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REVIEW ARTICLE

TRENDS IN LABORATORY DIAGNOSTIC METHODS IN PERIODONTOLOGY

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Summary: This work presents a summary of current knowledge on the laboratory diagnosis of periodontitis. It focuses on the theoretical foundations and is supplemented with new knowledge. It subsequently describes specifically the laboratory diagnosis methods of periodontitis: the protein expression of inflammation, oral microbiology and molecular diagnostics. Periodontitis is a serious disease worldwide and its confirmed association with systemic diseases means its severity is increasing. Its laboratory diagnosis has the potential to rise to the level of clinical and diagnostic imaging. The transfer of diagnostic methods from laboratory to clinical use is increasingly used in the prevention and monitoring of the exacerbation and treatment of periodontal disease, as well as of its impact on systemic disease.

Keywords: *Periodontitis; Diagnosis; Biomarkers; Saliva; Gingival crevicular fluid; Point-of care testing; Markers of bone remodelling*

Introduction

Periodontitis is generally defined as an inflammatory disease of the supporting tissues of the tooth, caused especially by a particular microorganism or group of microorganisms, resulting in progressive destruction of the supporting tissues of the tooth – the periodontal tissue. The undeniable role of bacterial infection in the pathogenesis of the disease is now known to be accompanied by the individual's immune and inflammatory response under the influence of external (e.g. dental plaque) and internal factors (genetic makeup of the individual).

It is believed that over 50% of the European population suffers from various forms of periodontitis, and in more than 10% this condition is serious. The population of those 60 to 65 years old has a prevalence that runs as high as 70–85% (24). Periodontal disease appears to be more common in men than in women (44). It has repeatedly been demonstrated that especially periodontitis, may affect the course of a number of systemic diseases, such as coronary heart disease and stroke, diabetes mellitus, osteoporosis, respiratory diseases and also increases risk of low birth weight (31). The threats posed by periodontal diseases to individuals with chronic diseases is caused by three principal mechanism: (i) metastatic spread of infection from the oral cavity as a result of transient bacteremia, (ii) metastatic injury from the effects of circulating oral microbial toxins, and (iii) metastatic inflammation caused by immunological injury induced by oral microorganisms (43).

The diagnostic possibilities of periodontal diseases are based on knowledge of their aetiology and pathogenesis. In periodontitis methods to date have focused primarily on the protein expression of inflammation and tissue destruction and oral microbiology. Molecular biochemistry has also brought more recent knowledge about this disease (see Fig. 1).

An element of dentistry is the diagnosis of periodontal disease and monitoring of traditional parameters, which includes the probing depth (PD) of the gingival sulcus, the gingival index (GI), clinical evaluation of insertion (clinical attachment level – CAL), gingival recession (GR), bleeding on probing (BOP), the plaque index (PI) and radiodiagnostical analysis. These parameters, however, have their limitations in fact, disadvantages. These include, in particular:

1. these diagnostic parameters are an excellent indicator of history of the disease, however, if we do not have standardized long-term measurements, only limited opportunities for determining the further development of the disease is provided;
2. the damage must be significant in order for these parameters to provide information about the severity of the disease.

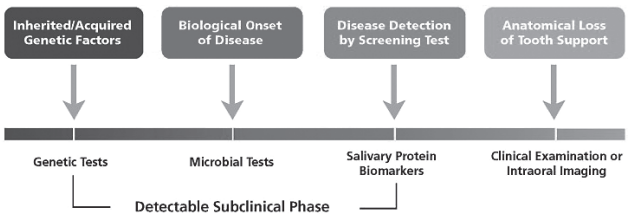

Fig. 1: Timeline of periodontal disease progression.
(Source: Giannobile WV. Salivary diagnostics for periodontal disease. JADA 2012; 143(suppl 10): 6S–11S.)

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As a result of these disadvantages, laboratory molecular-biochemical processes are increasingly used for the needs of early diagnosis and predicting the worsening of the disease with emphasis on their usefulness in routine outpatient practice.

Biological material

The use of conventional biological materials, such as whole blood (serum/plasma) and urine, are disadvantageous, because of the initially local nature of the disease. A specific marker characteristic only for periodontal tissues has not yet been found. Based on these facts, the most appropriate biological materials are therefore considered to be gingival crevicular fluid and saliva.

Gingival crevicular fluid

Sulcus fluid (GCF – Gingival crevicular fluid) penetrates into the gingival sulcus from the gingival tissue. It arises as a plasma transudate or more often as a result of inflammatory exudation. Its physiological function is to clean the gingival sulcus; penetrated proteins facilitate the adherence of the free gingiva to the tooth, and the fluid also exhibits antimicrobial properties. In a healthy periodontal tissue it forms in a minimum amount; however, under pathological circumstances formation harply increases (up to thirty-fold).

The main advantage of using it is the site specificity, significantly visible in orthodontic therapy (20). In addition, GCF may be collected as peri-implant sulcular fluid (PISF) from the gingival cuff that surrounds a dental implant (3). The most commonly used devices for collection are strips of filter paper and micropipettes.

Methods for measuring only the volume of the fluid are nowadays considered insufficient and have been replaced with the composition analyses, which can identify individual proteins of inflammatory and immune responses, proteinase inhibitors, hydrolytic enzymes, intracellular proteins, construction proteins of the cytoskeleton and apoptotic and signalling proteins (6, 40). Based on the above-mentioned analyses, it is possible to determine the current level of periodontal damage, and the future course of the disease can be anticipated. Although analysis of gingival crevicular fluid helps provide an explanation of the inflammatory response in periodontitis, multiple collection of samples with the assistance of traditional filter papers is currently considered to be impractical in clinical practice (22, 27). In recent years gingival crevicular fluid been revived as an excellent indicator of the current local state of the periodontal tissue during the testing of local application of biodegradable nanoparticles of drugs (50).

Saliva

Biological material such as saliva, given its natural presence in the oral cavity, is also available for research on potential markers of periodontitis. It has already been used in diagnostics for a number of systemic diseases, and the use of saliva is also commercially available through many tests. Saliva is formed by the mixing of liquid products of the large and small salivary glands and also includes components of gingival crevicular exudate, expectorated bronchial secretions, serum, blood cells from the oral micro-wounds, bacteria and their products, viruses, fungi, peeled epithelial cells and food particles (10). The main advantages of saliva as a biological material are the painless, non-invasive collection, the ability to repeat sampling and the easy transport and storage. In addition, the collection of saliva does not require a trained person; delivery is safer for staff and saliva is considered to be a "real-time" material. This attribute makes saliva suitable for monitoring children, the elderly and non-cooperative patients and not only in circumstances where the collection of blood or urine is not possible. The most appropriate method for collecting whole (glandular non-specific) saliva is considered to be the drainage method (drip off the bottom lip, spitting directly into the container) (37).

Potential biomarkers of periodontitis in saliva

The main groups of potential markers for periodontitis include: inflammatory markers, markers of connective tissue destruction and bone remodelling markers.

Markers of inflammation

Inflammation is often seen as something noxious for the body, but its current definition is completely the opposite. Every case of inflammation has a primary defence mission. This can manifest itself by an acute cell response, and if the complaint persists for a long enough time, it may turn into a chronic response. Objectively identifiable mediators are used as inflammatory biomarkers. The most commonly used markers for detection in periodontitis are mainly β-glucuronidase (GUS), C-reactive protein (CRP), interleukin-1 (IL-1), interleukin-6 (IL-6), macrophage inflammatory protein 1α (MIP-1α) and tumour necrosis factor (TNF) (36). The level of β-glucuronidase, which signals the influx of neutrophils, was confirmed in correlation with the increasing severity of periodontitis (28). Similarly, this correlation was confirmed in IL-1β, an important pro-inflammatory cytokine that is the predominant form of periodontitis (two forms: IL-1 alpha and IL-1 beta) and also in TNF-α (7, 8, 48). The newly identified anti-inflammatory interleukin IL-35, which is a member of the IL-12 family, is secreted by regulatory T-cells and suppresses the inflammatory response of immune cells. In one study it had significantly the highest level in saliva in a healthy group compared with groups with periodontal disease (gingivitis, chronic periodontitis), which shows its important role in the suppression of periodontal inflammation and maintaining of periodontal health (25).

Markers of connective tissue breakdown

The breakdown of connective tissue is responsible for pathogenesis of chronic inflammatory conditions and this also occurs in periodontitis. Matrix degradation is initiated by proteases produced locally at the site of inflammation and is balanced with their inhibitors. The degree of balance appears to be decisive for the progression of chronic periodontitis. The most commonly assessed are α2-macroglobulin, matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs) and alanine aminotransferase (ALT) levels. Levels of α2-macroglobulin, which is intended for the inactivation of different proteases including metalloproteinases and collagenase, are recorded as reduced in adult patients with periodontal disease, which under these conditions shows an imbalance between proteases and their inhibitors (1). This idea is supported by findings of an increase in tissue inhibitors in saliva after conventional treatment of periodontitis (13). The most important proteolytic enzymes are the matrix metalloproteinases MMP-8 (collagenase-2) and MMP-9 (gelatinase), a significant increase of which was confirmed in the saliva of patients with periodontitis. Monitoring of the level of MMP-8 in particular has potential for clinical use (41, 33).

