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REVIEW

An Update on the Genetic Aspects in Congenital Ventricular Septal Defect

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Ventricular septal defects (VSDs) are the most common type of heart malformation and may occur like a part of a syndrome or as an isolated form. Clinical manifestations are related to the interventricular flow, which is determined by the size of the defect. Aiming at the identification of genetic causes is important in both syndromic and non-syndromic forms of VSD, to estimate the prognosis and choose the optimal management. Other reasons of the identification of genetic factors in the etiopathogenesis include the assessment of the neurodevelopmental delay risk, recurrence in the offspring, and association with extracardiac malformations. The diagnostic process has been improved, and currently the use of the most suitable and accessible technique in the clinical practice represents a challenge. Additional advantages in genetic testing were brought by next-generation sequencing technique, various testing panels being available in many laboratories.

Keywords: ventricular septal defects, genetic analysis, updates

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Introduction

According to the current studies, the most common congenital anomalies at birth, are represented by congenital heart diseases (CHDs). The prevalence of CHDs is between 2% and 3% [1,2]. It is reported that 20-30% of CHDs are associated with non-cardiac anomalies, defined as syndromic CHD, and approximately 70-80% of CHDs are an isolated form of congenital anomalies, also called non-syndromic CHDs [3].

The ventricular septal defects (VSDs) can be present as part of other anomalies, like tetralogy of Fallot or diagnosed as isolated form, in approximately 0.4% of patients [4]. Cornoet et al. reported in their study, performed on patients aged between the first week of life and three decades of life, the frequency of 32.1% of VSDs in CHDs patients [5].

Genetic and environmental factors play an important role in the pathogenesis of CHD. The genetic variants have a major role in the development of these anomalies being identified in up to 30% of CHDs [6,7]. Based on sequencing technology, in a large study on CHDs, it was identified that 8% of mutations are de novo, and approximately 2% are inherited [8]. On the other side, in 2% of cases, the environmental factors are presumed to be the etiology for CHDs [7]. Similar, the genetic etiology of VSDs is complex and heterogeneous. Furthermore, the genetic anomalies in VSDs can be classified in different categories depending on the amount of the genetic material involved: (1) abnormal number of chromosomes (aneuploidy), (2) large deletions/duplications, (3) copy number variations (CNVs) and (4) single gene mutations. According to literature, large chromosomal abnormalities and aneuploidies are detected in 9% of patients with CHDs, CNVs in 3-10% of patients and small genetic variations in 4% of cases [7,8]. On the other hand, in patients with VSDs, chromosomal anomalies are reported in 36.5% of cases, CNVs in 16.9% and small genic variations in 2.8% [9-11].

Depending on the type of the anatomical severity in patients with isolated form of CHD, overall survival rate after cardiac surgery was improved in the last decades, but long-term complications remain a major problem [12]. Besides that, the short-term complications after surgery have a high incidence and are represented by cardiac failure, renal complications, lung, brain and vascular complications [13]. It was reported that long-term survival after surgery is strongly associated with the presence of CNVs or single nucleotide polymorphisms (SNPs) [14].

New strategies, consisting of personalized medicine according to the needs of each patient with CHD, are based on the use of the most efficient target therapies and improve the long-term outcome [15,16]. An experimental study performed on mice models with RASopathies, demonstrated that after administration of Rapamycin and threonine protein kinase inhibitors, an improvement on cardiac hypertrophy and left ventricular function was obtained [17]. Moreover, new genome editing techniques could be theoretically a therapeutic method based on their capacity to recognize and restructure the target sequence [18]. A recent publication sustains this perspective in inheritable mutations and describes the correction of a gene mutation in human embryos with hypertrophic cardiomyopathy [19]. Induced Pluripotent Stem Cells (iPSC) represent another important resource for study in CHD and allows to create human models of disease. Several experimental studies using human iPSC derived cardiomyocytes allow generating cardiac tissue like endothelial cells

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and vascular mural cells with safely and with feasible results in children with CHD [20]. Additionally, to generate disease model in iPSC the most highly efficient technique is CRISPR/Cas9 which can induce specific mutation and provides the possibility to study the underlying cause of human disease [21].

As a consequence, the advantages in genetic research need to be introduced into the clinical practice of CHDs and should evaluate the possible major risks to estimate the overall survival rate and be a part of future decision-making in assessment for these patients.

In conclusion, the results of genetic research need to be introduced in the clinical practice of CHDs, however, the potential risks must be evaluated, which should be a part of future decision-making in these patients. An additional benefit of clarifying the genetic origin is the possibility of recurrence risk assessment in the family in inherited forms.

For practical considerations, it is essential to understand the limitations and benefits of the existing genetic techniques and apply the most efficient genetic analysis in the investigation of CHDs patients [22].

We performed this study to summarize the latest genetics research and highlight the potential applications of genetic aspects in patients with VSDs.

Chromosomal aneuploidy and large deletions/duplications

The aneuploidies that survive to term include trisomy of 13, 18 and 21 and the heterosomal monosomy Turner syndrome. Clinical management of aneuploidies requires an accurate assessment including the cardiac anatomy, trisomy 21 being frequently associated with VSD.

Down syndrome (trisomy 21) is frequently associated with CHDs. In this syndrome, the prevalence of CHDs is between 40% and 50% [23]. It is reported that in 50% of cases, the mortality rate of Down syndrome patients is directly associated with CHDs and the associated complications [23]. Atrioventricular septal defects (ASVDs) are reported in approximately 30% of cases, followed by VSDs with a variable percentage between 21.5 - 35% [24]. Maslen et al. [24] reported the highest incidence of VSDs (35%) on Down syndrome patients and suggest that Down syndrome patients with hemodynamically significant lesion must be surgically treated to prevent the development of pulmonary vascular lesion, respectively Eisenmenger's syndrome [24]. The overall survival rate is directly associated with Eisenmenger's syndrome development, and according to literature, the prognosis of pediatric Eisenmenger's syndrome patients is influenced by mutations in the *ACVRL1* and *TBX4* genes [25].

Jacobsen syndrome is caused by the deletion in the long arm of chromosome 11, the length of the deletion being estimated at 7-16 Mb, which can include the telomeric region as well [26]. Clinical features include cognitive impairment, platelet dysfunction, CHDs, ophthalmological, gastrointestinal, and genitourinary anomalies [27]. Cardiovascular features are present in more than 50% of patients and one-third of them have a membranous VSD [23].

Also, VSDs are frequently identified in syndromes with aneuploidies, including trisomy 18, trisomy 13, and Klinefelter syndrome [28]. Other chromosomal abnormalities such as deletion in 4p24, duplications in 16p13, 22q11, 8q21 and partial monosomy of chromosome 18 were identified in a recent study that evaluated 151 cases diagnosed prenatally with VSDs [29].

Copy number variations

The presence of CNVs, such as chromosomal microdeletions or microduplications, play an important role in genetic variability. There are several CNVs in the human genome, but only a small part of them is being considered pathogenic for a disease. The rest are considered benign or with an unknown or uncertain significance. The pathogenic CNVs for non-syndromic CHDs were reported by Carey et al. being present in 10 to 20% of patients [30]. In the last decades, the number of pathogenic CNVs for CHDs increased as a result of the research studies published. Future studies with a large number of patients are needed because a high number of potential pathogenic or uncertain significant CNVs are currently being reported. Russel et al. reported that potential pathogenic CNVs can be considered in patients with CHD in the absence of other extracardiac malformations and are frequently encountered compared with the healthy population [31]. Besides the fact that not all deletions are considered pathogenic, currently it is accepted that a deletion is more frequently pathogenic compared to a duplication, having more often a phenotypic impact and being associated with a genetic syndrome. Herein, we describe the pathogenic CNVs associated with different syndromes which include VSDs.

Deletion 1p36 is the most common deletion in humans. Clinically the syndrome caused by a 1p36 deletion associates: developmental delay, vision problems, hearing loss, distinctive facial features, brain anomalies, CHD, cardiomyopathy, and renal anomalies. This deletion was reported frequently in patients with VSDs. The clinical features caused by 1p36 deletion syndrome are caused by the pathogenic deletion of the *RERE*, *CASZ1*, *ECE1*, and *LUZP1* genes [32].

22q11.2 microdeletion syndrome (DiGeorge syndrome) is characterized by hypoplasia of the thymus and parathyroid glands, cardiac malformation, and facial dysmorphisms.

Moreover, in chromosome 22, the deletion of a region in the long arm is described in literature to have the main role in the clinical appearance in 10 to 20% of cases with DiGeorge syndrome [33]. The frequency of the deletion confirmation increased to 45% in patients who also present VSDs and aortic arch anomaly [33]. Clinical features of this syndrome may also be a consequence of a small deletion in the short arm of chromosome 10 [23]. Also, it is known that maternal comorbidities, like diabetes mellitus,

or behavior factors like alcohol use, are directly involved in the appearance of various clinical features in the offspring [23, 34]. In 22q11 deletion syndrome, cardiovascular disease is one of the most common features, and it is estimated that 10% of patients with VSDs present this deletion [34].

Currently, for this genetic locus, reciprocal CNVs are described. Duplication of 22q11.2 region is associated with a variable disorder with a phenotype which includes learning disability and heart defects in 15% of cases, but opposite to deletion, the duplication syndrome had a protective effect for schizophrenia [35, 36].

An atypical phenotype of 22q11.2 microdeletion syndrome is described in cases with a mutation outside this region, in *GP1BB* gene. A mutation with loss of function of *GP1BB* is reported in Bernard-Soulier syndrome (BSS) being associated with a higher risk of bleeding during surgery or other invasive procedures [33].

1q21.1 deletion includes clinical features such as microcephaly, intellectual disability, short stature, eye abnormality, and less commonly associated skeletal malformation, genitourinary anomalies, and CHDs [37]. There are many types of CHD described in 1q21.1 deletion, including VSDs. One possible etiology for VSD is a mutation the *GJA5* gene (1q21.1) which encodes for connexin 40 - a cardiac gap junction protein [38]. Opposing, the reciprocal CNVs, such as **1q21.1 duplication,** are associated with the same features, but the opposing phenotype includes macrocephaly [35,37,38].

8p23.1 deletion frequently associate mild to moderate intellectual disability, behavioral problems, microcephaly, diaphragmatic hernia, and CHDs. *GATA4* encodes a transcription factor involved in heart development, and its absence/imbalanced expression due to 8p23.1 deletion often leads to CHDs (>90%) [39].

Small genetic variation - Syndromic single gene disorders

Alagille Syndrome, caused by a mutation in *JAG1* and *NOTCH2* gene, is an autosomal dominant syndrome characterized by mild motor and intellectual development delay, prominent forehead, hypertelorism, chronic cholestasis or even liver failure, orthopedic complications, and cardiovascular features. Most frequently, in approximately 60% of cases with Alagille syndrome branch pulmonary stenosis is described followed by arterial narrowing and structural cardiac defects (tetralogy of Fallot, VSDs and ASDs) [23, 40]. Some features, mainly the liver failure is influenced by specific types of mutations in *JAG1* and *NOTCH2* genes, but this mechanism is not similar for CHDs. Considering Alagille syndrome is recommended in the presence of characteristic cardiovascular features [41,42].

Holt-Oram syndrome is frequently caused by the presence of the null allele of the *TBX5* gene and in 17% of cases by a heterozygous variant of the same gene. In less than 1%, the Holt Oram syndrome is caused by a partial or complete deletion of the *TBX5* gene [43]. The phenotype consists mostly of two common features: (1) radial ray abnormalities and (2) CHDs. In 75% of cases, a cardiac anomaly is confirmed and involves atrial or ventricular septum defects and conduction system abnormalities [44].

Char syndrome is caused by mutations in the *TFAP2B* gene, and in 50% of cases a heterozygous variant of this gene is detected with familial inheritance [45]. Clinical aspects consist of flat midface, broad nasal tip, flat nasal bridge, hypertelorism, hand abnormalities with a shorter middle section of the fifth finger and patent ductus arteriosus as the most common heart anomaly [46]. Also, heart defects such as VSDs and complex CHDs have been reported as associated anomalies in Char syndrome [47].

CHARGE syndrome. In most cases causal pathogenic variants of the *CHD7* gene, and large deletions in this gene were identified [48]. The phenotype of this condition includes coloboma, heart defect, choanal atresia, delay in growth/development, genital, and ear anomalies. The CHDs reported in CHARGE syndrome are heterogeneous; conotruncal defects and septal defects are the most frequently described CHDs [49]. The study performed by Corsten et al. described truncating variants of *CHD7* in 80% of CHD patients and missense and splice site variants in 58% of patients, identified in subjects with CHD with the presence of both genetic anomalies [50].

Kabuki syndrome in 75% of cases is caused by pathogenic variants of the *KMT2D* gene and in approximately 5% in the *KDM6A* gene with an X-linked and autosomal dominant inheritance [51]. Common recognizable features are intellectual disability, long palpebral fissures, depressed nasal tip, arched eyebrows, large dysplastic ear, and cleft palate. Coarctation of the aorta, atrial septal defect, and VSDs are the most common CHDs described in Kabuki syndrome [52].

Noonan syndrome is a part of RASopathies group disorders with autosomal dominant inheritance. In 50% of cases multiple missense variants in the *PTPN11* gene were reported, and in other 30% of cases different genes variants involved in RAS pathways were described [53]. Facial features are age-dependent, and it is difficult to correlate the genotype with the phenotype, even that in 80% of cases, structural eye abnormalities, hypothyroidism, and short stature were described. A large number of patients with Noonan syndrome (80% to 90%) have CHDs, of which 40% cases have pulmonary stenosis [54]. Other CHDs are tetralogy of Fallot, atrial septal defect, and arterial defects [54]. In Noonan syndrome cases with septal defects commonly pathogenic variants in the *SOS1* gene were described [55].

Other syndromes commonly associated with VSD

In Table 1 are enlisted syndromes frequently associated with VSD, including the genes usually involved and other common clinical features.

