, Angelo C, Zambruno G, et al. The Italian registry of inherited epidermolysis bullosa. G Ital Dermatol Venereol. 2005;140(4):359-72.
9. Castori M, Floriddia G, De Luca N. Herlitz junctional epidermolysis bullosa: laminin-5 mutational profile and carrier frequency in the Italian population. Br J Dermatol. 2008;158(1):38-44.
10. Danescu S, Has C, Senila S, Ungureanu L, Cosgarea R. Epidemiology of inherited epidermolysis bullosa in Romania and genotype-phenotype correlations in patients with dystrophic epidermolysis bullosa. J Eur Acad Dermatol Venereol. 2015;29(5):899-903.
11. Pavicić Z, Kmet-Vizintin P, Kansky A, Dobrić I. Occurrence of hereditary bullous epidermolyses in Croatia. Pediatr Dermatol. 1990;7(2):108-10.
12. Meester I, Igoucheva O, Alexeev V, South A, Moreno-Treviño MG, Salas-Alanis JC. High concordance between clinical diagnosis of epidermolysis bullosa and immunofluorescence with a small, well-matched antibody panel. Australas J Dermatol 2018;59(1):73-6.
13. Shinkuma S, Natsuga K, NishieW, Shimizu H. Epidermolysis bullosa in Japan. Dermatol Clin. 2010;28(2):431-2.
14. Abahussein AA, al-Zayir AA, Mostafa WZ, Okoro AN. Epidermolysis bullosa in the Eastern Province of Saudi Arabia. Int J Dermatol. 1993;32(8):579-81.
15. Barzegar M, Asadi-Kani Z, Mozafari N, Vahidnezhad H, Kariminejad A, Toossi P. Using immunofluorescence (antigen) mapping in the diagnosis and classification of epidermolysis bullosa: a first report from Iran. Int J Dermatol. 2015;54(10):416-23.