Markers of bone remodelling

Salivary markers of alveolar bone remodelling (bone resorption/bone formation) are still not described as being as good as the biomarkers of the two above-mentioned groups. And the episodic nature of this process, which occurs during the progression of periodontal disease (predominantly resorption), also participates in this. This subject still requires longitudinal studies, but such research is much more expensive than cross-sectional studies. Longitudinal studies would be especially suitable for patients with aggressive periodontitis (36). The main problem with these biomarkers lies in their extremely low concentrations at the time of remission and at the time of exacerbation of premature degradation in saliva (8). The most important such biomarkers are alkaline phosphatase (ALP), C-terminal telopeptide (carboxy-terminal collagen crosslinks or CTX) and a recent receptor activator of nuclear factor kappa-B ligand (receptor activator of NF-κB or RANKL) and osteoprotegerin (OPG). Consequently, the historical performance of studies is often contradictory to longitudinal studies, and larger groups of patients could provide beneficial data on these bone markers in the context of periodontal disease.

Creating of diagnostic panel for periodontitis

In general, one could say that accurate diagnostic information about this disease can be obtained if a combination of appropriate biomarkers with the necessary sensitivity and specificity is created. For the purpose of threshold determination for periodontitis, several combinations of parameters were used; in one case the combination of MMP-8 and IL-1β showed an association with a significantly higher risk for periodontal disease. Combinations of these parameters also more often exhibit a positive predictive value for confirmation of the disease (36, 8).

Oral microbiology

An inseparable part of laboratory diagnosis of periodontal disease is indisputably microbiological diagnosis. The oral cavity has two special features with regard to microorganisms: it includes various micro environments which are contained in one complex, and microorganisms do not live as single species, but in colonies. Characteristic is the colonization of opportunistic microorganisms, which un-

Tab.1: Typical prevalent bacterial composition in selected cases of periodontitis.
(Source: Lamont RJ., Jenkinson HF. Oral microbiology at Glance. 2010, Wiley-Blackwell – modified.)

Healthy periodont	Gingivitis	Chronic periodontitis	Agressive periodontitis
G^+ *S. oralis, mitis, S. gordonii, sanguinis, Actinomyces gerencseriae, Actinomyces naeslundi*	**G^+** *Lactobacillus species, Actinomyces naeslundii, Peptostreptococcus micros, Streptococcus onginosus, Fusobacterium nucleatum*	**G^+** *Eubacterium brachy, Eubacterium nodatum, Peptostreptococcus stomatis*	
G^- *Fusobacterium species, Prevotella nigrescens, Veillonella species*	**G^-** *Prevotella intermedia, Fusobacterium nucleatum, Campylobacter species, Haemophilus species, Selenomonas species, Treponema species*	**G^-** *Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, Campylobacter rectus, Prevotella intermedia, Fusobacterium nucleatum*	**G^-** *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia, Prevotella intermedia, Prevotella nigrescens, Fusobacterium nucleatum, Campylobacter rectus*

der certain conditions are capable of causing disease (26). Equally characteristic is the presence of certain bacterial species at certain stages of periodontal diseases (see Table 1). Microorganisms, especially in the subgingival area, can activate a cascade of defence mechanisms associated with the production of factors causing inflammation and tissue destruction.

Options for microbiological diagnosis of periodontal diseases

The culturing of oral bacteria is the gold standard that is generally used, but this method has its pitfalls, especially tracking the presence of anaerobic species. Their culture result is often underestimated and only living bacteria can be culture, of course; their transport is also difficult, and many require specific conditions for growth. Finally, this method also requires special laboratory equipment and experienced staff and is very time consuming. All of these drawbacks led to the introduction of PCR (Polymerase Chain Reaction) for the identification of periodontal pathogens by their species-specific DNA sequence (42).

Qualitative methods

Qualitative methods (PCR based methods, enzymatic methods) are useful for confirming the presence of a certain type of bacteria, but say nothing about their quantity. They should be used for monitoring changes in the composition of bacteria after treatment in comparison with situation before treatment, which would help to assess its effectiveness (52).

Quantitative methods

Real-time PCR is used for measuring the amount of DNA. It can be used for both qualitative and quantitative analysis. This method has also been applied to measure the number of bacteria in periodontal diseases in samples such as saliva and GCF. The results showed, for example, a significant correlation between the number of bacteria and a deep pocket. Specifically, the number of *Porphyromonas gingivalis* increased to ten-times each growing millimetre of pocket depth (21, 52).

Molecular diagnosis in periodontology

Destructive periodontal diseases in the generally accepted view are initiated by changes in the bacterial flora, which triggers an immune response in susceptible individuals. This immune response is dependent on the nature and virulence of the pathogen. However, in most cases, the presence of a particular microorganism alone is not sufficient to initiate the disease. These findings suggest that environmental and genetic factors may influence the development of the disease. Specific genetic mutations that are responsible for causing periodontal diseases are rare and fortunately do not characterize the most common forms of periodontitis. Family cumulation of this disease is uncommon, and finding it can mean the impact of genetic predisposition but also exposure to the same external environmental influences (38). According to the studies conducted in twins, it appears that less than half of such variability is accounted for by genetic variability (35). Currently, studies are focusing on genetic polymorphisms of candidate genes associated with disease susceptibility (45).

Gene polymorphism in periodontitis

Genetic polymorphism is a form of a gene (allelic variant) which is found in an amount of at least 1% of a population, which is the border between a polymorphism and a mutation, while a mutation is referred to as an allele frequency of less than 1% in the population (32). Genetic polymorphism is more frequent in the population than mutation, and correlation between genetic polymorphism and disease is generally weaker than the functional relationship between mutation and disease (47).

The most studied polymorphisms in relation to periodontitis include the genes for cytokines (particularly IL-1 and IL-6), the genes for the receptors (Fc gamma receptor, Toll-like receptor), the genes for the RANK/RANKL/OPG and genes that encode enzymes (cathepsin C, matrix metalloproteinases, cyclooxygenase 2, myeloperoxidase, N-acetyl-transferase). The results to date are not clear, because they have not been obtained from a larger groups of patients, but some results are already known, e.g. the polymorphisms in Fc receptor III b, which serves (Fc receptor III b) first and foremost as a binding site for IgG on phagocytic cells, affects their mutual binding affinity and some of which are considered to be susceptible to periodontal disease. It has two polymorphisms, referred to as NA1 and NA2. The FcγRIIIb-NA2 allele and NA2/NA2 genotype occurred more frequently in controls and NA2/NA2 again more frequently in patients with the generalized aggressive form of periodontitis (GAgP) (16). The distribution of genotypes was significantly different among different races, and it seems that the relationship between FcγR polymorphisms and periodontal disease is associated with racial affiliation (9, 23, 49). Homozygous carriers of the polymorphism of a myeloperoxidase (−463 G / G) are at increased risk of periodontitis they are at the same time smokers (34). The situation is reversed e.g. in one polymorphism in the gene for COX 2 (cyclooxygenasis 2). Representation mostly covers a single nucleotide polymorphism (−756 G → C) and its protective effect in particular from aggressive periodontitis (15).

Several studies of the relationship between gene polymorphisms (for example matrix metalloproteinase 8, toll-like receptor 4, apolipoprotein E, interleukin 8) in patients with periodontitis were performed also in Czech population (4, 5, 18, 19).

To date, major gene mutations, which result in the periodontitis phenotype in otherwise systemically healthy

individuals, have not been identified and no specific genetic risk factor for the disease has been identified. The aim of the test is to determine several relatively common high-risk polymorphisms, that may mean cumulative high susceptibility genetic profile (47).

Epigenetic changes in periodontal diseases

In term of genetics, not only a mutation or polymorphism in a gene impacts an individual. Epigenetics is the science that deals with the study of changes in gene expression, which do not involve changes in the DNA sequence. Epigenetics is applied, for example, in the chemical modification of DNA and its proteins by blocking the binding of transcription factors, including the modifications of histones and DNA methylation (2). In subjects with a severe form of periodontitis hypomethylation of the gene for IL-6 was observed, which incites its increased expression in tissue affected by inflammation. Of interest are the speculations that long-standing inflammation and bacterial infection can also result in the methylation of DNA, which inactivates the suppression of cytokine signalling and contributes to the exaggeration of the inflammation (46). The number of studies on epigenetic changes in periodontitis is rapidly increasing (29)

Genetic testing

For the detection of polymorphisms PCR-RFLP (Restriction Fragment Length Polymorphism) is frequently used, which is based on the existence of restriction endonucleases that cleave the DNA polynucleotide chain within the area of the phosphodiester bonds in certain specific sequences. However, today many other methods for detecting polymorphisms and modification of DNA are known. For example, a genetic test is available for general public which is focused on severe chronic periodontitis (for IL-1 and IL-6 genetic variations). It focuses on specific polymorphisms that are associated with the disease in the respective country and by race (11).

Recent trends in the diagnosis of periodontal diseases

The transfer of possibilities for diagnostics of periodontal diseases that are easily incorporated into routine dental practice could mean earlier, simpler and more intensive treatment, which would likely bring even more cost-effective oral healthcare. Patients would benefit from tests carried out at home, according to the demands of conducting them, and sold without a prescription. The introduction of such measures would likely increase personal interest in treatment and overall compliance with the proposed therapeutic recommendations.