Table 1. Other syndromes frequently associated with VSD

Syndrome	Gene(s)	Loci/Region	Other cardiac disease	Other Clinical Features	References
Cornelia de Lange	*NIPBL* *SMC1A* *HDAC8* *SMC3* *RAD21*	5p13 Xp11.22 Xp13.1 10q25.2 8q24.11	PS, ASD, AoCo, HCM	Growth retardation, intellectual disability, gastroesophageal reflux	56
Williams-Beuren	*ELN*	7q11.23	AS, PS, Systemic hypertension, MVP, AoCo	Facial features, intellectual disability, difficulty in visual-spatial tasks	57,23
Costello	*HRAS*	11p13.3	Arrhythmia, HCM, PS, aortic dilatation	Growth retardation, intellectual disability, rhabdomyosarcomas	58
Rubinstein-Taybi	*CREBBP* *EP300*	16p13.3 22q13.2	MVD, ASD, TOF, PS, MS	Growth retardation, microcephaly, micrognathia, broad halluces, intellectual disability	59
Beckwith-Wiedemann	*CDKN1C*	11p15.5	ASD, PS	CNS malformations, abdominal wall defects, macroglossia	60
Smith-Lemli-Opitz	*DCHR7*	11q12-13	Complex CHD, ASD, PDA,	Hypotonia, CNS malformations, hypospadias, adrenal insufficiency	61
Nance-Horan	*NHS*	Xp22.13	TOF, PDA	Epileptic encephalopathy, congenital cataract, dental anomalies, hypotonia	62
Bardet-Biedl	*BBS1* *BBS10*	4q27	AS, PS, ASD PDA	Obesity, anosmia, polydactyly, retinitis pigmentosa, anosmia	23

PS = pulmonary stenosis; ASD = atrial septal defect; AoCo = aortic coarctation; HCM = hypertrophic cardiomyopathy; AS = aortic stenosis; MVP = mitral valve prolapse; MVD = mitral valve dysplastic; TOF = tetralogy of Fallot; ASD = atrial septal defect; MS = mitral stenosis; PDA = patent ductus arteriosus; CNS = central nervous system.

Non-syndromic single gene disorders

Genetic aspects of non-syndromic single gene disorders of VSDs are complex. A new correlation between the phenotype, genotype, and the prognosis of the patients is needed, especially in those cases where the etiology remains unknown. We admit that these correlations seem to be very difficult to be realized because of a multifactorial mechanism, with more than 50 genes involved in heart development [63]. In a study performed by Pang S et al. mutations in the *GJA1*, *SMAD2* (encode proteins involved in cell signaling), *TBX20*, *TBX5*, *GATA4*, *GATA6, CITED2*, (encode transcription factors involved in cardiac development) were described as pathogenic for VSDs [64].

The most important connexin proteins is connexin-43 (Cx43) encoded by the *GJA1* gene (MIM 121014) expressed mainly in the heart and liver [65]. Kosuke et al. identified a missense de novo mutation in the *GJA1* gene, c.145C>G in one patient with VSD and syndactyly type III [66]. Wang et al. screened 418 CHD patients from which 44.5% were diagnosed with VSD and they identified three heterozygous missense mutations (c. 458G>A, c. 781G>T, c.968 C>T) in the *GJA1* gene [67]. In the *SMAD2* gene (MIM 601366), two de novo point mutation involved in methylation pathway were identified by Zaidi et al. [68].

TBX20 (MIM 606061) is implicated in cardiovascular morphogenesis. Anomalies of this process were associated with mutations in this gene. In a study performed by Rai-Tai et al. [69], a loss-of-function mutation (c.820A>T) in a familiar form of CHD with autosomal dominant inheritance was described [60]. Akiko et al. identified a novel mutation (c.991A>G) in exon 7 of the same gene in one patient with VSD and in other two patients with the same pathology they identified a variant in exon 8 (c.791G>A) of *TBX5* gene [70].

Another gene involved in CHDs, specifically in VSD1, is *GATA4* (MIM 600576), anomalies of this gene being reported in the familial form of CHD, especially the pathogenic variant c.899 A>C [71]. Additionally, an experimental study on mice models confirmed that mutations in *GATA4* can be involved in CHD pathogenesis [72]. Variants in the *GATA6* (MIM 601656) gene, a member of GATA gene family, have also been reported in familial CHDs [73]. Additionally, Allen et al. identified a de novo heterozygous inactivating mutation in 50% cases with pancreatic agenesis, and 90% of them were also diagnosed with CHDs [74]. Other findings revealed the potential role of mutations of the *CITED2* (MIM 602937) gene in CHDs, especially in VSD2; Xu et al. described 3 mutations in this gene (c.550G > A, c.573-578del6, c.574A > G) with possible pathogenetic implications [75].

Mutations in *NKX2-5* (MIM 600584) are associated with VSD3 and in a study performed in 150 Egyptian children with CHDs it is reported that two polymorphisms (rs2277923, rs28936670) may be involved in the pathogenesis [76].

Technical approach

Briefly, we can summarize the genetic techniques in three categories: (1) conventional karyotype used to identify aneuploidy, large deletions or duplications, translocations, etc.; (2) chromosomal microarray/array comparative genomic hybridization (aCGH), Fluorescent in situ hybridization (FISH) and Multiplex Ligation-dependent Probe Amplification (MLPA) useful for identification of CNVs of the DNA such as microdeletions, microduplications but also for aneuploidies, small supernumerary chromosomes and point mutations [77]; (3) gene testing, sequencing and next generation sequencing with whole exome sequencing (WES) or whole genome sequencing (WGS).

New platforms for genetic testing have expanded options with an increased resolution. Thus, choosing the best type of analysis to identify the cause of disorders can be a challenge and can lead to waste of the resources. It is confirmed that some techniques, including WGS and WES are essential tools in diagnosis and establishing the pathogenesis in CHDs and VSDs, but with a high cost for a routine practice [78].

According to the resolution of genetic tests, the pathogenic mechanisms in VSDs can be detected using all these methods. However, based mainly on new higher resolution techniques (WES, WGS), the number of pathogenic and probably pathogenic variants increased. It is important to determine the genetic etiology for VSD patients to offer additional information about diagnosis and evolution of the disease. Moreover, only after identification of the pathogenic variant, the genetic counseling for the patient and his family can be adequate. Furthermore, based on this idea, Kelle et al. analyzed 152 family members of patients with Hypoplastic left heart syndrome caused by known mutations, and they identified 11% of family members with mutations and cardiovascular malformations that have been previously undiagnosed [79]. Also, the benefit for genetic testing is higher for patients entering reproductive age because the recurrence risk is described among 3% to 8% [80].

The cytogenetic investigation in VSDs, similar with CHDs, must be considered in patients with specific phenotype for a chromosomal syndrome; patients with developmental delay, dysmorphic features or multiple malformation syndrome; patients with abnormal prenatal screening (echocardiography) which revealed a major visceral malformation or a major cardiac anomaly.

In the detection of chromosomal abnormalities including CNVs, Monteiro et al. proposed the MLPA technique as a first genetic test based on low costs, efficiency in detection, and because it is easier to perform and analyze [81]. Additionally, it has been proved that in syndromic CHD cases with abnormal cytogenetic analysis, the MLPA was a fast and efficient method for establishing the origin of a small supernumerary marker chromosome [82].

Based on a large number of genes involved in non-syndromic VSDs patients, the sequencing techniques are increasingly used and according to Pierpont et al. there are several hundreds of genes which contribute to approximately 10% of cases with severe CHD [23]. Recommendations for WGS or WES techniques in VSDs are based on (1) multiple genes that can cause or contribute to VSD; (2) the possible existence of de novo variants associated with syndromic VSD and inherited variants for non-syndromic type [83]; (3) the fact that both sporadic and inherited sequence variants can cause VSD; (4) the heterogeneity of the phenotype (type and dimensions of VSD) caused by different variants in the same gene; (5) incomplete penetrance and inheritance especially in familial form of VSD [23].

Conclusions

Based on the new molecular techniques, the knowledge in the pathogenesis of VSD increased and had a major effect on medical care and genetic counseling. Currently, next-generation sequencing panels are available, and CNVs detection is accessible for many laboratories. All the techniques mentioned are useful for syndromic and non-syndromic VSD patients' diagnosis, increasing the accuracy of the diagnosis, but each of these techniques has different benefits and limitations. The identification of the genetic variants in some cases also had direct implications in the clinical care of the VSDs patients, in the recognition of other extracardiac anomalies, estimation of the recurrence family risk and improving the decision of the medical therapy.

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Authors' contribution

GAC designed the study, wrote the manuscript and approved the final manuscript. FT review the manuscript and edit the final version. MA designed the study, read and approved the manuscript. KBB read the draft, revised the manuscript. CB designed the study, performed the critical revision and approved the final version.

Conflict of interest

None to declare.

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REVIEW

Considerations on the Use of Organic Substances in Chemical Peels: A Systematic Review

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Chemical peel is a dermato-cosmetic procedure used to destroy and remove, in a controlled manner and under the supervision of the specialists, the degraded parts of the skin, in order to allow acceleration of the skin regeneration process. Based on their depth of skin penetration chemical peels are classified into superficial, medium and deep peels. The substances used in the chemical peels differ from each other depending on the effective action depth. Different peel agents with an appropriate peel depth should be selected based on the problem to be treated, considering also the nature of skin pathology. To achieve the best results other factors, such as skin type and characteristics, region to be treated, safety issues, healing time, and patient adherence, should also be considered. The present review focuses on the particularities of the substances used in various peel types, highlighting recent advances in chemical peel technology and explaining suggested application of certain substances in different peel types.

Keywords: chemical peels, peel agents, alpha hydroxy acids, betha hydroxy acids, trichloracetic acid, phenols

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Introduction

Peeling is a dermato-cosmetic method used to remove dead cells from the skin surface in order to stimulate the function of the ones from the deeper layers of the skin. Most peeling techniques do not dramatically alter the architecture of the skin, because they mainly work at the level of the epidermis (superficial layer of the skin), but there are also more aggressive methods by which even the structure of the skin proteins can be altered [1].

Peels can be classified into two categories, depending on the mechanism of action: mechanical peels and chemical peels. Mechanical peels use the exfoliating action of the granules and abrasive particles contained in various creams or masks, which by easy and superficial massage removes the dead cells from the epidermis. Chemical peels are method of rejuvenation for facial care that involves exfoliation of the epidermis with the help of chemical substances; depending on the particularities and problems of the skin, it can be achieved using different substances [2].

Chemical peels can be classified in three categories [3,4]:

– superficial peels - the mildest form of peel, has the advantage of being able to be applied for all skin types; this method uses a weak acid (diluted acids, most often glycolic acid);
– moderate or medium peels - substances used in this type have the property of penetrating deeper into the skin than those used in superficial peels and cause the appearance of second degree burns (most often trichloroacetic acid peels);
– deep peels - it also determines the appearance of a second degree burns, but it differs through the substances that are used, in this case it is preferred the application of the solution based on phenols or higher concentration of trichloroacetic acid; this method is recommended only for people with serious dermatological problems and should only be performed by a dermatologist.

Superficial peels, which penetrate only the epidermis, can be used to improve treatment of different skin conditions, including acne, melasma, photodamage, dyschromias and actinic keratoses. Medium peels, that reach the papillary dermis, may be used for multiple solar keratoses, dyschromia, superficial scars, and pigmentation disorders. Deep peels, that affect reticular dermis, may be used for severe photoaging, deep wrinkles or scars [2,5].

The agent used in the chemical peels should be selected taking into account a series of variables such as application method, application area, contact time with the skin, skin type, medical history and patient's lifestyle and the formulation and concentration of the chemical agent. Among the factors that influence the penetration capacity of the tissues by chemical substances are the acidity of the peel agent, the number of layers applied, and the time given for the action, before the substance is neutralized. The depth of penetration is determined by the type, concentration and pH of the peel agent [6].

The mechanism of action of acids used in chemical peeling consists in the coagulation of dermal and epidermal proteins. In superficial peeling the necrosis occurs up to the level of the basal layer of the epidermis, while in me-

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dium and deep peeling the necrosis extends to the papillary and reticular dermis. The necrosis process is followed by re-epithelialization and improvement of the skin's appearance [5,7].

The current review presents the main chemical substances used in chemical peels used in cosmetics and aesthetics focusing on the peculiarities of substances used in various peeling types, highlighting recent developments in chemical peeling technology.

Alpha hydroxy acids (AHA)

AHA are a class of hydroxy-carboxylic acids in which a hydroxyl group is attached at the alpha position of the carboxyl group. AHAs are naturally occurring acids found in different fruit such as apples (malic acid), citrus (citric acid), grapes (tartaric acid), bitter almond (mandelic acid), sugar cane (glycolic acid) or milk (lactic acid) [8]. The chemical structures of AHA used in peels are presented in Figure 1.

Topical application of AHAs (in concentration higher than 25%) decreases the cohesion of corneocytes immediately above the granular layer, resulting in the stratum corneum detachment and layer desquamation from the first 24 hours of treatment. After application of an appropriate concentration of AHA on an aging skin, histological changes occur without edema or inflammation of the skin. Due to their stable molecular structure AHAs are not considered potential allergens, consequently their preliminary testing is not required before the peel operation [9].

The most commonly used acid in superficial peels is **glycolic acid** (2-hydroxyethanoic acid, hydroxyacetic acid). Glycolic acids have the smallest molecular size among all AHAs, it is a highly hydrophilic molecule and has the greatest bioavailability among AHAs as it penetrates the skin easily. It is usually used in concentrattions between 25% - 70% at a of pH 2-3; tolerance is generally good. The peel will be more intense with the increase of concentration and at lower pH values. Glycolic acid has a keratolytic, germinative layer and a fibroblast stimulating action. At low concentrations (<25%) produces only superficial exfoliation, at concentrations of 25-50% causes the discontinuity of keratinocytes, and at concentrations of 50-75% epidermolysis [10].