16. Fine JD. Epidemiology of inherited epidermolysis bullosa based on incidence and prevalence estimates from the national epidermolysis bullosa registry. JAMA Dermatol. 2016;152(11):1231-8.
17. Yuen WY, Lemmink HH, van Dijk-Bos KK, Sinke RJ, Jonkman MF. Herlitz junctional epidermolysis bullosa: diagnostic features, mutational profile, incidence and population carrier frequency in the Netherlands. Br J Dermatol. 2011;165(6):1314-22.
18. Kho YC, Rhodes LM, Robertson SJ, Su J, Varigos G, Robertson I, et al. Epidemiology of epidermolysis bullosa in the antipodes: the Australasian Epidermolysis Bullosa Registry with a focus on Herlitz junctional epidermolysis bullosa. Arch Dermatol. 2010;146(6):635-40.
19. Horn HM, Priestley GC, Eady RA, Tidman MJ. The prevalence of epidermolysis bullosa in Scotland. Br J Dermatol. 1997;136(4):560-4.
20. Hiremagalore R, Kubba A, Bansel S, Jerajani H. Immunofluorescence mapping in inherited epidermolysis bullosa: a study of 86 cases from India. Br J Dermatol. 2015;172(2):384-91.
21. Hernandez-Martín A, Aranegui B, Escámez MJ, de Lucas R, Vicente A, Rodríguez-Díaz E, et al. Prevalence of dystrophic epidermolysis bullosa in Spain: a population-based study using the 3-source capture-recapture method: evidence of a need for improvement in care. Actas Dermosifiliogr. 2013;104(10):890-6.
22. McKenna KE, Walsh MY, Bingham EA. Epidermolysis bullosa in Northern Ireland. Br J Dermatol. 1992;127(4):318-21.
23. Müller FB, Küster W, Wodecki K, Almeida H Jr, Bruckner-Tuderman L, Krieg T, et al. Novel and recurrent mutations in keratin KRT5 and KRT14 genes in epidermolysis bullosa simplex: implications for disease phenotype and keratin filament assembly. Hum Mutat. 2006;27(7):719-20.
24. Vahidnezhad H, Youssefian L, Saeidian AH, Mozafari N, Barzegar M, Sotoudeh S, et al. KRT5 and KRT14 Mutations in Epidermolysis Bullosa Simplex with Phenotypic Heterogeneity, and Evidence of Semidominant Inheritance in a Multiplex Family. J Invest Dermatol. 2016;136(9):1897-901.
25. Rugg EL, Horn HM, Smith FJ, Wilson NJ, Hill AJ, Magee GJ, et al. Epidermolysis bullosa simplex in Scotland caused by a spectrum of keratin mutations. J Invest Dermatol. 2007;127(3):574-80.
26. Pfendner EG, Sadowski SG, Uitto J. Epidermolysis bullosa simplex: recurrent and de novo mutations in the KRT5 and KRT14 genes, phenotype/genotype correlations, and implications for genetic counseling and prenatal diagnosis. J Invest Dermatol. 2005;125(2):239-43.
27. Irvine AD, McKenna KE, Bingham A, Nevin NC, Hughes AE. A novel mutation in the helix termination peptide of keratin 5 causing epidermolysis bullosa simplex Dowling-Meara. J Invest Dermatol. 1997;109(6):815-6.
28. Wertheim-Tysarowska K, Sobczyńska-Tomaszewska A, Kowalewski C, Skroński M, Swięćkowski G, Kutkowska-Kaźmierczak A, et al. The COL7A1 mutation database. Hum Mutat. 2012;33(2):327-31.
29. Varki R, Sadowski S, Uitto J, Pfendner E. Epidermolysis bullosa. II. Type VII collagen mutations and phenotype-genotype correlations in the dystrophic subtypes. J Med Genet. 2007;44(3):181–92.
30. Murata T, Masunaga T, Ishiko A, Shimizu H, Nishikawa T. Differences in recurrent COL7A1 mutations in dystrophic epidermolysis bullosa: ethnic-specific and worldwide recurrent mutations. Arch Dermatol Res. 2004;295(10):442-7.
31. Gardella R, Zoppi N, Zambruno G, Barlati S, Colombi M. Different phenotypes in recessive dystrophic epidermolysis bullosa patients sharing the same mutation in compound heterozygosity with two novel mutations in the type VII collagen gene. Br J Dermatol. 2002;147(3):450-7.
32. Gardella R, Castiglia D, Posteraro P, Bernardini S, Zoppi N, Paradisi M, et al. Genotype-phenotype correlation in italian patients with dystrophic epidermolysis bullosa. J Invest Dermatol. 2002;119(6):1456-62.
33. Murata T, Masunaga T, Shimizu H, Takizawa Y, Ishiko A, Hatta N, et al. Glycine substitution mutations by different amino acids in the same codon of COL7A1 lead to heterogeneous clinical phenotypes of dominant dystrophic epidermolysis bullosa. Arch Dermatol Res. 2000;292(10):477-81.
34. Tamai K, Murai T, Mayama M, Kon A, Nomura K, Sawamura D, et al. Recurrent COL7A1 mutations in Japanese patients with dystrophic epidermolysis bullosa: positional effects of premature termination codon mutations on clinical severity. Japanese Collaborative Study Group on Epidermolysis Bullosa. J Invest Dermatol. 1999;112(6):991-3.
35. Youssefian L, Vahidnezhad H, Barzegar M, Li Q, Sotoudeh S, Yazdanfar A, et al. The Kindler Syndrome: A Spectrum of FERMT1 Mutations in Iranian Families. J Investigative Dermatol. 2015;135(5):1447–50.
36. Has C, Castiglia D, del Rio M, Diez MG, Piccinni E, Kiritsi D, et al. Kindler syndrome: extension of FERMT1 mutational spectrum and natural history. Hum Mutat. 2011;32(11):1204-12.
37. Mansur AT, Elcioglu NH, Aydingöz IE, Akkaya AD, Serdar ZA, Herz C, et al. Novel and Recurrent KIND1 Mutations in Two Patients with Kindler Syndrome and Severe Mucosal Involvement. Acta Derm Venereol. 2007;87(6):563-5.
38. Burch JM, Fassihi H, Jones CA, Mengshol SC, Fitzpatrick JE, McGrath JA. Kindler syndrome: a new mutation and new diagnostic possibilities. Arch Dermatol. 2006;142(5):620–4.
39. Thomson MA, Ashton GH, McGrath JA, Eady RA, Moss C. Retrospective diagnosis of Kindler

syndrome in a 37-year-old man. Clin Exp Dermatol. 2006;31(1):45-7.
40. Herz C, Aumailley M, Schulte C, Schlotzer-Schrehardt U, Bruckner-Tuderman L, Has C. Kindlin-1 is a phosphoprotein involved in regulation of polarity, proliferation, and motility of epidermal keratinocytes. J Biol Chem. 2006;281(47):36082–90.
41. Ashton GH, McLean WH, South AP, Oyama N, Smith FJ, Al-Suwaid R, et al. Recurrent mutations in kindlin-1, a novel keratinocyte focal contact protein, in the autosomal recessive skin fragility and photosensitivity disorder, Kindler syndrome. J Invest Dermatol. 2004;122(1):78–83.
42. Youssefian L, Vahidnezhad H, Uitto J. Kindler Syndrome. In: Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LJH, Bird TD, Ledbetter N, Mefford HC, Smith RJH, Stephens K, editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2018. 2016.

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