For this purpose devices that are generally used in the place where the patient is located should serve and permanently allocated space and tests performed outside the central laboratory should not be required for them. These tests will reduce the cost of transportation, packaging, handling, storage and tracking samples to the central laboratory, thus reducing the likelihood of sample contamination or sample confusion, loss or degradation (30). The device should combine and use modern nanomaterials, microfluidic engineering and microelectronics for the practical creation of miniature sensors. Importantly, the results of the use of these miniature sensors thus far correlate with standard methods used at present (36). In terms of using an oral fluid, several tests are now commercially available, for example, that allow detection of antibodies to HIV, steroid hormones, alcohol and drugs as well as forensic and genetic analysis (14).

Nano-biochips, which integrate various laboratory procedures in a single cartridge (device), are currently considered to be the most appropriate for this type of diagnosis. A saliva sample (100–300 microlitres) or a drop of blood is sufficient for the diagnosis. A network of liquid components ensures complete transfer and processing of salivary samples for multiple analyses in order to provide quantitative and qualitative information on the target biomarkers of disease. Starting the analysis is automatic, without the need for human intervention, and the internal flexibility of the software allows further modifications. The biochips used are disposed of as solid organic waste (36). The principle of the analysis may like that for immunoassays, the most common of which are the LF strip and the ELISA method.

More complicated was the problem of using biochips in molecular diagnostics. It is now possible to carry out analysis of a nucleic acid in a continuous flow simultaneously with the necessary temperature control (see Fig. 2). All the reagents are present on the chip; the temperature is controlled

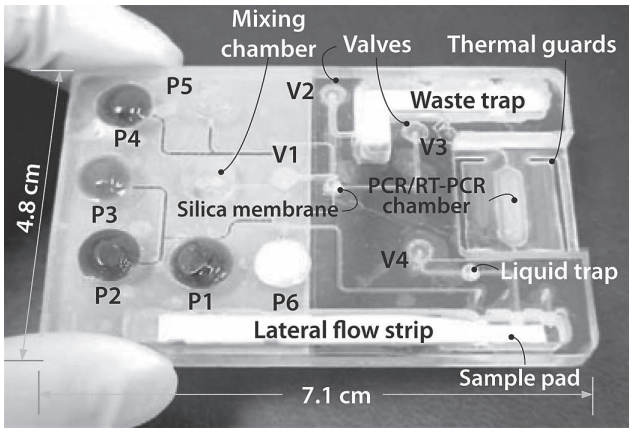

Fig. 2: Disposable cartridge for PCR.
The plastic case includes a network for microfluidic lysis, isolation of nucleic acids on a solid phase, PCR and PCR-detection products labelled with phosphorus on an LF strip.
(Source: Hart RW, Mauk MG, Liu C, Qiu X, Thompson JA, Chen D, Malamud D, Abrams WR, Bau HH. Point-of-care oral-based diagnostics. Oral Dis 2011 Nov; 17(8): 745–52.)

by thermal chamber, and the individual reagents are placed in a wax which melts during the initial heating and reagents are hydrated in the kit. The amplification process can be observed in real time, when using an appropriate colour, e.g. SYBR Green, a fluorescent dye that emits light only when the intercalate with double-stranded DNA (17). A modified standard PCR method, which is called the LAMP (Loop-mediated isothermal amplification), may be at approximately the same temperature to carry out the amplification (multiplication of the DNA) in a short time (approx. 1 hour), and the result is visible to the naked eye.

Perspective of PoCT in periodontics

With the convergence of microfluidic techniques and diagnosis of oral fluids, it would be possible to diagnose and monitor a patient with on-site testing, in an outpatient department, at home, or even in remote areas. The purpose is to support individualized treatment, or "treatment tailored for each patient". And oral fluids (saliva, GCF) are ideal for such measurements. Point-of-care testing (PoCT) may be of particular interest in the dental community because patients usually visit a dentist more frequently than a general physician (39).

Conclusion

Oral health does not mean only an attractive smile; the term encompasses a comprehensive view of the oral cavity under physiological conditions. Today we know that pathologies in this area can affect the overall condition of the body, and the connection is evaluated in the context of many systemic diseases.

Periodontal diseases are still living issue. Although periodontal diagnostic testing initially served to delimit patients at higher risk for developing this disease, the future of these laboratory tests is now extended to patients at risk of developing systemic diseases caused by periodontitis, and if this risk is confirmed, the disease may be reduced by effective treatment. Finding suitable markers, whether for early diagnosis, exacerbation or other consequences of this disease would mean not only a reduction of the suffering of more than tens of millions of people around the world, but also cut the cost of their treatment.

Currently, saliva has come to the forefront as a biological material. Equally high hopes are placed on molecular diagnostics. Some tests are already available commercially, but their acceptance in medical practice is slow, in part due to the lack of treatment algorithms that would give clear guidance for their use in the provision of health care. Scientists see the future of complex scientific research in this area in a comprehensive approach to the examination of biological materials using equipment that would be able to simply, specifically and sensitively investigate suitable parameters, even in outpatient clinic, and perhaps include additional screening parameters for other diseases.

The question is whether we are separated from such results by a great deal of research work or only a few small steps.

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ORIGINAL ARTICLE

IS ALLERGIC RHINITIS A FACTOR THAT AFFECTS SUCCESS OF TYMPANOPLASTY?

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Summary: Objective: The aim of the present study was to investigate the effect of allergic rhinitis on the success of the operation in chronic otitis surgery by using score for allergic rhinitis (SFAR). Materials and Methods: In the present study; 121 patients, who underwent type 1 tympanoplasty were examined retrospectively. SFAR of all patients were recorded. The graft success rates of 26 patients with allergic rhinitis (AR) and 95 patients with no allergic rhinitis group (NAR) were compared. Results: While the graft success rate in NAR group was 89.5%, this rate was 80.8% in the AR group. However, the difference between groups was not statistically significant (p = 0.311). Conclusion: These findings suggest that allergic rhinitis decreases the graft success rate of the pathologies occurring in eustachian tube, middle ear and mastoid although statistically significant difference wasn't found. Prospective studies with larger patient groups are required in order to evaluate this pathology.

Keywords: *Allergic rhinitis; Tympanoplasty; Graft success rate; Chronic otitis surgery; Middle ear pathology*

Introduction

Since the first application of tympanoplasty in chronic otitis surgery by Zöllner (1) and Wullstein (2) in 1952, various graft materials and techniques have been used. Today, despite different operation techniques and grafts, success rates still vary widely (3–5). Graft success rates are affected by various factors such as perforation size and the type of middle ear pathology (chronic tubal dysfunction, pathological middle ear mucosa).

Negative effects of nasal mucosa pathologies and eustachian tube dysfunction on middle ear and mastoid are known (6–10). It has been shown in various studies that allergic rhinitis affects nasal mucosa and eustachian tube functions (6–10). Just as allergic reaction affects the nasal mucosa and nasopharyngeal mucosa, it can also affect the middle ear and eustachian tube mucosa (8–11). In numerous studies, it was shown that there is an increase in allergic rhinitis prevalence in the patients with chronic otitis media with effusion (11, 12). Allergic rhinitis has a high prevalence (10% and 54%) and its effects on middle ear and eustachian functions are well recognized. However its effect on the operation success in chronic otitis surgery has not been investigated (13–15). Allergic symptom history, in vivo and in vitro tests are used in the diagnosis of allergic rhinitis (16). However in the studies performed, it has been shown that SFAR (17, 18) correlates with standard diagnostic tests and it can also be used in the diagnosis and treatment of allergic rhinitis.

In patients undergoing tympanoplasty operation, demographical characteristics and middle ear pathologies were similar. Graft success rates were compared in the patients with and without allergic rhinitis by using the SFAR score.

Materials and Methods

In the present study, 121 patients with type 1 tympanoplasty were retrospectively examined between 2008 and 2013. Detailed history was taken from the patients and micro otoscopic physical examinations and laboratory tests were carried out. After the evaluations of all patients were completed, SFAR was recorded. Allergic rhinitis patient group consists of the patients with the score of 7 or more, as defined (Table 1). The patients, who did not have medical treatment of allergic rhinitis before operation, were included in the study. Treatment of allergic rhinitis was not given in postoperative early period.

There were 26 patients in the allergic rhinitis group (AR) and 95 patients in no allergic rhinitis group (NAR). Perforations in these patients consist of subtotal perforations with over 75% of the tympanum membrane surface area. The patients with ossicular chain defect, pathological middle ear mucosa, cholesteatoma, tympanosclerosis and otorrhea are excluded from the study. All of the patients had preoperative

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Tab. 1: SFAR (Score For Allergic Rhinitis) (17).

Items/discriminators	Score	Cumulative score
Blocked nose, runny nose, sneezing in past year (nasal symptoms)	1 for each symptom	3
Months of the year	1 for perennial 1 for pollen season	5
Nasal symptoms plus itchy eyes (rhinoconjunctivitis)	2	7
Triggers: Pollens, house dust mites, dust Epithelia (cat, dog)	2 1	9
Perceived allergic status	2	11
Previous positive allergic tests	2	13
Previous medical diagnosis of allergy	1	14
Familial history of allergy	2	16
Total points		16

temporal CT examinations and those with mastoid pathology were excluded from the study.