Glycolid acid peel is a minimally invasive cosmetic procedure commonly used for treatment of acne, photoaging and pigmentary disorders such as melasma. It can be used in a treatment every 3-4 weeks, in a total of 4 to 6 applications; based on the tolerance and results of previous applications, application time can be gradually increased. Glycolic acid reduces cellular cohesion at the lowest level of the stratum corneum, exfoliating dead cells, encouraging younger, brighter cells to the surface; this superficial exfoliation guarantees a reduction in comedonal acne, pore size, and acne lesions [9,10].

Lactic acid (2-hydroxypropanoic acid) is structurally almost identical to glycolic acid except for an additional methyl group. Lactic acid has a lower pH than glycolic acid, therefore lower concentrations are used to achieve an equal depth of keratocoagulation. Lactic acid peels are best suited for people with very sensitive skin that may not tolerate the slightly more aggressive glycolic acid peel [9].

Lactic acid improves the photo-aging of the skin, being effective in treating post-inflammatory hyperpigmentation lesions, melasma and solar lentigo. Lactic acid inhibits melanin synthesis by inhibiting tyrosinase activity. The treatment starts with low concentrations of lactic acid, the concentrations is increased progressively to avoid post-peel hyperpigmentation of the skin and irritation [11].

Other AHA including mandelic acid, are used less frequently in peels than glycolic acid or lactic acid.

Pyruvic acid (α-ketopropionic acid) is an alpha-keto-acid used as a medium chemical peel agent in inflammatory acne, moderate acne scars, greasy skin, actinic keratosis. It causes a slight peel of the corneal layer of the epidermis; the erythema disappears within a few minutes after the procedure and does not cause post-procedure hyperpigmentation spots. Pyruvic acid can be associated with lactic acid for the treatment of melasma [12].

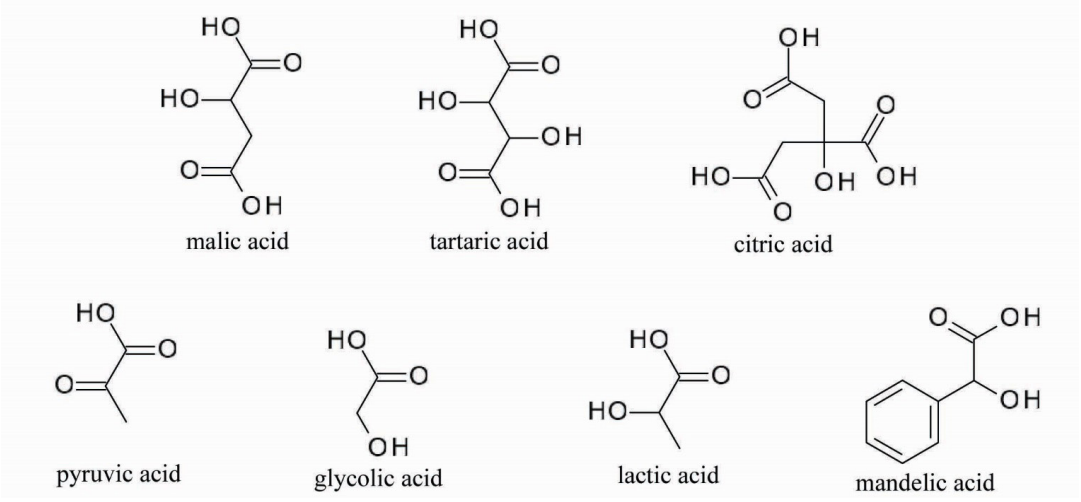

Fig. 1. Chemical structures of AHA used in chemical peels

AHA peels need neutralization to stop their action; this is achieved using usually sodium bicarbonate; however sodium hydroxide or ammonium salts solutions may also be used [9].

Beta hydroxy acids (BHA)

BHAs differs from AHAs by the presence of a hydroxyl group at the second carbon atom position (beta position).

Salicylic acid (2-hydroxybenzoic acid) is a naturally occurring aromatic hydroxyacid found in bark of the meaning white willow and in wintergreen leaves [13].

The chemical structure of salicylic acid is presented in Figure 2.

It is a lipophilic agent that produces the peel of the upper corneum layer and stimulates the basal cells and the subadiacent fibroblasts layer. Its antibacterial, anti-inflammatory, antifungal, and anticomedogenic properties can be explained as it prevents the synthesis of pantothenic acid, necessary for the life of microorganisms. It destroys intercellular lipids that are covalently bound to the cornified envelope surrounding cornified keratinocytes, thus desquamating the stratum corneum and activating basal keratinocytes and fibroblasts [13].

The therapeutic action of salicylic acid depends on the formulation, the solvent chosen in the preparation but also on the pH of the application site. Concentration formulas of 20% or 30% salicylic acid in ethanol, are used as a superficial peel agent. Superficial salicylic acid peels can be done at home with over the counter (OTC) products; stronger salicylic acid peels that penetrate more deeply should be performed by the dermatologist. It is applied for 3–5 minutes, causing a transient burning sensation; evaporation of the hydroethanolic solvent leaves a white precipitate which can be mistaken for frosting. In contrast to glycolic acid peels, salicylic acid peels do not induce frosting patterns or do not require neutralization [14].

It can be used in the treatment of skin damage due to sunburn, oily skin with large pores, comedogenic or inflammatory acne, rosaceous, epidermal melasma, postinflammatory hyperpigmentation. Salicylic acid peels are preferred to be used in comedonal and inflammatory acne, as well as for oily skin [15].

A lipophilic derivative of salicylic acid, lipohydroxy acid has been used in chemical peels. Lipohydroxy acid targets corneodesmosome protein structures to differentiate uniformly corneocytes; its higher lipophilicity results in a more complex mechanism of action in the epidermis and a higher keratolytic effect compared with salicylic acid. Lipohydroxy acid is used in concentrations of 5–10% and does not need neutralization [16].

Fig. 2. Chemical structures of salicylic acid

Due to the fact that BHAs are liposoluble and AHAs are hydrosoluble, it has been established that BHAs are more suited to the mixed, oily, normal skin that exhibits acne or has acne trends, also for skin with rosacea, while AHAs are more suitable for normal and dry skin. In recent years, more and more studies have emerged proving that AHAs have a beneficial effect on skin with excess sebum, in improving the appearance of post acne spots or caused by sun, post-acne scars, and in particular helps to smoothen color and texture skin [17].

A complex care routine for skin conditions is presented in Table I [18].

Trichloroacetic acid (TCA)

TCA is a structural analogue of acetic acid in which the hydrogen atoms in the methyl group have been replaced with chlorine atoms [19].

TCA precipitates epidermal proteins and causes destruction of upper dermis. Histologically, TCA is responsible for superficial coagulation of skin proteins and degradation of epidermis, followed by the rejuvenation of the epidermis and dermis with formation of new collagen storage and normalization of the elasticity of the tissue [19].

It is soluble in water and is used in concentration between 10-50%. The degree of the peel depends on the concentration of TCA and on the application time. Superficial TCA peel is performed at concentrations of 10–30%, medium-depth peel is performed at TCA concentrations of 35–50%, higher TCA concentrations (>50%) should be used with caution because of the risk of post-inflammatory hyperpigmentation and scarring. It may be used alone or as part of a combination peel, with other peel agents [20,21].

The superficial TCA peeling is used for the restoration of the skin appearance, for the treatment of wrinkles, in actinic keratosis and in the treatment of benign pigmented lesions. TCA is among the most used agents for treating scars caused by atrophic acne. Combinations of 40% TCA

Table I. Correlation between skin conditions and type of peels [18]

Skin condition	Recommended peel type
comedonal acne (black dots), all skin types	BHA
comedonal acne (white dots), all skin types	AHA
papulo pustulous acne, all skin types	AHA
cystic nodulous acne, all skin types	BHA
rosacea acne, all skin types	BHA
fine wrinkles, all skin types	AHA
spots and post acne scars, all skin types	AHA
superficial sunspots or melasma, all skin types	AHA
demodex, all skin types	BHA
eczema, all skin types	AHA
seborrheic dermatitis, all skin types	BHA
psoriasis, all skin types	BHA
improving skin texture, all complexion	AHA, BHA
skin color uniformity, all types of complexion	AHA

with 70% glycolic acid gel are used to reshape skin on the neck and chin, and combinations of 20% TCA with 5% ascorbic acid are effective in the treatment of melasma [22].

TCA cannot be neutralized and is not associated with allergic reactions or systemic toxicity. During TCA peels frosting can occur; frosting disappears in 20-30 minutes and is replaced by erythema, which can last 1-2 days. The redness can last for several days up to a week, and peels with TCA can be repeated after 2-6 weeks [21].

Retinoic acids

The family of retinoids comprises of vitamin A (retinol) and its natural derivatives such as retinoic acid, retinaldehyde, and retinyl esters, as well as many synthetic derivatives. Retinol is a 20-carbon molecule containing of a cyclohexenyl ring, a side chain with four double bonds (all in trans configuration forms), and an alcohol end group, hence the term all-trans-retinol. The oxidation of the alcohol end group of retinol results in the formation of an aldehyde (all-trans retinaldehyde or retinal), that can be further oxidized to a carboxylic acid (all-trans retinoic acid or tretinoin) [23].

Tretinoin (all trans-retinoic acid) is a retinoic acid derivative used in superficial chemical peels. Tretinoin has been shown to improve photoaged skin. A 1% tretinoin peel proved to be as effective as 70% glycolic acid in the reduction of the pigmentation in melasma in dark-skinned patients, being also less irritating and therefore better tolerated. There are still question marks about the advantage of highly concentrated tretinoin peels (5-10%) versus its continuous use at lower concentrations (1%) [24].

The chemical structure of the main retinoic acid derivatives are presented in Figure 3.

Phenols

Phenol (hydroxybenzene, carbolic acid) is an aggressive peel agent used usually in deep peels. Its use is limited by its high toxicity; it is strongly caustic, coagulates albumin and destroys mucous membranes and skin; it is recommended to be handled with rubber gloves [25].

Phenol peel effects vary depending on its strength and the surface area to which it is being applied. Phenol induces rapid and irreversible denaturation and coagulation of epidermal keratin and proteins at concentrations above 80%, this results in the creation of a barrier that prevents the peel agent from penetrating into the deep dermis. A 50% phenol solution acts as a keratolytic agent; disrupting sulfur bridges and allowing further penetration into the dermis, causing greater destruction and systemic absorption. Systemic absorption via the skin into the systemic circulation may cause serious adverse effects including renal failure and hepatotoxicity, cardiac arrhythmias, making it a potentially dangerous agent in inexperienced hands [26].

Phenol peel is used to treat facial wrinkles, skin spots caused by pregnancy, birth control pills, viral illnesses, sun exposure including precancerous skin conditions. Another disadvantage is represented by the fact that it stains the treated areas of the skin used for peel; consequently, is used for the peel of the whole face or on limited areas of the face where the contrast with the untreated areas is not so obvious. Phenol peels are used primarily on the face, since it can cause scarring if used on the neck [26].

Resorcinol (m-dihydroxy benzene, 1,3-dihydroxi benzene) is a phenol derivative, the isomer of catechol and hydroquinone.

It is a bactericidal, reducing agent with keratolytic properties; by stimulating prostaglandin E_2 formation and disrupting the hydrogen bonds of keratin. Resorcinol is used in concentrations of 10–50% for chemical peel in the treatment of acne, acne scars, epidermal melasma, mild photoaging and freckles. Side effects linked with resorcinol peels include allergic dermatitis, irritant contact dermatitis and post-inflammatory hyperpigmentation [27].

Hydroquinone (p-dihydroxy benzene, 1,3-dihydroxi benzene) is usually used as topical depigmenting agent effectively clearing melanin pigments in melasma. It can be used to treat skin condition associated with hyperpigmentation [28].

The chemical structures of phenols used in peels are presented in Figure 4.

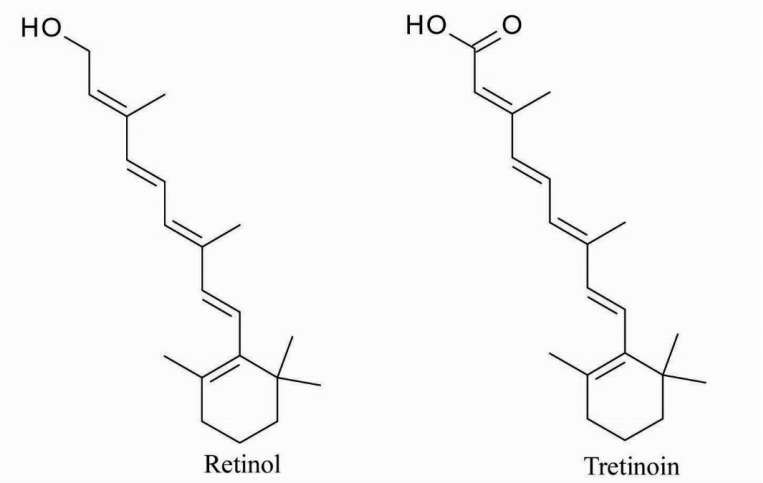

Fig. 3. Chemical structures of retinoic acids derivatives

Several other composed phenol formulas have been used for deep chemical peels. These formulas consist of phenol in varying concentrations together with other ingredients such as croton oil (Baker-Gordon phenol peel, Hetter phenol-croton oil peel) [29].

In practice more and more emphasis is placed on combining various peel agents, with advantages related with the decrease of the complication and risks of the peel by using lower concentration of substances, accelaration of the tissue regeneration and subsecent time for recovery and extension of the application range.

Jessner solution

The most frequently used peel combination formula is Jessner solution. Jessner solution contain of a mixture of 14 g salicylic acid, 14 g resorcinol, 14 mL lactic acid 85% in 95% ethanol q.s. Modified Jessner solution which do not contain resorcinol (considered to be responsible for side effects such as irritant and allergic contact dermatitis and skin discoloration) are also used. It is a clear, amber-colored photo sensitive solution that needs to be kept in a brown bottle to prevent oxidation [30].