When all the patients were evaluated, follow-up period was determined to vary between 1 and 4 years. Average follow-up period of the patients in AR group was 2.5 (1–4) years and average follow-up period of the patients in NAR group 3 (1–4) years. The graft success rates of type 1 tympanoplasy in 26 patients with allergic rhinitis (AR) (14 F, 12 M average 36.9 ± 14.7) and 95 patients with no allergic rhinitis group (NAR) (51 F, 44 M, average 31.6 ± 13.3) were compared.

Type 1 tympanoplasty operation was performed on 27 patients by using chondroperichondrial island graft and on 94 patients by using temporal muscle fascia. Operation procedure was standardized for both groups. Under general anesthesia, over-underlay technique was used by a postauricular approach. We used classical "over underlay" technique with cartilage and fascia. The graft was placed over malleus and under the annulus.

The patients were called for the controls in the postoperative first, second weeks and in the first month. In postoperative second week, spongostane and pomades with antibiotics in the external ear way of the patients were aspirated. No antibiotics were given preoperatively to the patients in neither of the groups. But oral penicillin was given to all patients postoperatively for 7 days in both groups. Then patients were followed with monthly follow-up.

The repair of tympanic membrane perforation was considered as success criterion of the operation. Reperforation was observed in 5 patients in AR group and in 10 patients in NAR group.

Statistical analysis

Data analysis was performed by using SPSS for Windows, version 11.5 (SPSS Inc., Chicago, IL, United States). Whether the distributions of metric discrete variables was normal or not was determined by Kolmogorov Smirnov test. Data were expressed as mean ± SD or median (min-max), where applicable. While the mean differences between groups were compared by Student's t test, otherwise, Mann Whitney U test was used for comparisons of the median values. Categorical data were analyzed by Pearson's Chi-square or Fisher's exact test, where appropriate. A p value less than 0.05 was considered statistically significant.

Results

Graft success rates of 26 patients in AR group who underwent type 1 tympanoplasty and 95 patients in NAR group were compared. When the groups were examined in terms of demographical properties; no statistically significant difference was observed ($p > 0.05$) (Table 2). No statistically significant difference was found between the groups in terms of gender, age, control period etc.

Tab. 2: Demographical characteristics.

Variables	NAR (n = 95)	AR (n = 26)	p-value
Age (years)	31.6 ± 13.3	36.9 ± 14.7	0.079†
Gender			0.988‡
Male	44 (46.3%)	12 (46.2%)	
Female	51 (53.7%)	14 (53.8%)	
Follow-up times (years)	3 (1–4)	2.5 (1–4)	0.406¶

† Student's t test, ‡ Pearson's chi-square test, ¶ Mann Whitney U test.

Average allergic rhinitis symptom score of 26 patients with allergic rhinitis was calculated as 10 (7–15) while that of 95 patients with no allergic rhinitis was 4 (1–6) (Table 3).

When the fascia and cartilage graft usage rates were compared in both groups, no statistically significant difference was found ($p = 0.151$) (Table 4).

Tab. 3: Descriptive statistics for SFAR scores.

SFAR score	NAR	AR
Number of cases	95	26
Mean	3.6	10.5
SD	1.34	2.32
Median	4	10
Minimum	1	7
Maximum	6	15

Tab. 4: The types of tympanoplasty in groups.

	NAR (n = 95)	AR (n = 26)	p-value
Tympanoplasty			0.151†
Fascia	75 (78.9%)	17 (65.4%)	
Cartilage	20 (21.1%)	9 (34.6%)	

† Pearson's chi-square test.

When the graft success rates were evaluated, while perforation ratio in the patients with allergic rhinitis (±) was 19.2%, it decreased to 10.5% in the patients with no allergic rhinitis. However, there was no statistically significant difference between groups (p = 0.311) (Figure 1).

No statistically significant difference was found between AR and NAR groups in terms of average age in patients with successful grafts (p = 0.146). No statistically significant difference was found between AR and NAR groups in terms of average age in patients with perforated grafts (p = 0.251).

Average age of patients with perforated grafts was statistically significant lower than patients with successful grafts in NAR group (p = 0.007). No statistically significant difference was found between patients with perforated grafts and patients with successful grafts in terms of average age in AR group (p = 0.727) (Table 5).

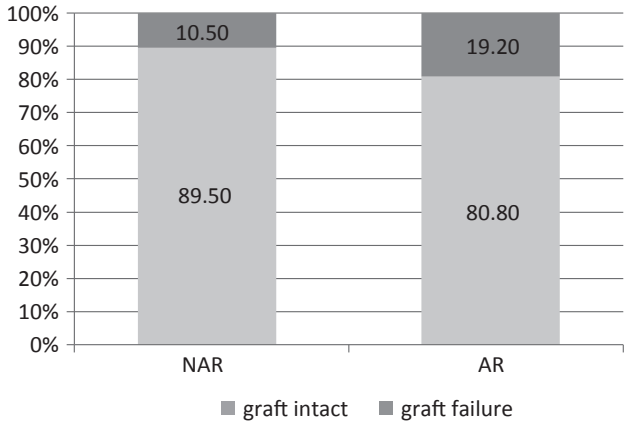

Fig. 1: Comparison of the graft success rate between the AR and NAR groups.

Tab. 5: Mean ages regarding for both allergic rhinitis and graft success.

	NAR	AR	p-value †¶
Intact	32.6 ± 13.5	37.4 ± 14.0	0.146
Perforated	23.2 ± 8.3	34.8 ± 19.0	0.251
p-value ‡¶	**0.007**	0.727	

† The comparisons between NAR and AR groups, according to the Bonferroni Correction p < 0.025 was considered as statistically significant, ‡ The comparisons between Intact and Perforated groups, according to the Bonferroni Correction p < 0.025 was considered as statistically significant, ¶ Student's t test.

Discussion

Prevalence of allergic rhinitis in the population varies between 10% and 54% (13–15). Despite its high prevalence and its negative effects on the middle ear and mastoid, its effect in chronic otitis surgery has not been investigated. It is not considered in surgery planning and evaluation of success criteria. To our knowledge, the present study is the first one in the literature investigating the effect of allergic rhinitis on tympanoplasty operation success.

Success rates in tympanoplasty still show variance despite various operation techniques and different grafts (4, 5, 19). Success rates in the literature vary depending on various factors such as the perforation size, weight of the middle ear pathology (chronic tubal dysfunction, pathological middle ear mucosa), technique applied, monitorization period, change in the number of cases etc.

Chronic tubal dysfunction has an important role between such factors. Effect of allergic rhinitis on nasal mucosa and eustachian tube functions is shown in various studies (6–10). Mediators and cytokines released during allergic reaction cause nasal and nasopharyngeal edema and hyper secretion, leading to eustachian dysfunction (8–11). In the studies performed so far; it has been shown that there is an increase in allergic rhinitis prevalence in the patients with chronic otitis media with effusion (11, 12, 20). In the study of Pelikan et al. (11), it was shown in 87 patients with chronic secretory otitis media that nasal allergy affects eustachian tube functions and middle ear pressure changes, causing deterioration of hearing functions. In the study of Alles et al. (12) performed in 209 children with chronic otitis media with effusion; prevalence of allergic rhinitis was found to be 89%. The role of allergy in otitis media with effusion can be correlated to various mechanisms. Exposure of middle ear mucosa to allergic reaction, nasal and nasopharyngeal inflammation and obstruction of the edema occurring in the eustachian tube and transmission of the bacteria from nasopharynx to the middle ear via hyper secretion due to allergic reaction are the essential factors.

In the diagnosis of allergic rhinitis; typical allergic symptom history and diagnostic tests are used (16). Diagnostic laboratory tests are in vivo (specific IgE etc.) and in vitro (skin tests) tests (16). SFAR (17) is an efficient test in the

determination of allergic rhinitis prevalence defined in 2002. In the studies performed; it was shown that SFAR correlates with standard diagnostic tests and that it can be used in the diagnosis and treatment of allergic rhinitis (17, 18). Ologe et al. (18) has stated that 94.8% sensitivity and 95.1% specificity can be obtained in allergic rhinitis diagnosis by using SFAR. In the present study; average allergic rhinitis symptom score of 26 cases with allergic rhinitis was 10.46 ± 2.32.

Graft success rates in the literature show variability (4, 19, 21). After 24-months of follow-up; Cabra et al. (4) found a success rate of 82% in the patients subjected to palisade cartilage tympanoplasty and 64% in the patients subjected to fascia tympanoplasty. Locovou et al. (19) have reported a success rate of 97.2% in their study in 2014 performed by using cartilage graft. Cavaliere et al. (5) have reported 100% success ratio in tympanoplasty performed by using cartilage shield graft in the study consisting of 236 patients. Such variation in success rates can be due to the technique applied, follow-up period and the variability in the number of cases. In the present study; while the graft success rate was 89.5% in the NAR group, it was found to be 80.8% in the AR group ($p = 0.311$). These findings suggest that allergic rhinitis decreases the graft success rate of the pathologies occurring in the nasal mucosa, eustachian tube, middle ear and mastoid, although statistically significant difference wasn't found. Studies with higher number of patients can show statistically significant difference. This pathology should be investigated in chronic otitis media surgery because of its active role in pathogenesis of secretory otitis media which has high prevalence (11–13). Studies with larger number of cases are required in order to evaluate this issue more thoroughly.

Conclusion

These findings suggest that allergic rhinitis decreases the graft success rate of the pathologies occurring in nasal mucosa, eustachian tube, middle ear and mastoid although statistically significant difference wasn't found. Prospective studies with larger patient groups are required in order to evaluate this pathology that influences middle ear and mastoid bone considerably.

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ORIGINAL ARTICLE

A STUDY COMPARING THE EFFICACY OF MONOPOLAR RADIOFREQUENCY AND GLYCOLIC ACID PEELS IN FACIAL REJUVENATION OF AGING SKIN USING HISTOPATHOLOGY AND ULTRABIOMICROSCOPIC SONOGRAPHY (UBM) – AN EVIDENCE BASED STUDY

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Summary: Background: Radio frequency (RF) and chemical peels have been used for nonablative skin rejuvenation. Both of these cause collagen remodeling in the dermis and neo-collagen formation resulting in facial rejuvenation. There is limited literature on the evaluation of collagen remodeling by objective methods. Objective: To compare the benefits of monopolar radiofrequency and glycolic acid peels in facial rejuvenation with regards to histopathology and Ultrabiomicroscopic sonography (UBM). Methodology: In this study, forty patients with mild to moderate photoaging received four treatments with 3 weeks interval of monopolar radiofrequency on one side of face and glycolic acid peels in increasing concentrations (Neostrata^{R}) on the other side. Pre and post treatment, 2 mm biopsies were taken from both preauricular areas and Ultrasonography using a 35 MHz probe was done from outer canthus of eye and nasolabial folds from both sides of face. A blinded assessment was done to measure the increase in the grenz zone and dermal thickness.
Results: In 35/40 patients there was a significant increase in the grenz zone on histopathology and decrease in subepidermal low-echogenic band (SLEB) on UBM of the nasolabial folds on both sides of the face ($p < 0.05$). Conclusion: Radiofrequency and chemical peels showed equal efficacy in the treatment of facial rejuvenation.

Keywords*: Radiofrequency; Chemical peels; Grenz zone; SLEB*

Introduction

There are two clinically and biologically distinct aging processes affecting the skin (1). The first is intrinsic aging, which affects the skin by slow, irreversible tissue degeneration. The second is extrinsic aging or photoaging, which is the effect of chronic exposure to ultraviolet radiation on skin. There are a myriad of therapeutic modalities that can improve photoaging. These modalities may be divided into topical agents and procedural agents. Topical agents include retinoids, hydroquinones, and combination therapies. Procedural agents include chemical peels, microdermabrasion, lasers, and intense pulse light and nonablative radiofrequency. Chemical peeling is a cosmetic procedure that involves the application of one or more exfoliating agents to the skin to wound the epidermis and dermis in a controlled manner. Monopolar radiofrequency (RF) is emerging to be a novel treatment in the field of cosmetic dermatology (2). Radiofrequency is the number of oscillations (or waves) per second of the electric and magnetic fields within the radio waves portion of the electromagnetic spectrum. When RF energy is applied to the skin, resistance encountered by the energy flow causes heat to be produced which causes tightening to the underlying tissue structures. When comparing RF to other non-ablative (procedures that do not involve the destruction of the outer layer of the skin) techniques, RF energy differs from laser energy in that lasers tends to scatter or absorb into the upper layers of the skin, making it difficult to deliver sufficient heat into the deeper layers without damaging the skin's surface while RF energy is able to penetrate deeper into the skin and affect the deeper dermis and subcutaneous layers causing skin tightening. RF energy produced is not affected by tissue diffraction or absorption by epidermal melanin. As such, RF-based systems are appropriate for any skin type. Both radiofrequency and chemical peels produce subsequent collagen remodeling and skin rejuvenation. The purpose of this study was to assess and objectively quantify the benefits of both the treatments in facial rejuvenation.

Materials and Methods

Subjects with mild to moderate signs of photoaging in terms of freckles, wrinkles and mild skin laxity in the age group of 35–55 years were enrolled in the study. The study was approved by the institutional review board. Treatment

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and study details were fully explained to subjects, and all signed an informed consent form. Inclusion criteria were bilateral facial changes due to sun damage. Exclusion criteria were photosensitivity to sunlight, any sign of infection or inflammatory skin disease, history of hypertrophic scars or keloids, use of oral isotretinoin or any photosensitizing drugs in the past 6 months. Monopolar radiofrequency was done on the left half of the face and glycolic acid peels in increasing concentrations (35, 50, and 70%) were done on the right half at an interval of three weeks. A total of four sittings of each modality were done. Findings were recorded in a clinical proforma.

Device and technique: The radiofrequency machine used in our study was a monopolar radiofrequency, Surgitron Ellman® generating a radiofrequency upto 6 MHz, consisting of a hand piece, rejuvenation probe, antenna and foot plate. A ball tip rejuvenation probe of 0.5 cm by 0.5 cm was used. A coupling gel as a cooling agent was applied to the skin. When the machine was on, the tip was placed against the skin. Radio frequency current passes from the tip to the return pad and back again. Holding the tip against the skin for the whole treatment cycle which consisted of 10 seconds, caused heating of the skin and tissue beneath it, causing the required contraction and tightening. Therefore in one cycle (10 sec), area of skin measuring 1 cm by 1 cm was treated. Each application to an area lasts for 10 seconds and then the probe was moved to the next area. The treatment was continued till mild erythema developed. The entire left half of the face was treated in this manner. Initially a constant frequency of 2.5 MHz was used and then increased according to the patient's tolerability in each visit. A maximum frequency of 4 MHz was used. On the right side of the face, glycolic acid peels (Neostrata^{R}) were applied for a total of 4 sittings. The peel cleansing solution (which is generally an alcohol based product) was applied to remove any final debris. Subjects were instructed to keep their eyes closed during the procedure. The peel procedure began by applying the glycolic acid to the face, beginning at the forehead and working it down over the cheeks, chin, nose, and upper cutaneous lip within 20 seconds. If blanching or frosting was encountered in any particular areas, then immediate neutralization was performed at that site. Once the skin achieved a uniform degree of erythema, full face neutralization was done with cool water. In each sitting increasing concentrations were applied (35, 50, 70%) on the right half of the face. After the procedure a constant sunscreen was given to the patient.

Assessment: During the treatment sessions, subjects were monitored for heat discomfort, edema, and intense erythema. Photographs were taken before and after treatment. Assessments were done on the basis of histopathology, UBM and subjective evaluation.

Histopathology: Punch biopsies (2 mm) were done at baseline and after the last treatment from the pre-auricular areas on both sides of the face. In photoaged skin, elastotic changes and thin collagen bundles are seen in the upper dermis called the "grenz zone" (3, 4) (Figure 1). After treatment, neo-collagen is deposited in the upper dermis which causes widening of the grenz zone. In our study, the thickest portion of the grenz zone was measured. This parameter was measured using an eye-piece micrometer. An eye-piece micrometer has a series of numbered lines inside of it which make it look like a ruler (x). After placing the special eyepiece, a calibration slide is used which is a glass slide 0.01 mm engraved on to its top surface(y). The eyepiece and the slide are mounted on the microscope on 40× and the number of lines are counted. The distance between each line of the eyepiece is calculated using an equation: y/x ×10 = measurement between two lines.

Ultrabiomicroscopic sonography (UBM): A 35 mHz ultrasonography probe of Paradigm® was placed against the outer canthus of eye and nasolabial folds from both sides of

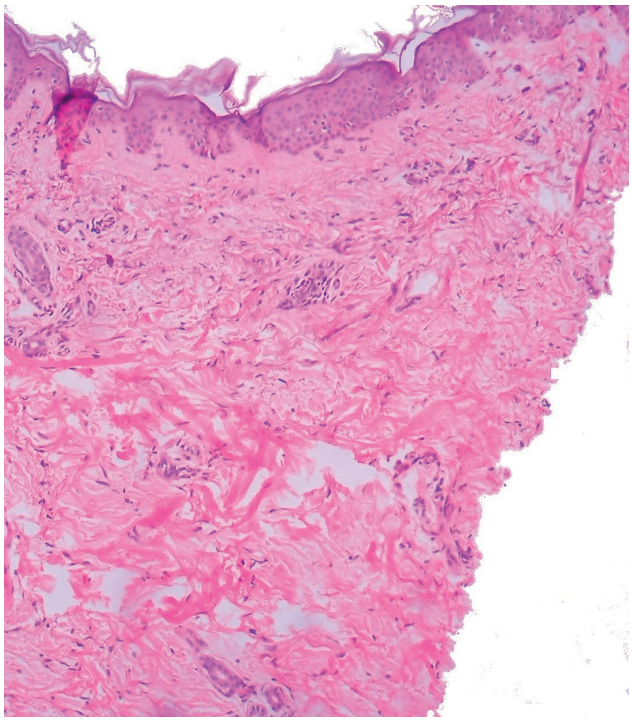

Fig. 1: 40× H&E: Histology of aging skin showing grenz zone.