Jessner solution is used in peels to treat acne, melasma, lentigines, post-inflammatory hyperpigmentation, freckles, and photodamage. Application of Jessner leads to loss of corneocyte cohesion and induces intercellular and intracellular edema [29].

Jessner solution is generally applied in two coats, additional application increases the depth of the peel. It does not require neutralization. After application mild erythema and patchy frosting develops. It is also used as a preparatory peel to enhance the penetration before a peel with TCA [31].

The factors affecting the depth of the peel, and thus its effects on the skin, include the properties of the chemical agent used (e.g., concentration and pH), the application technique, and the patient's skin condition and sensitivity. Also, the depth of the peel is cumulative and dose-dependent; as a monolayer of application can lead to a superficial peel, while subsequent multiple layers application will result in an additive deeper peel [32].

A classification of the chemical peel agents based on the depth of penetration is presented in Table II [33].

Recently, the mechanism and techniques used in peels suffered a slight change, as lasers are more and more frequently used in deep peels due to their enhanced ablative depth modulation and relative lack of adverse effects and toxicity. However, the popularity of superficial peels also increased in popularity, demonstrated by the large number of OTC cosmetics on the market [34].

Statistically currently chemical peels are among the most frequently performed noninvasive cosmetic procedures together with botulinum toxins and soft tissue fillers [35].

Conclusions

Chemical peeling is a slightly invasive dermatoesthetique procedure that consists in the application of chemical substances on the skin that follows the controlled destruction of the skin layers, having the effect of peeling the skin to varying degrees. In this way, dead cells are removed from the superficial layers of the skin and at the same time cells from the deep layers are stimulated to regenerate and produce collagen and elastin.

Although there can be slight variation between the categories of peel agents and their desired cosmetic effects, a chemical peel ultimate purpose is to enhance the clinical appearance of the skin by minimizing the quantity and consistency of wrinkles and acne scars, decreasing inflammatory acne lesions, enhancing dyspigmentation and giving an overall more youthful look.

Chemical peels are non-invasive intervention with the ability to solve a personalized problem of skin imperfection,

Fig. 4. Chemical structures of phenols used in chemical peels

Table II. Peeling agents used in different peel types [33]

Peel type	Peel agent	Depth of penetration
Superficial	AHA - glycolic acid (30–50%), lactic acid (10–30% mandelic acid (40%) BHA - salicylic acid (30%) pyruvic acid (50%) resorcinol (25-50%) Jessner solution (3-7 coats) TCA 10-35 % (1 coat)	superficial/stratum corneum exfoliation/epidermal necrosis
Medium	BHA – salycilic acid (>30%) AHA - glycolic acid (>70%) – with or without pretreatment Jessner solution TCA (30-50%) – with or without pretreatment Jessner solution TCA 35% + glycolic acid 70%	medium depth/papillary dermal necrosis
Deep	TCA (>50%) - pretreatment Jessner solution Phenol 88% Baker-Gordon phenol peel (50–55% phenol)	deep/reticular dermal necrosis

to allow the choice of the level of epidermal penetration: superficial, medium or deep. Regarding the characteristics of the skin, one can choose one of the three variants and the peel agent. When used for the appropriate indication by professionals, noninvasive chemical peel solutions demonstrated very good clinical efficiency in improving skin tone and texture and are cost-effective solutions compared to invasive procedures.

Authors' contributions

Soimita Emiliana Măgerusan (Conceptualization; Data curation; Formal analysis; Methodology; Project administration; Resources; Writing – original draft; Writing – review & editing)

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Conflict of interest

None to declare.

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RESEARCH ARTICLE

Enumerating the Yield and Purity of *Pf*DNA from Archived, Newly Used mRDTs and Comparison with DBS from a Malaria-Endemic Focus

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Objective: Archived malaria rapid diagnostic test strips (mRDTs) serves as an important source of plasmodium Deoxyribose Nucleic Acid (DNA) in epidemiological studies. The presence of *Plasmodium falciparum* DNA (*Pf*DNA) in mRDTs (yr. 2016-2017) and newly used ones (yr. 2018) were enumerated with a view to establish the parasite's optimum genomic DNA volume. **Methods**: A retrospective study to determine the yield and purity of used mRDTs was carried out on randomly selected mRDTs (2016 – 2018). Both positive and negative mRDTs samples were analyzed with nested Polymerase chain reaction (nPCR). Dried blood spots (DBS) were obtained from study enrolments and analyzed molecularly. nPCR and Agarose gel electrophoresis were used to determine *P. falciparum* DNA. **Results**: Agarose gel electrophoresis results showed that only 26 out of the 50 samples eligible for screening were PCR positive for *P. falciparum*. The following was observed; yrs.: 2016 - 17(34%) with 2.06 X 103 yield, 1.7235 purity; 2017 - 16(32%) with 1.03 X 103 yield, 1.7619 purity and 2018 - 17(34%) with 1.42 X 103 yield, 1.6194 purity. Molecular analysis (P.f. 18Ss rRNA) was determined to ascertain positive result that appeared negative using mRDTs or microscopy. The DNA yield of the DBS for 2018 was 1.66 X 103 and a purity (Optical Density 260/280) of 1.69. The purity was higher than that of the mRDTs with a DNA yield of 1.42 X 103 and 1.62 purity. **Conclusion**: *Pf*DNA extraction is an important process for malaria PCR screening and the reliability is dependent on pureness and concentration.

Keywords: *Plasmodium falciparum*, mRDTs, DNA, purity, yield

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Introduction

The discovery of suitable malaria genetic markers for the establishment of a promising elimination technique is paramount at this time when it appears all efforts at combating the scourge is fast becoming elusive [1]. Archived mRDTs are important source of *Plasmodium falciparum* DNA (*Pf*DNA) in epidemiological studies, but a number of limiting factors affect the yield and purity [2-5]. The analysis for genetic diversity of malaria among human and *Plasmodium falciparum* populations is akin to monitoring control and elimination [4, 6]. The species *P. falciparum* is said to be the most virulent among the other four types of malaria parasites known to cause physio-pathological syndromes in humans with the transmission rate and the migration of infected and carriers differing from one endemic area to the other [3, 7]. It poses great challenges to malaria elimination, as effective tools are required to detect genomic changes in the parasite populations and subsequently becomes necessary for monitoring the spread of genetic variants [8]. In the past few years, several methods have been developed for eliciting parasite DNA, these methods have allowed the introduction of exogenous DNA into the human malaria parasite [9]. The discovery of parasite transfection as an important technical tool is providing more insights for understanding the function of plasmodium proteins and their roles in biology and disease. This has allowed the analysis of promoter function and helped to establish the role of particular molecules and/or mutations in the biology of plasmodium species. The ability of *P. falciparum* to transfect plasmids and replicate episomally during the merogonic stages is paramount to the survival of the parasite in the presence of lethal antimalarial drugs [9, 10]. Recently, the epidemiology of malaria revealed the discovery of several multiple loci variable tandem repeat analysis (MLVA) aided with microsatellite markers and observed that *P. falciparum* genetics correlate with malaria transmission intensity [1, 6, 11, 12]. In malaria endemic zones that have an uneven atmospheric temperature distribution across the year, collection of occult blood for analysis of DNA probes can prove inconsistent [11], but the advent of malaria rapid diagnostic test (mRDTs) kits for determining clinical malaria infections may provide desired measures to establish past malaria infections and determine variability in DNA sequence of the plasmo-

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dium parasite [4]. Additionally, ethical issues surrounding patients consent are easily resolved as ordinarily these used strips/cassettes are discarded once interpreted. The World Health Organization (WHO) [13] advocated the use of mRDTs to detect the presence of circulating malaria parasite specific antigens such as the histidine rich protein 2 (*Pf*HRP2) and lactate dehydrogenase (*Pf*LDH). Earlier studies indicated that used mRDTs potentially harbor *Pf* DNA [5, 14-17). With this development, it is imperative that the detection of antimalarial drug-resistant genes can be achieved through the single nucleotide polymorphism [17]. The abundant contamination of clinical malaria samples for plasmodium species DNA sequencing with human DNA is a major constraint militating against effective and quality *P. falciparum* genome studies [18]. The removal of leukocytes and other blood constituents from infected blood samples [19-21], the use of frozen blood samples [22, 23] and the isolation, genotyping and sequencing of *P. falciparum* from archive clinical blood samples using flow cytometry [18] are germane contributions to its genome sequencing and evolution. In the light of the aforementioned obstacles, our study aims to determine and compare the yield and purity of *Pf* DNA in archived and newly used mRDTs and DBS collected from an area with high malaria transmission.

Methods

Study Design

A retrospective study was carried out on randomly selected used mRDTs in the last three years (between February 2016 and March 2018) at the Molecular Parasitology laboratory of the Department of Zoology, University of Ilorin, Nigeria. Both positive and negative mRDTs samples were analyzed with nPCR. The study was designed to compare and evaluate *Pf*DNA yield and purity of archived and newly used mRDTs using current molecular techniques. The study also evaluated and compared the yield and purity of *Pf*DNA simultaneously derived from 50 samples of dried blood spot (DBS) and newly used mRDTs (2018), respectively. These samples were freshly obtained from study enrolments in private and public hospitals in Kwara State, Nigeria. Peripheral blood finger prick samples (5 – 10ul) were absorbed into mRDTs. Two to three drops of blood were placed on Whatman FTA card (Whatman Inc., Brentford, UK) and stored in air tight desiccant containers and consequently preserved at -80ºC.

Study Area

The study was carried out in Ilorin, an urban location that is the capital of Kwara state, which lies in the North-Central part of Nigeria, located on the longitude 4.33°E - 4.45°E and latitude 8.30°N. It covers an area of about 38 square miles, with an estimated population of 3,192,893million people (2016 projection) [24]. Ilorin is associated with intense rainfall from April to October and daily temperature between 23°C and 37°C with humidity of 70% [25].

Ethical Clearance

Approval for this study was obtained from the University of Ilorin Ethical Consideration Committee. The Directors of Children Specialist Hospital, Centre Igboro and the University Health Services, University of Ilorin, Ilorin were briefed with the details of the aim and objectives of the study. Thereafter, with the assistance of the hospital's laboratory staff, written consent and cooperation of the patients were sought.

DNA Extraction – Qiagen Minikit

Parasite DNA extraction was performed on DBS samples using a QIAamp DNA Mini Kit and the DNeasy Blood and Tissue Kit (Qiagen, Venlo, Netherlands) with strict compliance to the manufacturer's instructions. Extracted genomic DNA was recovered in a final elution volume of 50 µL.

DNA Quantification by Nanodrop 1000

DNA quantification was performed by measuring the UV-induced emission of fluorescence and nucleic acid light absorption at 260nm in two-stranded DNA, having a concentration of 50ng/µl. This was correlated with an Optical Density (OD) of 1 at 260 nm, thereby making it easy to calculate DNA from OD measurements. The light absorbed in the 260nm region was used to determine the concentration of DNA in solution by applying the Beer-Lambert law (Beer-Lambert equation is only linear for absorbance between 0.1 and 1.0 while using the Nanodrop).

DNA concentration was estimated by measuring the absorbance at 260nm; adjusting the A260 measurement for turbidity (measured by absorbance at 320nm), multiplied by the dilution factor, and using the relationship that an A260 of 1.0 = 50µg/ml pure dsDNA.

Concentration (µg/ml) = (A260 reading – A320 reading) × dilution factor × 50µg/ml

Total yield was obtained by multiplying the DNA concentration by the final total purified sample volume.

DNA yield (µg) = DNA concentration × total sample volume (ml)

Polymerase Chain Reaction (PCR) Amplification

PCR is a technique used in molecular biology for amplifying a single copy or a few copies of a segment of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence. PCR amplification was carried out after the methods described in Oyola et al. [26] and Papa Mze et al. [2].

Agarose Gel Electrophoresis

The separation of the *P. falciparum* DNA fragments (100 bp to 25 kb) was carried out after the methods of Pie et al., (2012) [27]. After separation, the resulting DNA frag-

ments appeared as clearly defined bands on standardized ladder and separated to a degree that allows for the useful determination of the sizes of sample bands (Figure 1).

Data Analysis

Statistical analysis was performed using Statistical Package for Social Science (SPSS) for windows version 22.0 full version. The mean±SD was also analyzed by comparing means of yield and purity of the samples. Molecular analyses for the determination of yield and purity of *Pf*DNA were carried out in the first part on fifty randomly selected archived mRDTs (2016 – 2018) and same evaluation was simultaneously done on fifty fresh samples of Dried Blood Spots (DBS) and newly used mRDTs (2018). For all tests, the significance level was $\alpha = 0.05$ measured at 95% confidence level.

Results

A total of fifty (50) valid samples were examined for *P. falciparum* DNA obtained from clinically used mRDTs (2016 – 2018). Out of these samples; 24 (48.0%) were truly positive for *P. falciparum* with the following distribution with respect to 2016 – 2018 viz 10 (58.8%); 5 (31.3%) and 9 (52.9%). The distribution for *Pf* DNA positive samples for nPCR was 26 (52.0%) (Table I).

The yield and purity of the sampled *Pf*DNA determined with spectrophotometry (Nanodrop ND-1000) for three consecutive years are as depicted in Table II. The following yield and purity were consecutively recorded in the years' 2016 – 2018, viz; 17(34%) samples with 2.06 X 103 ± 824.55 yield; purity 1.7235 ± 0.2067 (2016), 16(32%) samples with 1.03 X 103 ± 1065.16 yield; purity 1.7619 ± 0.1158 (2017) and 17(34%) samples with 1.42 X 103 ± 1096.64 yield; purity 1.6194 ± 0.1163 (2018). It was observed that archived mRDTs from 2016 had a higher yield when compared to other recently stored mRDTs (i.e. 2017and 2018), but the purity results however showed no obvious differences between 2016 and 2017. A marked downward difference in purity was observed with year 2018 samples as compared to year 2016 and 2017 respectively (Table II).