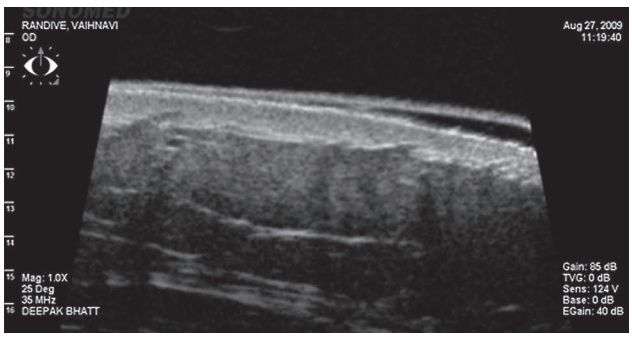

Fig. 2: Normal sonography skin showing epidermal entrance echo and dermal thickness (hyperechoeic region).

face to assess SLEB. The SLEB (subepidermal low-echogenic band) is defined as a clearly visual low echogenic band in the upper dermis immediately below the epidermal entrance echo. It is usually present in aging skin and is due to the degenerative changes in upper dermis. SLEB is seen in aging skin in 50% of individuals (3). After treatment the SLEB decreases and may disappear also because of collagen deposition (5, 6). The SLEB was measured with calipers inbuilt in the software. The images were captured on the software (Figure 2).

Subjective evaluation: All patients were asked to grade their clinical response after the treatment according to the treatment response scale (0 = no improvement, 1 = 1–25% improvement, 2 = 26–50% improvement, 3 = 51–75% improvement, and 4 = 76–100% improvement).

Statistical analysis: Quantitative and qualitative measurements were analyzed using the software package for statistical science (SPSS for Windows). Data were analyzed using the Greenhouse-Geisser test and Mann-Whitney test for parametric and non-parametric data respectively. Statistical significance was defined as P less than or equal to 0.05.

Results

Clinical evaluation: Out of 40 patients, only 35 patients completed the study. All 35 patients showed some clinical improvement of skin tightening (Figure 3) All subjects were asked to grade the improvement after the last treatment according to the treatment response scale. Only 3 patients had > 50% improvement on the chemical peel treated side and 24 patients had > 50% improvement on the radiofrequency treated side. The mean subjective evaluation was more on the radiofrequency treated side than the chemical peel treated side. However the comparison was not statistically significant.

Histopathological evaluation: On microscopic examination of hematoxylin-eosin stained sections various parameters like epidermal thickness and grenz zone were assessed. The mean epidermal thickness on the chemical peel treated side decreased from 41.07 μ to 35.50 μ and the mean epidermal thickness on the radiofrequency side decreased from 43.57 μ to 37.85 μ. This decrease within the chemical peel and radiofrequency group was statistically significant by applying the Greenhouse-geisser test of significance. The mean grenz zone increased from 23.75 μ to 57.94 μ after 4 sittings on the chemical peel treated side. The mean grenz zone increased from 25.92 μ to 57.50 μ after 4 sittings on the radiofrequency treated side. This increase within the chemical peel and radiofrequency group was statistically significant. However the comparison of the above parameters between the radiofrequency and chemical peel side was not statistically significant by applying the same test. (Figure 4a,b).

The treatment response on histopathology was assessed by an independent observer also. There was a correlation of more than 98% between the investigator and blinded observer.

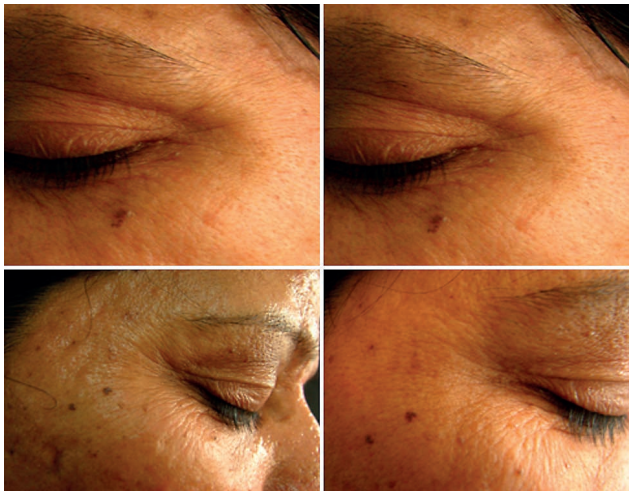

Fig. 3: Pretreatment (left) and posttreatment (right) on radiofrequency and chemical peel treated side.

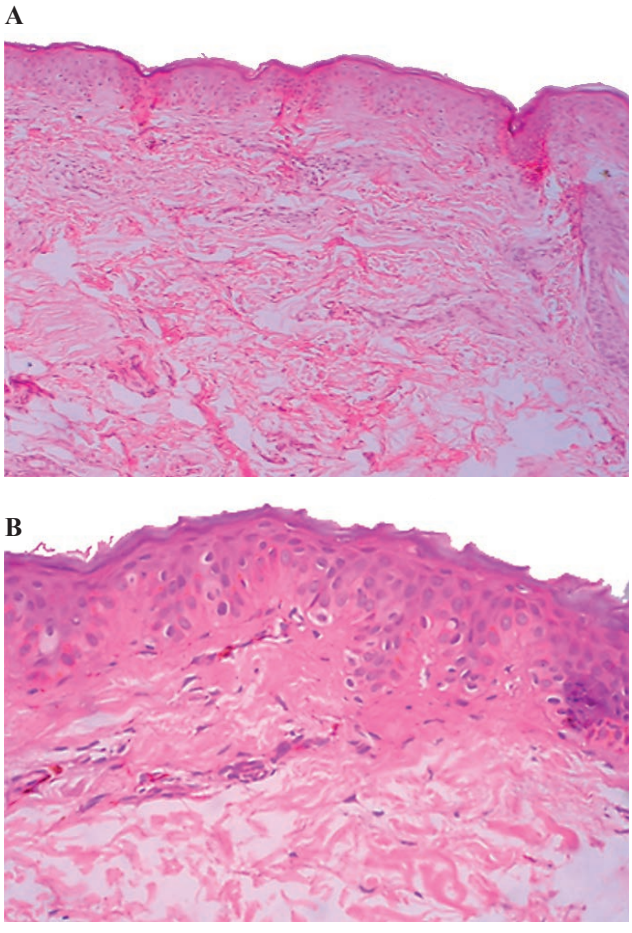

Fig. 4: Increase in the grenz zone after treatment.
(a) 10× H&E: Baseline: Histology of aging skin showing epidermal hyperplasia and disorganization of collagen bundles.
(b) 40× H&E: Box showing a well developed grenz zone seen.

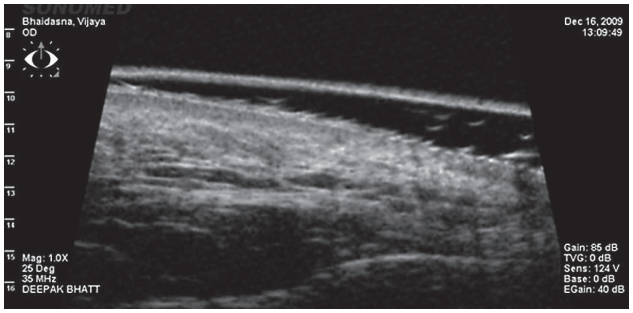

Fig. 5: Sonography shows absence of SLEB.

Ultrasonography evaluation: On UBM, subepidermal low-echogenic band (SLEB) was assessed. The mean SLEB from lateral canthus on the chemical peel treated side decreased 0.34 mm to 0.31 mm and decreased from 0.31 mm to 0.30 mm on the radiofrequency treated side. The decrease in SLEB pre and post treatment was not statistically significant by applying the Greenhouse-geisser test of significance. The mean SLEB from nasolabial fold from the radiofrequency treated side decreased 0.32 mm to 0.25 mm and from 0.30 mm to 0.21 mm on the chemical peel treated side. The decrease in SLEB was statistically significant on both sides. However the comparison of the above parameters between the radiofrequency and chemical peel side was not statistically significant by applying the same test (Figure 5).

Discussion

Facial rejuvenation is a science which focuses on the use of various techniques: ablative or non-ablative to yield impressive results. In the past, ablative techniques were used to achieve the same but due to disadvantages like increased downtime and prolonged recovery, a search for nonablative techniques were done. There is now an increased interest in a wide range of nonablative treatments of skin aging, which are used to rejuvenate skin with minimal downtime and complications. There are numerous studies on nonablative facial rejuvenation in literature documenting treatment response by subjective methods like photography (7).

There are a very few studies where objective parameters have been used to assess collagen remodelling. We used quantitative evaluation on histopathology and UBM at baseline and at the end of treatment to objectively evaluate the efficacy of anti-ageing procedures.

On histological analysis of pre and post procedure biopsies, we documented a significant mean decrease in the epidermal thickness on the chemical peel and radiofrequency treated side. Actually in reality, chronic UVB irradiation produces an irregular hypertrophy of the epidermis and rejuvenation treatment leads to normalisation of the epidermis (8). We postulate that the decrease in the thickness of the epidermis in our study is due to the normalisation of the epidermis and not true atrophy. There was a significant increase in the mean grenz zone on both sides of the face indicating a collagen deposition induced by radiofrequency and glycolic acid peels. There are no studies evaluating treatment response by measuring grenz zone in literature. Although radiofrequency and chemical peels have been used in various studies for facial rejuvenation few have histologically analyzed the skin of the subjects treated.

On sonography, we documented a decrease in SLEB between both the treatment groups on the nasolabial folds and the lateral canthus, however we found a significant decrease only on the nasolabial folds. There is no published study which has measured decrease in SLEB in literature. We found an increase in dermal thickness on the radiofrequency treated side. Ultrasonography assessment studies of radiofrequency and glycolic acid peels for facial rejuvenation have not been reported. There is no published study which has measured decrease in SLEB in literature. However we conclude that SLEB and Grenz zone are significant assessment parameters to study the effects of anti ageing pocedures on ageing skin.

In conclusion, both monopolar radiofrequency and glycolic acid peels are equally efficacious in facial rejuvenation. In our study we have made a sincere effort to quantitate improvement objectively.

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CASE REPORT

GASTRIC AND COLORECTAL METASTASES OF LOBULAR BREAST CARCINOMA: A CASE REPORT

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Summary: Background: Occurrence of gastric metastasis as the first symptom of breast carcinoma with a long period of latency before presentation of the primary breast carcinoma is rare. Case Report: A patient with gastric metastasis as the first symptom of lobular breast carcinoma, treated by neoadjuvant preoperative chemoradiotherapy and total gastrectomy, with complete local control. Fourteen months after presentation of the gastric metastasis a primary lobular breast carcinoma was discovered, treated by radiotherapy, chemotherapy and hormonal treatment with complete local response. Twenty-three months after diagnosis of breast cancer multiple colorectal metastases from the breast cancer occurred, which were treated by chemotherapy and hormonal treatment. Eighty-six months after diagnosis of gastric metastasis the patient died due to progression of cancer. Conclusions: Metastases to gastrointestinal or gynaecological tracts are more likely in invasive lobular carcinoma than invasive ductal cancer. The pathologist should determine whether or not they check estrogen and progesterone receptor status not simply by signet ring cell morphology but also by consideration of clinic-pathological correlation of the patient, such as the presence of a past history of breast cancer, or the colorectal localization of poorly differentiated carcinoma, which may occur less frequently than in the stomach.

Keywords: *Lobular breast carcinoma; Gastric metastasis from breast carcinoma; Multiple colorectal metastases from breast carcinoma*

Introduction

McLemore et al. reported that cases of gastrointestinal metastasis from primary breast cancer are as rare as 73 cases among 12,001 cases. From these, only 24 cases with colorectal metastasis were recorded (1). The occurrence of gastric metastasis as the first symptom of breast carcinoma with a long period of latency before presentation of the primary breast carcinoma in this case report is even more infrequent. We report a patient with gastric metastasis as the first symptom of lobular breast carcinoma, who was treated by neoadjuvant preoperative chemoradiotherapy and total gastrectomy, with complete local control. Fourteen months after presentation of the gastric metastasis a primary lobular breast carcinoma was detected, and then treated by radiotherapy, chemotherapy and hormonal treatment with complete local response. Twenty-three months after diagnosis of breast cancer multiple colorectal metastases from the breast cancer occurred, which were treated by chemotherapy and hormonal treatment. Thirty-eight months after diagnosis of multiple colorectal metastases ascites occurred, and subsequently edema of the lower extremities and bilateral pleural effusion. Eighty-six months after diagnosis of gastric metastasis the patient died due to progression of cancer.

Case report

A 58-year-old woman was examined for abdominal pain and weight loss of 6 kg. The biopsy from gastroscopy was assessed as a common dissociated gastric carcinoma with numerous signet ring cells (Figs. 1 and 2). Pretreatment stage was assessed as T2N0M0 according to endosonography, abdominal ultrasound, abdominal CT scan and chest X-ray, with normal pretreatment levels of CEA 3.39 µg/l, CA 19–9: 18.45 U/ml and CA 72–4: 3.12 U/ml. The patient was treated by neoadjuvant preoperative chemoradiotherapy which consisted of two 3-week cycles: 5-fluorouracil 300 mg continuously days 1–21, weekly cisplatin 40 mg and weekly paclitaxel 90 mg. Concomitant with the second course of chemotherapy, radiotherapy of stomach and regional lymph nodes was administered with 30 Gy in 15 fractions from a linear accelerator. Radiotherapy was potentiated by ultrasound hyperthermia once weekly. Surgery was performed within

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5 weeks after completion of chemoradiotherapy. Total gastrectomy with concurrent cholecystectomy and splenectomy was performed. The histological examination of the resected stomach revealed coarse mucosa, which was densely infiltrated by the cells of dissociated gastric carcinoma with numerous signet ring cells. These cells penetrated through the muscularis mucosa and were dispersed throughout the wall, including the subserosa. Examined lymph nodes were without metastasis. Gallbladder and spleen were also without pathological changes. After surgery 6 cycles of adjuvant chemotherapy tegafur and calciumfolinat were administered.

Fourteen months after presentation of the gastric metastasis, lump in the right breast was detected during follow-up.

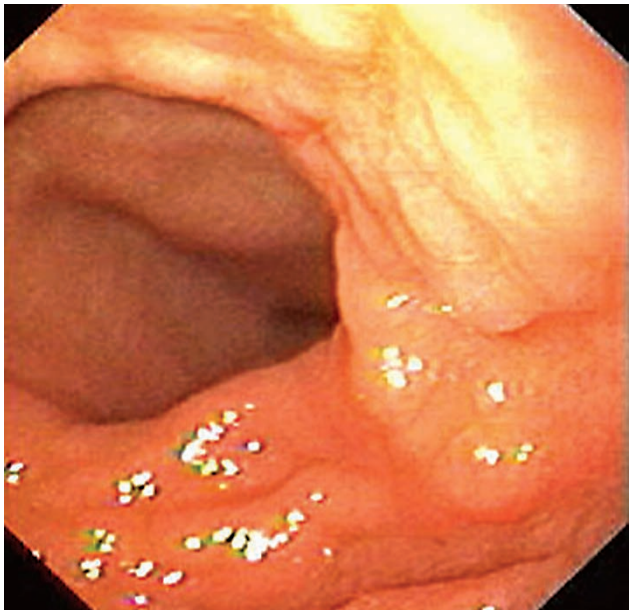

Fig. 1: Gastroscopy: There is a polypoid infiltration of the gastric mucosa in the dorsal wall in the region of the transition of the fundus and antrum of the stomach on the righthand side of the figure.

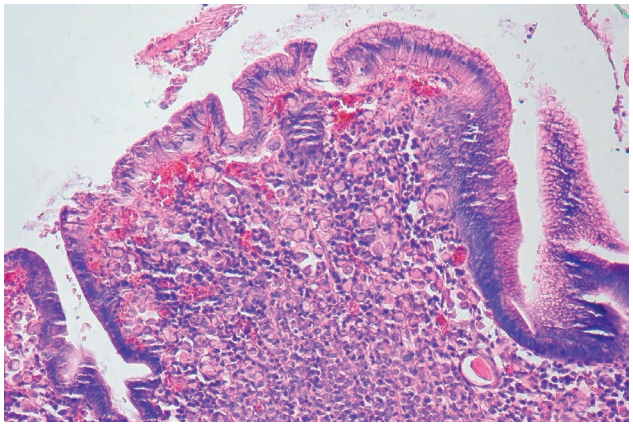

Fig. 2: Histological examination of biopsy from the stomach: coarse mucosa that is densely infiltrated by cells of a dissociated carcinoma with numerous signet ring cells.

Following mammography, this lump was evaluated as T3N0 breast carcinoma. Chest X-ray, abdominal ultrasound and bone scan did not indicate any distant metastases. Core biopsy demonstrated invasive lobular cancer, provisional grade 2, ER 6/8, PR 8/8 (modified Allred score) her 2 negative. Six cycles of chemotherapy in combination with docetaxel, epirubicin and cyclophosphamide were administered. After chemotherapy was started hormonal treatment with tamoxifen (20 mg) once daily. After 6 cycles of chemotherapy, the control mammography showed no focal changes in the right breast or lymphadenopathy in the ipsilateral axilla or supraclavicular region. According to the multidisciplinary team the decision not to perform surgery was made. Radiotherapy was administered from a linear accelerator to the region of the right breast, the ipsilateral axillary and supraclavicular lymph nodes.