In another vein, a total of fifty (50) valid samples were separately simultaneously obtained by DBS and mRDTs in 2018 to compare *Pf*DNA yield and purity (Table III). It was discovered that the yield of *Pf*DNA (4.51 X 103 ± 2986.34) obtained was higher than that obtained through the DBS (1.96 X 103 ± 666.15) for the same sample of blood. In fact, the purity obtained through the same means was higher with mRDTs (5.1048 ± 0.4388) than DBS (1.6312 ± 0.09) (Table III).

Agarose gel electrophoregram of *P. falciparum* 18Ss rRNA (205 bp) resolved on 1.2% agarose gel from 50 selected positive mRDTs samples (Fig. 1). Lanes 1= 100bp DNA ladder, lanes 2, 3, 4, 5, and 10, 11, 12, 13, 14, 15 are PCR positive for *P. falciparum*, lane 16 is no template control. Although, the RDT tested negative for samples 9, 12, 13, 17 for year 2016 likewise for year 2017 with samples number 19, 20, 21, 22, 23, 31 and 38, 44 all tested

Table I. Retrospective distribution of *P. falciparum* DNA in used mRDTs stored at room temperature

mRDTs	Years of Enrolment			Total (%)
	2016	2017	2018	
Total No. examined/year	17 (34.0%)	16 (32.0%)	17 (34.0%)	50 (100%)
No. positive	10 (58.8%)	5 (31.3%)	9 (52.9%)	24 (48.0%)
No. negative	7 (41.2%)	11 (68.8%)	8 (47.1%)	26 (52.0%)
Positive PCR for *P. falciparum*, n (%)	10 (58.8%)	9 (56.3%)	7 (41.2%)	26 (52.0%)

mRDTs – malaria rapid diagnostic tests; nPCR – nested Polymerase Chain Reaction

Table II. Comparison of the *Pf*DNA yield and purity obtained by Nanodrop 1000 with respect to the years under study (2016 – 2018)

Year under study	Sampling type	No. Examined	No. Positive	Mean ± St. Dev	Minimum	Maximum
2016	mRDTs					
	Yield(ng/µL)	17	10	2.06 X 103 ± 824.55	1152.70	3833.30
	Purity			1.7235 ± 0.2067	1.15	1.91
2017	mRDTs					
	Yield(ng/µL)	16	5	1.03 X 103 ± 1065.16	102.90	3638.70
	Purity			1.7619 ± 0.1158	1.58	1.97
2018	mRDTs					
	Yield(ng/µL)	17	9	1.42 X 103 ± 1096.64	487.90	5466.00
	Purity			1.6194 ± 0.1163	1.51	1.96

mRDTs – malaria rapid diagnostic tests

Table III. Comparison of *Pf*DNA yield and purity obtained by Nanodrop 1000 with respect to mRDTs and Dried Blood Spot (DBS) from the study enrolments in 2018

Sampling type	No. Examined	No. Positive (%)	Mean ± St. Dev	Minimum	Maximum
mRDTs	50	26 (52.0)			
Yield(ng/µL)			4.51 X 103 ± 2986.34	1743.5	12938
Purity			5.1048 ± 0.4388	4.24	5.84
DBS	50	22 (44.0)			
Yield(ng/µL)			1.96 X 103 ± 666.15	1021.20	3424.90
Purity			1.6312 ± 0.09	1.52	1.76

mRDTs – malaria rapid diagnostic tests, DBS - dried blood spots.

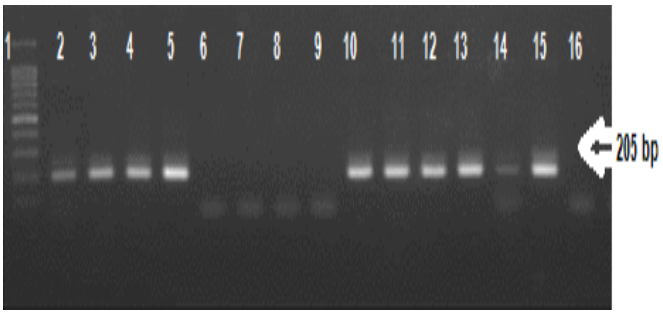

Fig. 1. Agarose gel electrophoregram of P. falciparum 18Ss rRNA resolved on 1.2% agarose gel

negative with mRDT but tested positive during agarose gel electrophoresis which is an indication of the low sensitivity of RDT in diagnosis. A total number of 50 Dried Blood Spot (DBS) for year 2018 were analyzed in agarose gel electrophoregram having 20 positives 2, 3, 4, 15, 17, 21, 22, 23, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 50.

Discussion

In many malaria endemic regions, mRDTs have gradually replaced routine microscopy for the clinical detection of suspected symptomatic malaria infections [5, 13]. The general acceptability of mRDT as a simple diagnostic procedure will certainly give credence to a wide array of molecular approach in plasmodium genome studies. A promising novelty in the current efforts for malaria elimination in Africa is the ability to elute malaria parasite DNA from archived, mRDTs and DBS. From our findings, we established that used mRDTs harbour *Pf*DNA, as reported by other authors [4, 5, 14, 15, 17]. The majority of the eluted DNA were positive for *P. falciparum*, which makes the detection of antimalarial drug-resistant genes achievable even in our own settings. The aforementioned is made possible through the identification of the parasite's strains from single nucleotide polymorphism, an important finding earlier reported by Morris et al. [17]. Again, a major hindrance to the progress observed in the present study was the contamination of human DNA with PfDNA during sequencing which definitely affected its quality for further genomic studies [18]. Earlier, the yield and purity of archived blood samples were used for determining the amount of protein contamination left from nucleic acid isolates [4, 28]. The yield and purity of the sampled specimen for *Pf*DNA however was dependent on the storage facility and the longevity of the storage bearing in mind the ambient temperature of the room where the archived mRDTs were kept. From the foregoing, the highest yield and purity of the *Pf*DNA was obtained from the samples obtained in 2016. However samples analyzed from year 2018 showed a decrease in yield and purity, a value lower than the standard purity value (1.8-2.0) which may due to excess of PCR inhibitors [29, 30]. The current findings are dissimilar to that of Sylwia et al. [31] that reported 57-94 ng/µl of yield and -1.76-1.86 purity. Similarly, other studies were able to identify certain limitations in the yield and purity of *Pf*DNA which the authors suggested might be as a result of one or more of the following; viz, sample collection by in-experienced technicians, infection risk, presence of certain interfering proteins and PCR inhibitors in blood [32].

The variation observed between the samples that tested positive by mRDTs and negative for qPCR may be interpreted as false-negative due to the presence of PCR inhibitors or errors in the DNA extraction process as it was the case in other regions [4, 5, 14, 15]. Inadequate parasitic material can also lead to false-negative result due to low volume of blood dropped inside the RDT kit compared to higher volume of blood spotted on the filter paper which is comparable to earlier reports [33]. However, it was observed that increased parasitaemia demonstrated a significant association with increased *Pf*DNA. A similar case was reported by Auburn et al., (2011) where the authors observed that lower human DNA levels resulted in greater relative representation of *Pf*DNA [19]. From the foregoing, it then suggests that there is a strong correlation between parasite density and specific DNA probes. Barka et al. (1986) enunciated a general method for the development of highly species specific DNA probes for the diagnosis of *P. falciparum* and established sequences that recognize the difference between drug-resistant and drug-sensitive strains [34].

Conclusion

The assessment of *Pf*DNA quality and integrity is strongly dependent on both the storage medium and conditions. Obtaining an optimum parasite yield and purity in laboratory/field conditions is certainly a way to go in genomic and malaria vaccine studies. Eliminating malaria in endemic populations can be achieved when there is an understanding of the evolution of the malaria parasite in response to the human immune system and interventions such as drugs and vaccines. It is however difficult to draw general conclusions regarding the suitability of extracted genomic DNA for molecular biological analysis. Indeed, the assessment of DNA quality and integrity may depend on the techniques used for the evaluation. The current findings may provide multifaceted platforms in guiding malaria control programs and also facilitate the monitoring of drug resistance via genotyping of resistance genes. mRDTs are becoming more popular during the course of evaluation in the case management of malaria in hyperendemic regions and our study identified with these kits as potential source of *Pf*DNA in diverse and large population studies. In situations where there are difficulties associated with ethical and clinical approval for blood samples for genomic studies, used mRDTs may just be the ideal, as they are in conformity with the recommendations of the World Health Organization. mRDTs also provide easy transportation from field to standard laboratories with minimal to no health associated challenges. Genetic diversity is easily achievable with mRDTs collected from diverse environmental foci, in order to guide and monitor current control strategies.

Authors' contributions

OS (Conceptualization; Data curation; Writing – original draft)
OAI (Formal analysis; Methodology; Writing – review & editing)
OAO(Conceptualization; Data curation)
OA (Formal analysis; Investigation)
GIC (Methodology; Project administration)
MIA (Methodology; Project administration)
OF (Investigation; Methodology; Supervision)
EA (Formal analysis; Investigation; Methodology)
MRF (Investigation; Methodology; Project administration)

Conflict of interest

None to declare.

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RESEARCH ARTICLE

A Study of Heat Generation in Orthopaedic Bone Drilling Process

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Reconstruction and repair of a complete bone fracture requires surgical drilling of bone in order to create holes which support easy insertion of screws. The objective of the research is to optimize kinematic parameters when drilling bone in order to avoid bone necrosis and increase the capacity of bones to retain the surgical screws. In literature there are presented attempts to measure the temperature of bones by introducing thermocouples into bone near the drill path which is not a satisfactory method. In this research it is proposed a new method for measuring temperature by means of a digital infrared thermometer oriented on bone surface where holes are made. We have drilled animal bones and represented the experimental curves of temperature for a wide range of kinematic parameters that are supposed to be used during orthopaedic operations. It is concluded speeds ranges that can be used when drilling bone holes, which ensures good cutting conditions and temperatures at a level which does not affect the quality of the assembly.

Keywords: orthopaedic surgery, surgical drilling, bone necrosis, temperature measurement, cortical screw

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Introduction

Bone fracture is common due to traumatic accidents or other causes. Broken bone produces cells that tend to re-establish connection between broken pieces. But the principal method for reconstruction and repair of a complete bone fracture is achieved by bone surgical drilling to create holes which support screws and pins easy insertion that allow adjacent bone fragments alignment, as well as application of fracture plates [1].

In current clinical practice, electrically-powered handheld drills are frequently used for bone drilling. This is somehow in contradiction with modern surgery where procedure can be conducted off-site by teleoperation [2], or drilling may be supported by haptic systems equipped with force and torque transducers [3], or with CO2 pulsed lasers [4].

In some situations depending on feed rates, mainshaft speeds, drill bit geometry and outer diameter, drill bit wear and type of bone nonoptimal forces and torques can be generated at interface between drill bit and bone which have the effect to raised temperatures that may cause bone necrosis and a poor quality of guide holes, resulting in poor screw fixation [5].

Thermal conductivity of cortical bone in body is located in the range of 0.64 ± 0.04 W/ mK for bovine cortical bone and 0.68 ± 0.01 W/mK for human cortical bone [6], which is quite poor. On the other hand it is not recommended to use a coolant during drilling because of the danger of causing infection to the area [7].

The phenomenon of high temperature generation is mainly due to friction between drill bit and bone likewise deformation of the bone, the bone chip being removed [8]. Even if the presence of blood fluids and tissues can dissipate some of the heat generated during drilling and chips can absorb some of the heat, the temperature that results at the cut edge of a cortical hole is significantly high [9]. About 40% of the heat generated is absorbed by treated bone while 60% dissipates in bone chips [10].

For these reasons, generation of high temperatures during drilling process becomes a problem due to the bone sensitivity to the temperature and the difficulty of evacuating temperature in the area [11].

The research purpose is to accurately measure the temperature at interface between cutting edge and bone surface when drilling bone, to determine variations of bone temperature during bone drilling depending on the geometric and kinematic parameters of the drill bit. The particular emphasis is to examine effect that temperature rise has on the bone structure physical aspect in order to optimize parameters which avoid bone necrosis and increase the bone capacity to retain a surgical screw.

Methods

We started the research from observations that the phenomenon of thermal generation may produce bone necrosis [12], especially if it is exceeded temperature of 45°C more than 5 hours, or temperature of 55°C more than 30 seconds, or even for a moment the temperature of 70°C [13,14].

Previous scientific research in literature indicates that for exceeding temperature of 55°C for longer than 30 seconds, the bone is severely damaged and recovery takes several weeks [15]. Consequently, temperature control at drill bite bone interface is a clinically relevant topic.

But, in the orthopaedic surgery temperature control at drill bite bone interface is not monitored. Orthopedic sur-

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geons usually use manual drilling machines, and adjust the mainshaft speed according to the surgical procedure performed. The drill advancement speed in the bone is subjectively controlled, depending on the surgeon's skill. One way to improve this process is by performing pre-operative BMD scans of the patient, which would allow for engineering predictions of kinematic parameters of bone drilling, supplemented with the use of manual drilling machines equipped with electronic display devices, which allow better control of kinematic parameters of bone drilling.

Because there is a great diversity of cutting geometries of the drill bit, in literature there is no consensus regarding influences generated by drilling forces, feed rate and rotational speed of the mainshaft on bone peak temperature [10].

In literature there are presented attempts to measure bones temperature by introducing thermocouples [16, 17, 18, 19] into the bone near the drill path, which give temperature indications as drill advances in hole. The research results concluded that cutting speed has a limited effect on temperature changes above 55°C, while the drill force which is driving drill bit causes a much higher temperature rise [20].

From literature it can be concluded that methods of measuring the temperature of drilled bone with thermocouples placed in cortical bone at a certain distance from drilled area have led to contradictory results without consent of researchers [11].

In conclusion, due to the bone structural inconsistency and its reduced conductivity, the heat transfer modeling is difficult and incorporation of thermocouples neighboring drilled area is not an effective way to investigate effects of high temperatures on bones.

In order to overcome this drawback and to develop a simplified method, easy to reproduce in laboratories, but with scientific relevance for research, in this study it is proposed a new method for measuring the temperature by means of a digital infrared thermometer oriented on the surface of the bone where holes are made. This allows real-time temperature measurement at hole surface and recording instantaneous temperature values.

After fitting thermometer properly with support of additional guidance devices, bone drilling experiments were performed on animal bones with cortical bone thickness of approx. 14 mm, which were obtained from a local slaughterhouse. These were kept before study in the freezer, wrapped in vacuumed plastic bags at temperatures that dropped below -20°C. Before experiment with more than 12 hours the bone specimens were removed from the freezer and kept on a flat surface in vacuumed plastic bags at room temperature. It was sprayed with saline water solution to prevent dehydration.

After that it was determined the drilling location. Bone specimens were mounting into a vice, the place where the hole was to be made was measured and it was drawn with a marker. Due to geometric and dimensional variability of biological bones, a common reference dimension has been established and calculated for all bone specimens. Thus relative position of the drilling site reported to one end of the bone was determined as a relative dimension 1/4,…,1/2%.

It was assembled the test setup for surgical bone drilling research. The vice rigidly hold bone specimens in proper position. Before actual drilling on the marked place it was created a point pressed which was later used for a good guidance of surgical drill bit during experiment. The bone was tight in vice with the point pressed on the axis of the drill bit chuck. The perpendicularity between the bone and drill bit axis was verified by means of a leveling gage. Then surgical drill bit was chosen which was used in drilling experiment. The choice was based on surgical procedure that requires a pilot hole, the type of the drilled bone, and the particular research question being asked about drill bit: drill bit diameter, drill bit geometry, etc. In our experiment we started from the raw data for hole drilling tests which evidence dimensions of the 4 screws that are usually used in orthopaedics (Table I, column 2) with thread diameter 1.5,…,4.5 mm. The drilling tests were performed with 4 drill bits of diameters 1.1 – 2 – 2.5 – 3.2 mm.

If hole length is same in the 4 situations, 14 mm, the Surface Area of the contact between drill bit and bone ($SA=\pi \cdot D \cdot L$) is computed in Table I, column 5. This allowed a normalization of the conditions for caring out testing.

Then the actual bone drilling experiment was conducted which led to the creation of pilot hole. The desired size drill bit was inserted in the chuck and positioned above bone specimen that was fixed in a vice at approximately 25 mm. After that, it was started the drilling process on the automatic drilling machine to which they were set feed rate and mainshaft speed within typical ranges used clinically.

Holes were practiced with a surgical drill whose rotation speed and feed rate were controlled by means of a professional drilling machine which allowed keeping constant the kinematic parameters of drilling. In the performed tests were used 4 speeds for mainshaft varying between 560,…,2,100 rotations/minute and 4 feed speeds between 0.5 and 2 millimeters/second. Drilling process was performed until drill bit reached the set depth. Then the drill bit was withdrawn to the starting position, drilling machine was stopped and bone was removed from the vice.

During the whole drilling process a digital infrared thermometer mounted on a support near vice measured temperature of bone during drilling (Figure 1). Temperature on thermometer was recorded on video, which allowed collection of results for later data analysis.

Table I. Raw data for hole drilling tests

No.	Thread diameter [mm]	Drill bit for threaded hole [mm]	Hole length [mm]	Surface Area [mm2]	Mainshaft speed [r/min]	Feed speed [mm/s]
1	2	3	4	5	6	7
1	1.5	1.1	14	48.35	560	0,5
2	2.7	2	14	87.92	900	1
3	3.5	2.5	14	109.9	1200	1,5
4	4.5	3.2	14	140.67	2100	2

Fig. 1. a. Experimental setup for drilling into bone; b. Temperature measurement in the guide hole during drilling; c. Temperature measurement in the guide hole after drilling

Pilot hole photos and videos, as well as bone fragments generated in drilling process, allowed examination of bone surface for evaluation if there is a change in color and if necrosis occurs.

Results

Variation law of drill diameter on bone temperature during drilling process was explored for speed of mainshaft 2,100 rot/min and feed speed 1.5 mm/s. Four drill diameters were used: 1.1 mm, 2 mm, 2.5 mm, 3.2 mm. The corresponding time-dependent variation curves of temperature are presented in figure 2.

From its analysis it can be seen that all four temperature variation curves have a ∧ shape during the drilling process. The maximal drilling temperature (the lowest) 36°C is accomplished earlier at 3 seconds for the 1.1 mm drill, while maximal drilling temperature (the highest) 47°C is accomplished later at 5 seconds for the 3.2 mm drill. This result reflects that bone temperature increases with incense of drill bit diameter if other drilling parameters are constant.

Variation law of mainshaft speed on bone temperature during the drilling process was explored for 3.2 mm drill under 0.5 mm/s feed speed. It was used four speeds of mainshaft: 560 r/min, 900 r/min, 1,250 r/min and 2,100 r/min. These kinematic parameters have been selected to exceed the speeds recommended in literature for bone drilling. The corresponding time-dependent variation curves of temperature are presented in figure 3.

From its analysis it can be seen that all four temperature variation curves have a ∧ shape during drilling process. After 15 seconds since drilling process started the maximal drilling temperature was achieved at measuring point. At same time for mainshaft speed of 560 r/min, 900 r/min, 1,250 r/min and 2,100 r/min the bone temperatures are 37°C,… , 45°C. This result reflects that bone temperature increases together with mainshaft speed if other drilling parameters are constant.

Variation law of feed speed on bone temperature was studied for a 3.2 mm drill bit diameter rotating with 2,100 r/min speed of mainshaft. It was explored four feed speeds: 0.5 mm/s, 1.0 mm/s, 1.5 mm/s and 2.0 mm/s. These val-

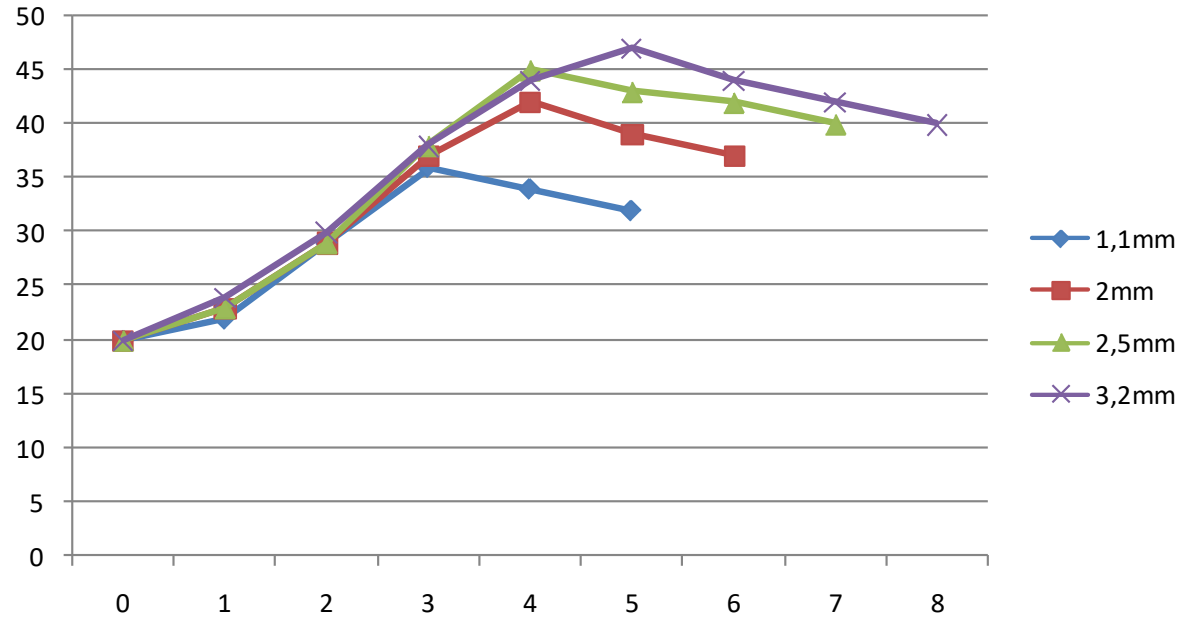

Fig. 2. The experimental curve of temperature change in bone versus time with different drill bit diameter for threaded hole (2,100 rot/min, 1.5 mm/s)

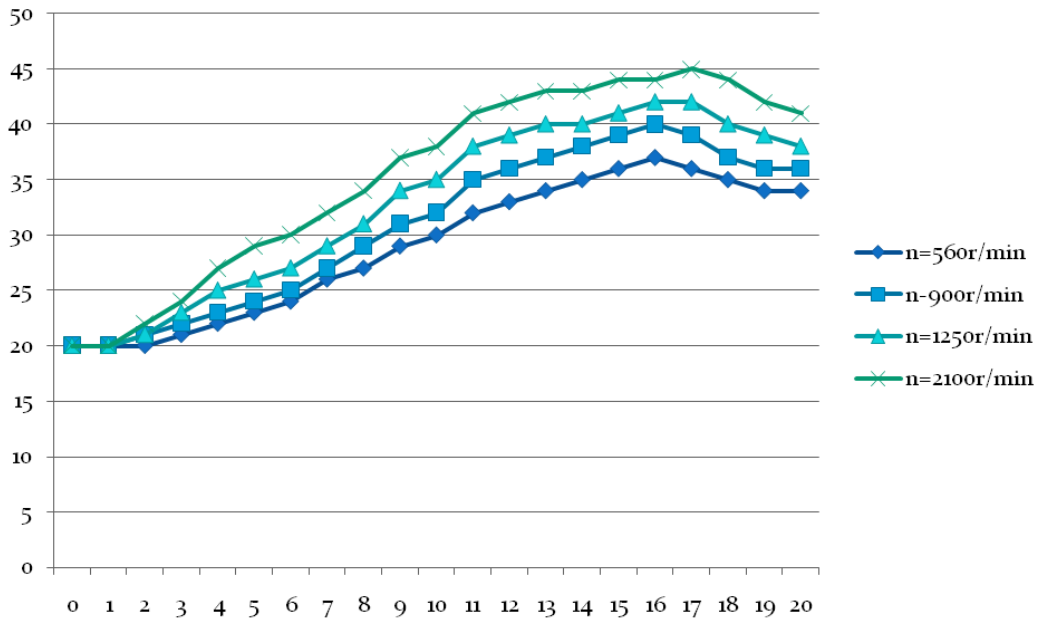

Fig. 3. The experimental curve of temperature change in bone versus time with different revolution (3,2 mm, 0.5 mm/s)

ues have been arbitrarily chosen over a wide range, because in orthopedic practice they are variable from surgeon to surgeon as drilling machine is manually controlled. The corresponding time-dependent variation curves of temperature are presented in figure 4.

From its analysis it can be seen that all four temperature variation curves, in first part of the drilling process, increase and then decrease. The maximal achieved drilling temperatures are 39°C,…,36°C. This demonstrates that drilling temperature and mainshaft feed speed is in an inversely proportional relationship.

Discussion

Orthopaedic surgeons use electrically operated drills and drill bit advancement is achieved empirically by skill. As a consequence, temperatures generated at contact between drill bit and bones are influenced by cutting parameters which may generate bone necrosis.

In order to study these influences we conducted in our research an experiment in which we performed hole drilling tests with 4 drill bits of diameters 1.1 – 2 – 2.5 – 3.2 mm and a wide range for kinematic parameters for the mainshaft speed 560,…,2,100 rot/min and feed speed 0.5,…,2 mm/s, that are usually used in orthopaedics.

The variation laws of bone temperature during bone drilling depending on geometric and kinematic parameters of drill bit reveal increase with drill bit diameter and the mainshaft speed but also decrease with feed speed. This can be an important observation with clinical relevance for orthopaedic surgeons when selecting these parameters.

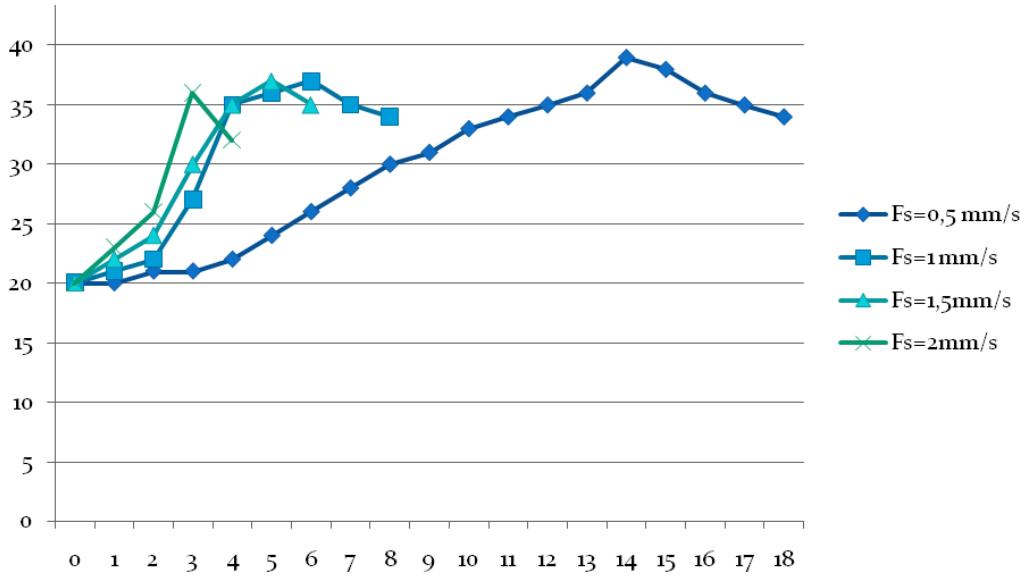

Fig. 4. The experimental curve of temperature change in bone versus time with different feed speeds (d=3.2 mm, 2,100 r/min)

The variation law shapes of various kinematic parameters as function of bone temperature during the drilling process: drill diameter, mainshaft speed, feed speed are of equivalent forms with similar studies [13, 16, 18] but with slightly different values of the measured temperatures, usually 10% lower due to the different conditions and limitations in which each of these studies was performed.

The proposed method employing a digital infrared thermometer is validated in practice, being able to measures bone temperature during drilling and predicts if raised temperature may cause bone necrosis.

Process optimization requires preoperative BMD scans of patients to allow engineering predictions about appropriate drilling parameters: drill bit diameter, speed of mainshaft and feed speed.

The study limitations occur because temperature is measured at bone surface. Inside it can be higher and further study is needed to determine temperature gradient in the vicinity of drilling wall as a function of bone surface temperature and its BMD characteristics. In this way a correction coefficient can be deduced for measurement of temperature inside the bone. Another limitation of the study is that bone drilling experiments were performed on devitalized animal bones. In vivo, the biological conditions are slightly different, for example, the blood flow may influence the local temperature.

Conclusion

In these circumstances we can conclude that temperature generated when cutting hole, has high values being located within the limits presented in literature, without visibly affecting the hole bone area and compromising cortical screw assembly.

It can be concluded that speeds of 560,…,2,100 r/min can be used when drilling holes with diameters of 1.1,…,3.2 mm, which ensures good cutting conditions while temperatures are at a level which does not affect quality of screw assembly.

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Authors' contributions

FM (Data curation; Investigation; Methodology; Writing – original draft)
TB (Conceptualization; Formal analysis; Supervision; Validation; Writing –
review & editing)

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CASE REPORT

Urinary Retention in Female after Augmentation Gluteoplasty: A Case Report

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Introduction: There are many well-known complications after gluteal augmentation surgery, such as: seroma, hematoma, capsular contracture, retraction, wound dehiscence etc., but there are some due to nervous damage (especially submuscular pockets with large implants) insufficiently recognized. The **aim** of this case report is to highlight a rare complication (urinary retention) after gluteal augmentation surgery with use of solid silicone implants in case of 41-year-old female. Woman aged 41 with a buttock augmentation with silicone implant (submuscular pocket, 300cc) performed 2 months before at plastic surgery service in Madrid, was admitted in our service, the Clinic of Urology from Tg. Mures, with permanent bladder catheter inserted for urinary retention. **Outcome**: At the admission, two months after the surgery the clinical examination revealed a permeable urinary catheter with clear urine and a fistulisated wound infection localized in the superior 1/3 of the incision in the intergluteal sulcus. Neither neurological or gynecological examination identified any pathology. After the removal of the catheter, next day the abdominal ultrasonography showed a distended bladder, with a postvoid residual urine volume of 320 ml. Urodynamic investigations (uroflowmetry, pressure flow studies) revealed a reduced Qmax. 7,6 ml/sec, underactive detrusor with a reduced BCI value of 60 (bladder contractility index), requiring self-intermittent catheterization, associated with alpha-blockers. **Conclusions**: Buttock implantation is a frequently used plastic surgery procedure with rather high rate of complications, some of them not well identified, unknown such as detrusor underactivity leading to urinary retention.

Keywords: uroflowmetry, urodynamic investigations, urinary retention, gluteal augmentation, buttock implantation

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Introduction

In our days more and more patients request body contouring with increasing applications for enhancing the gluteal region. Augmentation gluteoplasty is a surgical intervention completed in over 21.000 cases/ year in the USA (1) and is performed by using implants, gluteal flaps, lipografting and gluteal lifts (2). The most popular methods involve autologous fat grafting and silicone prothesis (1), that can be solid or semisolid. Buttock augmentation with solid silicone implants are more used in this increasingly popular procedure worldwide, since they cannot rupture, are soft and look and feel natural (3). The sizes of the implants may differ from 250 CC to 275 CC, 300 CC or 350 CC (4).

There still exist discussions regarding the safest pocket in which the implants can be positioned, since the intervention can be performed by placing the implant using a submuscular, sub-fascial, intramuscular or intramuscular XYZ method (5). As for the incisional access, different methods can be used, such as performing a single incision in the gluteal cleft or two separated incisions within the cleft (6).

Despite the popularity of this surgical intervention, buttock enhancement using gluteal silicone implants is a high risk (10 - 38%) procedure (1, 4, 7, 8). Some of the most frequent complications encountered are: wound infections, gluteal prosthesis rupture, seroma (9, 10), hematoma, capsular contracture, retraction (1), overcorrection (4), wound dehiscence, asymmetry (2), implant exposure, malposition, long term numbness of the buttock, implant rippling (2). Gluteal compartment syndrome and transient sciatic paresthesia (1) are rare, but possible complications of gluteal surgery. Concerning the innervation of the gluteus maximus, the motor innervation is performed by the inferior gluteus nerve, a ramification of the sacral plexus (11). Another risk of the surgery can be the dissection inside the muscle during the procedure, too near to the sacrum that can lead to denervation (11). As for the complications regarding micturition following buttock augmentation, the literature doesn't mention any.

Senderroff (2017) states that due to important complications there are cases where a revision should be considered including replacement of the implant, capsulotomy, capsulogrhaphy, site change. (12)

Case presentation

The aim of this case report is to highlight urinary retention following gluteal augmentation with silicone implant in a case of a 41 year old woman, a rather rare complication of gluteoplasty, not well presented by the literature.

A female patient, aged 41, was admitted in the Clinic of Urology Târgu Mureș, Romania having a bladder catheter set for 2 months for incomplete urinary retention, following a gluteal augmentation with 300CC silicone prosthesis implanted in submuscular manner and liposuction under rahianesthesia, performed in an aesthetic surgery clinic in Spain, Madrid. After the surgery, the patient accused voiding hesitancy, weak stream, sensation of incomplete

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voiding and hypogastric pain. Clinical examination and ultrasound revealed a large amount of residual urine and a bladder catheter was inserted. Before the surgery the patient had no urinary complaints.

Clinical examination revealed lumbar and hypogastric region with no pathological findings, a permeable urinary catheter with clear urine and a fistulisated wound infection localized in the superior 1/3 of the incision in the intergluteal sulcus (Figure 1).

The blood analyses had normal values but the urine tests revealed a urinary tract infection with E. coli for which an antibiotic treatment was indicated, according to the antibiogram. Nor the neurological or the gynecological examination revealed any pathological findings.

After the removal of the bladder catheter, an abdominal ultrasonography and uroflowmetry was performed the morning after. Ultrasonography revealed a bladder with high capacity and 320 ml of postvoid residual urine, with no dilatations of the kidney.

The uroflowmetry revealed an irregular, interrupted curve with a rather low Qmax (maximal flow rate) of 7,6 ml/ sec. (Figure 2).

In continuing of the urodynamical investigation, a pressure flow-study was performed. In the filling phase there were no detrusor contractions found and the patient had only a slight sensation of bladder filling at 300 ml. During the voiding phase the detrusor contraction was weak, prolonged with pdet=20 cm H^2O. The Bladder Contractility Index (BCI) (13,14) which characterizes very well the detrusor's ability to contract, was reduced to 60 units (the normal value being over 100 units) (Figure 3).

Based on the above findings a diagnosis of a hypoactive, hypocontractile detrusor, incomplete urinary retention associated with urinary tract infection (E. coli) was established.

At discharge a treatment with antibiotics (Ciprofloxacin), pelvic training, alpha blockers (Tamsulosin) for a month and self-intermittent catheterization was recommended. The follow up performed one month later showed the complete remission of the minor wound dehiscence. Uroflowmetry revealed the same prolonged, interrupted curve with a low Qmax value and 400 ml post-void residual urine. (Figure 4)

The patient was advised to have an aesthetic surgery reevaluation and a revision of the buttock implantation. Since she did not agree to take measures, she is still performing self-intermittent autocatheterisation.

Discussions

Gluteal augmentation still faces strong resistance as well from patients as some surgeons, due to several well-known complications such as seroma, dehiscence, infection, implant displacement, capsule contracture, pain and implant rupture. Sinno conducted a study on over 21.000 procedures and found the following complications in 2375 patients: wound dehiscence (9,6%), seroma (4,6%), infection (1,9%), transient sciatic paresthesia (1,0%) (1). In 2016, Vergara reported complications including: seroma (4%), asymmetry (2,66%), capsular contracture (2%), overcorrection (0,66%) and rupture of the implant in 0,66%. (4)

According to the study conducted by Sinno, the only study in literature that describes neurological complications: transient sciatic paresthesia in 1% of the cases with no references about the bladder activity (1). Moreover, urinary retention in women after gluteal augmentation surgery with silicone implants and liposuction is a complication that isn't analyzed by the literature. Possible causes of this complication can be: rahianestezia, local edema and compression (by large silicone implants of 300 ml) of the peripheral nerves, infection, profound implantation of the silicon, an unappropriated dissection, proximity of the implant to the sacrum which can lead to denervation, alike to the cauda equine syndrome (11). These causes altogether can lead to an acute distention of the bladder, with a high capacity and a large volume of postvoid residual urine, due

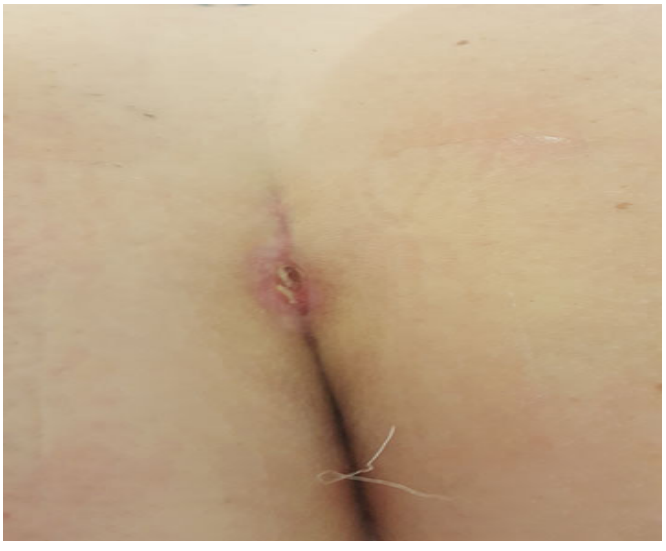

Fig. 1. Fistulisated wound granuloma in the intergluteal sulcus (picture taken 2 month after the gluteoplasty), PM female, aged 41

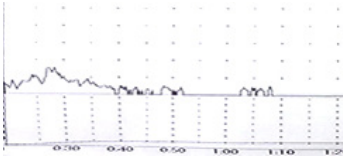

Fig. 2. Uroflowmetry result, PM female, aged 41

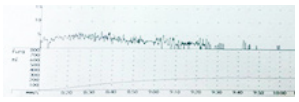

Fig. 4. Revaluation of the post-void residual urine after 1 month (PM female, aged 41)

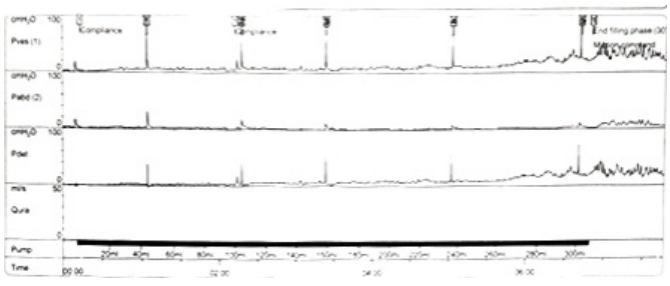

Fig. 3. Pressure-flow study (filling cystometry), PM female, aged 41

to the inefficient contraction of the detrusor. In spite of a careful dissection and an adequate surgical technique, it is still important to have a discussion with the patient about the possible risks during the preoperative consent (13) considering this possible complication.

In 2017, Senderoff published a study in which he denotes the importance of revision of the buttock implantation the replacement after removal, asymmetry or size change. The revision of the implant includes: implant removal (n=24), implant replacement (n=19), implant exchange (n=18), capsulotomy (n=6), site change (n=5), capsulorrhaphy (n=1) (3). In the case presented of the 41-year-old woman with gluteoplasty and secondary urine retention due to detrusor underactivity, a revision of the implant would have been of utmost indication, but unfortunately the patient refused it.

Conclusions

Buttock implantation is a frequently used plastic surgery procedure with a rather high rate of complications, some of which are not well identified, such as detrusor underactivity leading to urinary retention.

However, there is a multitude of technical procedures without any complications, the operative results need to be improved.

Authors' contribution

V.G.- conception and design of the article, acquisition, analysis and interpretation of data

A.F., D.P.H., C.C., T.M.C., V.O.- reviewed the final manuscript

O.M.- conception and design of the article, analysis and interpretation of data, reviewed the final manuscript

Conflict of interest

None to declare.

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CASE REPORT

Necrobiosis Lipoidica Diabeticorum

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Introduction: Necrobiosis lipoidica diabeticorum is a rare complication occuring mostly in diabetic patients appearing as a skin redness that soon transforms into an extensive necrosis. The usual aspect of such lesions is a deep ulceration with irregular borders developing especially on the lower limbs. **Case presentations**: This paper intends to present two cases of necrobiosis lipoidica diabeticorum focusing on the clinical aspect of this disease and the original treatment applied in the Plastic Surgery Department of the Clinical County Hospital of Targu Mures. Both patients suffered from type II insulin-requiring diabetes. The first case is a 63 year old female with different stages of necrobiosis lipoidica diabeticorum lesions developed on the anterior aspect of both legs. This patient was treated using only conservative methods. The second case is a 64 year old male who developed an extensive full-thickness necrosis on the right dorsal aspect of the hand and forearm. The lesions required conservative treatment and the surgical debridement of the extensive necrotic tissues. **Conclusions**: Necrobiosis lipoidica diabeticorum is a dramatic condition requiring a well informed approach in order to save the healthy tissues as much as possible. In both cases, the wounds healed spontaneously after a long period of time.

Keywords: necrobiosis lipoidica diabeticorum, conservative treatment, type II insulin-requiring diabetes

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Introduction

Necrobiosis lipoidica diabeticorum is a rare and chronic granulomatous skin disorder of little known etiology that affects 0,3% of diabetic patients. More than 50% of those diabetic patients require insulin treatment [1,2]. There are literature reviews mentioning cases occuring in non-diabetic patients but they suffered from other comorbidities such as essential hypertension, cardiac failure, obesity or dyslipidemia. The condition was first described in 1929 by Oppenheim [1].

Regarding its epidemiological aspect, it is three times more frequent in females than in males. The average age of onset is 30 years old, but there are cases reported in all age groups, including children [1,2,3].

The etiology is unknown, but there are several theories trying to explain the mechanism:

- Diabetic microangiopathy – the leading etiologic theory, due to the association between the disease and diabetes [4]. Similar changes can be found in diabetic nephropathy and retinopathy;
- The deposition of immunoglobulins, the third component of complement and fibrinogen in the blood vessel walls [1,4,5];
- Abnormal collagen formation and organisation [4];
- Abnormal glucose transport by fibroblasts [1];
- Inflammation, trauma and metabolic changes.

The diagnosis of necrobiosis lipoidica diabeticorum is mainly clinical. In rare cases, when the clinical exam is inconclusive, a biopsy of the lesion might be required for diagnosis.

The typical localisation for those lesions is the lower limbs (most of them on the pretibial area) and are usually multiple and bilateral, but they can also be unilateral. Other atypical presentations involve the face, trunk, scalp and upper limbs.

Patients usually present with a characteristic asymptomatic erythema which slowly enlarges over different periods of time ranging from several days-months to one year. The size of those areas can vary from 1-3 mm to huge dimensions, are well-delimited and initially red-brown in colour and progress to yellow, shiny, atrophic plaques [4]. Ulceration at the site of minor trauma (Koebner phenomenon) is a rare complication. [6]

Pain could be one of the principal symptoms of this disease, but due to the diabetic neuropathy many lesions are painless.

Marginal biopsy of the lesion and pathology examination of the sample shows characteristic granulomas in the dermis and subcutaneous tissue disposed in a layered fashion. Between the granulomas one can find degenerated collagen, cholesterol, mucin and fibrin deposits. The main components of the granuloma are histiocytes, lymphocites, occasional plasma cells and eosinophils [1]. There is also a decrease in the number of intradermal nervous ends, blood vessel wall thickening and endothelial cells swelling.

Direct immunofluorescence microscopy can reveal the presence of IgA, IgM, the third component of the complement or fibrinogen in the blood vessel walls [5].

Differential diagnosis can refer to dermatologic pathologies such as granuloma annulare, xanthomas, xanthogranulomas, amiloidosis and rheumatologic disorders (sarcoidosis, rheumatoid arthritis). [4]

This paper intends to present two cases of necrobiosis lipoidica diabeticorum focusing mostly on the clinical aspect of this disease and the original treatment applied in the Plastic Surgery Department of the Clinical County Hospital of Targu Mures.

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Case number 1

The first case is a 63 year old female with type II insulin-requiring diabetes, rheumatoid arthritis and operated colangiocarcinoma, who presented to our department with an extensive necrobiosis lipoidica lesion. The lesion showed a characteristic aspect – large size and well-defined borders and was located on the anterior aspect of the left leg. The onset and evolution were slow, finally reaching the aspect shown in Figure 1A.

The ulceration was treated with daily or every two days dressings (depending on the amount of exsudate), using nitrocellulose dressings. After aproximately 2 months, the upper 2/3 have been debrided and a good granulation tissue appeared, while the inferior 1/3 has shown the devitalised tendon of the anterior tibialis muscle (Figure 1B).

After 3 months, the initial ulceration has been completely debrided. The granulation tissue was covered by the new epithelium, excepting a small portion of the proximal part of the wound (Figure 1C). After about 2-3 more weeks, the entire ulceration has been debrided and healed by spontaneous epithelialization, showing a shiny new epithelium, which will slowly go through the wound contraction phase (Figure 1D).

During her last appointment for follow-up treatment and consultation (Figure 1E), the aspect of the lesion has improved a lot. New scarring tissue appeared along with a slight hiperkeratosis and desquamation. The next step is the daily application of different hidrating cremes which substantially improved the aspect and the evolution of the scar tissue, stabilizing the neoepithelium and preventing hiperkeratosis (Figure 1F).

Unfortunately, while the left leg was healing, a new ulceration appeared on the anterior aspect of the right leg with a characteristic aspect for necrobiosis lipoidica diabeticorum (Figure 1G). Fortunately, this lesion was limited to the superficial skin and suprafascial subcutaneous tissue.

This wound was also treated conservatively using thick absorbent nitrocellulose dressings that were changed every time they were soaked with exsudate. In the case of this second wound, the exsudate was not so abundant and the evolution towards healing was faster (Figure 1H). Unfortunately, the patient developed multiple metastases due to the intrahepatic cholangiocarcinoma, so she couldn't come to the hospital anymore for follow-up consultation and treatment.

Case number 2

The second case is a 64 year old male with type II insulin-requiring diabetes and chronic renal failure undergoing dialysis 3 times a week. He spontaneously developed an extensive necrosis on the posterior aspect of the right forearm and right hand (Figure 2A). The necrosis involved the superficial skin and suprafascial subcutaneous tissues. It could also be interpreted as a secondary necrosis (vascular type) due to the trombosis of one of the therapeutic fistulas used for dialysis, but the evolution and clinical aspect leans more towards necrobiosis lipoidica diabeticorum.

The pacient was admitted in the nephrology department but he was brought daily to the plastic surgery dressing room to undergo local treatment. Initially, the necrotic tissues were surgically debrided on behalf of scissors and forceps. The excision was made at the demarcation line between the necrotic and the healthy tissues. The large defect on the posterior aspect of the arm and fingers was then covered daily by alternating silver sulfadiazine and povidone-iodine dressings which led to the progressive autolytic debridement of the slough and fibrin deposits (Figure 2B).

Two-three weeks later, the necrotic lesions were slowly replaced by granulation tissue, with adherent slough and abundent fibrin deposits still present, along with an important exsudation with its characteristic foul odour. Slowly,

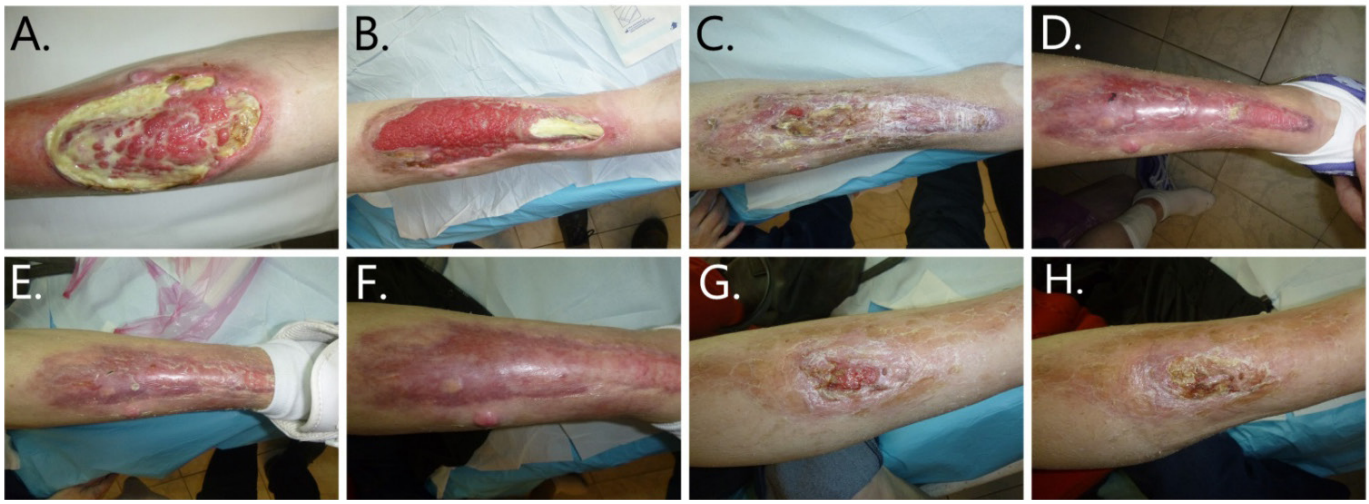

Fig. 1. Extensive necrobiosis lipoidica diabeticorum lesions in a 63 year old female. One can see the initial aspect of the left leg wound (A) and different stages of evolution during the treatment period: 2 months (B), 3 months (C), 4 months (D), after the formation of the scarring tissue (E) and after the application of hidrating cremes (F). While the lesion on the left leg was healing, another one appeared on the right leg (G). The same conservative treatment was used (H).

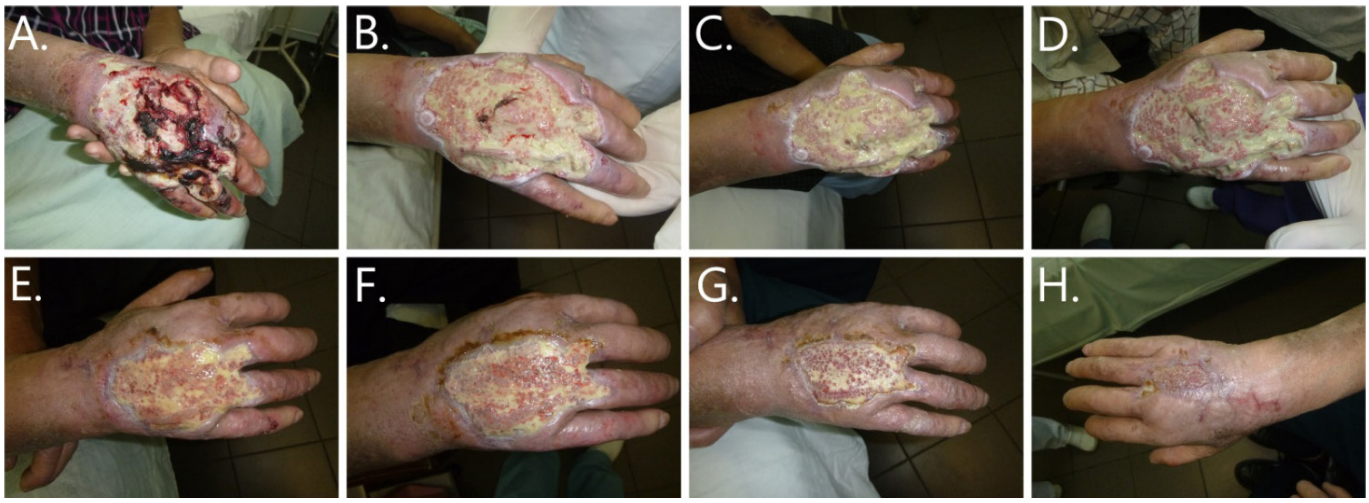

Fig. 2. Necrobiosis lipoidica diabeticorum lesions in a 64 year old male, who developed a spontaneous necrosis on the right forearm and hand (A). The surgical removal of the necrotic tissues and the autolytic debridement of the wounds was performed (B). Several stages of the wound evolution during the autolytic debridement can be seen in pictures B-G, leading to the final aspect (H).

the dressings were replaced by specific polyurethane foam dressings and nitrocellulose dressings (Figure 2C).

After the application of the absorbent dressings, which encouraged the autolytic debridement process, one can see that the exsudate and fibrin deposits have significally diminished and a granulation process started (Figure 2D).

The autolytic debridement and granulation development have been two long term processes due to the fact that deep vein thrombosis appeared on the right forearm and arm, which eventually led to the reccurence of the abundent exsudate and formation of new fibrin deposits but with a lower intensity. About 2-3 months have passed from the beginning to the wound aspect seen in Figure 2E.

In figure 2F, one can see how (when alternating the four therapeutic factors silver sulfadiazine, povidone-iodine, polyurethane foam dressings and nitrocellulose absorbent dressings), the initial lesion shrunk progressively and the granulation tissue islands increased in number.

About 4 months later, the wound margins started to get progressively smaller, the lesion reaching less than half of its initial size. A thin marginal epithelialization lining appeared which will lead to the reduction of the lesional surface (Figure 2G).

Finally, after 4-5 months of conservative treatment, the whole lesion healed completely with minimum scarring and about 70% of its initial functionality gained back. To be noted that conservative treatment was the only way to keep the right upper limb, since the already existing therapeutic fistulas and the frequent thrombosis would have been an indication for above elbow amputation. Even though the therapeutic period was long, the treatment was a success, since the final morphological and functional result was spectacular, saving the patient's hand (Figure 2H).

Discussions

In necrobiosis lipoidica diabeticorum, the surgical removal of slough and necrotic tissues is not suitable for the wound healing process because such aggressive procedures usually remove healthy tissues and new formed blood vessels which are extremely important for the proliferative phase of healing. So, we preferred to deal with it conservatively, excepting the full-thickness necrosis located on the distal part of the right upper limb as mentioned above.

Conclusions

Necrobiosis lipoidica diabeticorum is a dramatic condition, requiring a fully documented surgical approach in order to prevent unnecessary loss of healthy tissues. All cases treated in the last 20 years in the Plastic Surgery Department of the Clinical County hospital of Targu Mures healed spontaneously in a pretty long period of time but with no need for skin grafting of the extensive ulcerations and almost no need for mutilating amputations.

Authors' contribution

C.A. (Data collection and analyzing; Conceptualization; Writing – original draft)

B.A. (Data collection; Study coordinator; Conceptualization; Writing – supervision, review & editing)

Conflicts of interest

None to declare.

Informed consent

This paper is one of the chapters of my graduation degree for which data collection was approved by the Ethics Commitee.

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