The patient was in complete remission for 23 months. Then the first subjective problems started: flatulence, belching, and diarrhea, without spasms, pain, or weight loss. Twenty-three months after diagnosis of breast cancer, colonoscopy was performed: an incomplete examination to the colon ascendens sampled 9 specimens for biopsy (Figs. 3 and 4). Multiple biopsies throughout the colon demonstrated adenocarcinoma with signet ring morphology, positive for etrogen and progesterone receptors supporting diagnosis of metastatic invasive lobular carcinoma of breast (Fig. 5). Immunohistochemistry shows the loss of E-cadherin expression (Fig. 6). The histological specimens from the stomach, right breast and colon and rectum were compared and the examination of hormonal receptor status in the histological specimens from the stomach was added. Based on this new information the description of histology from the stomach was upgraded from the original description of primary gastric dissociated gastric carcinoma with numerous signet ring cells to gastric metastasis from lobular breast carcinoma. Thirty-seven months after diagnosis of gastric metastasis there were no oncological changes according to control gastroscopy, mammography, chest X-ray, abdominal ultrasound and bone scan. The multiple breast cancer metastases in the colon ascendens, colon transversum, colon descendens, colon sigmoideum and rectum were assessed as inoperable. Two cycles of chemotherapy using capecitabine were administered, followed by paclitaxel weekly. Hormonal treatment was changed from tamoxifen (20 mg) once daily to letrozole once daily, which was administered until progression 75 months after diagnosis of gastric metastasis. According to the control examinations, there were no other metastases in any other locations besides colorectal breast cancer metastases. The level of CEA was 5.32 µg/l, CA 19–9: 24.28 U/ml, CA 72–4: <3 U/ml and CA 15–3: 31.5 U/ml. Mild abdominal pain was noted, with weight loss of 2kg during the previous month, but with good appetite, regular brown stools with normal consistency, and no vomiting. Thirty-eight months after diagnosis of gastric metastasis abdominal ultrasound revealed small to mild ascites without liver metastases. Colonoscopy confirmed multiple

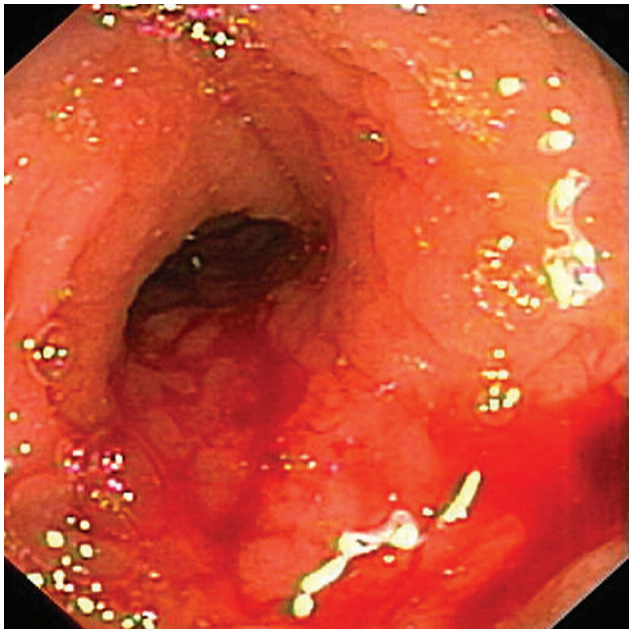

Fig. 3: Colonoscopy: Lumen of colon ascendens is narrowed; mucosa is saturated with deleted vessels. There is a bleeding spot on the righthand side of the figure. Histological examination showed large bowel mucosa focally infiltrated by the structures of dissociated adenocarcinoma.

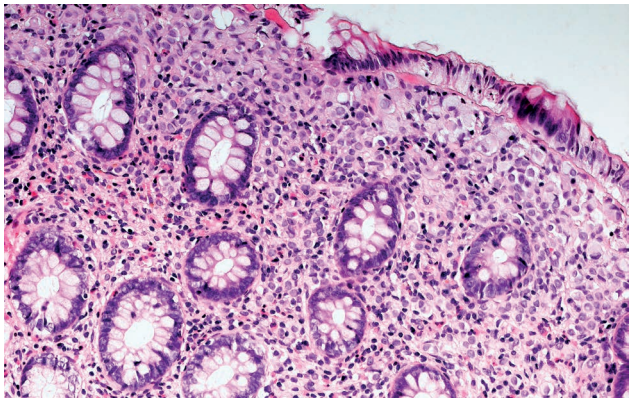

Fig. 4: Histological examination shows large bowel mucosa focally infiltrated by the structures of dissociated adenocarcinoma (hematoxylin-eosin stain).

colorectal metastases, histologically dissociated adenocarcinoma with signet ring cell morphology. The level of CEA was 7.12 µg/l, CA 19–9: 20.22 U/ml, CA 72–4: 16.09 U/ml and CA 15–3: 66.1 U/ml. Hormonal treatment changed from letrozole to fulvestrant (250 mg) i.m. once monthly, due to progression. According to the control mammography and gastroscopy there was no local recurrence of cancer. According to scintigraphy of the skeleton there were no skeletal metastases. The performance status of the patient progressively declined: ascites, bilateral edema of the lower extremities, little bilateral pleural effusion, fatigue, weakness, lack of appetite, weight loss, flatulence and abdominal pain. Patient was treated only with hormonal treatment of fulvestrant 250 mg i.m. once monthly; symptomatic treatment included analgesics and repeated punctures of ascites. The level of CEA was 49.89 µg/l, CA 19–9: 187.86 U/ml, CA 72–4: 153.20 U/ml, and CA 15–3: 136.6 U/ml. According to abdominal ultrasound there were extensive ascites, without liver metastases. Chest X-ray showed bilateral pleural effusion, more on the right side, without lung metastases. Eighty-six months after diagnosis of gastric metastasis the patient died due to progression of cancer.

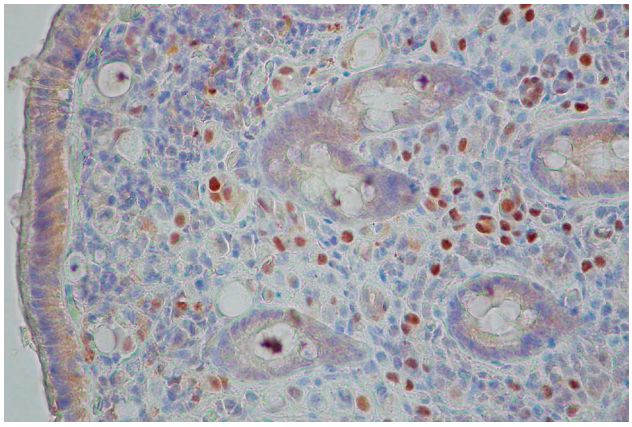

Fig. 5: Histological examination shows fragments of large bowel mucosa infiltrated by the elements of dissociated adenocarcinoma; cancer cells are positive for the expression of estrogen receptors, confirming the metastatic origin of tumor involvement of breast cancer metastases.

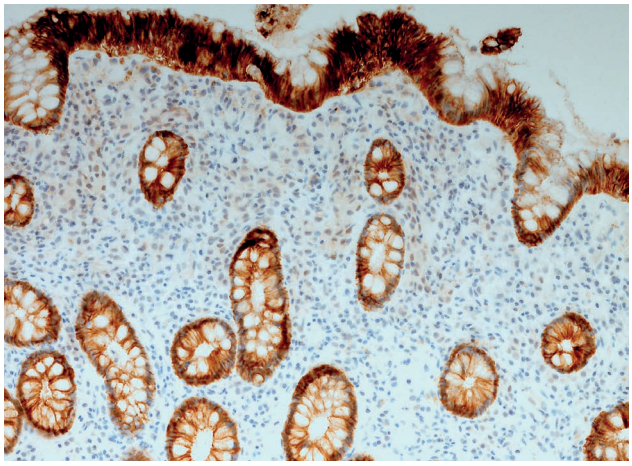

Fig. 6: Immunohistochemistry shows the loss of E-cadherin expression.

Discussion

From the point of view of the clinician the occurrence of gastric metastasis as the first symptom of breast carci-

noma with a long period of latency before presentation of the primary breast carcinoma is rare. Subsequent multiple metastases selectively to the colorectum are not so rare. From the point of view of the pathologist the first biopsy from the stomach looked like common dissociated gastric carcinoma from the signet ring cells. It did not cross our mind to search for origin from another site. The most common sites of distant metastases from breast cancer include bones, lungs, the central nervous system and the liver (1–4). The true incidence of breast cancer metastatic to the gastrointestinal tract is not known (3, 5). In an autopsy series, it was reported to occur in 4–25% of patients with known disseminated breast cancer. The liver is the most common site of breast cancer metastases in the abdomen (5). The stomach is more often involved with breast cancer metastases than the colon (3, 4, 6, 7). Solitary metastases to the stomach are more common than multiple metastases. Solitary lesions are mainly located in the middle or the upper third of the stomach (8). In this case there was a solitary lesion in the dorsal wall in the region of the transition of the fundus and antrum of the stomach. Most cases in the literature of metastatic breast cancer masquerading as primary gastric carcinoma described primary breast carcinoma first and consequently gastric metastasis (3, 9, 10). The opposite sequence – gastric metastasis first and after 14 months of latency presentation of primary breast carcinoma – makes this case unusual. The disease-free interval between primary breast cancer and gastrointestinal metastases may range from synchronous presentation to as long as 30 years (3). In this case colorectal metastasis from breast cancer occurred 23 months after diagnosis of breast cancer. In a retrospective study of 51 patients with gastric metastases of breast carcinoma median survival from detection of gastric metastases was 10 months, with a 2-year survival rate of 23% (11). In our case survival was 86 months. Although ductal carcinoma accounts for the majority of primary breast cancers, intestinal metastases are the most common lobular type (5, 6). Gastric metastases usually derive from lobular rather than ductal breast carcinoma (11). In this case, in accordance with the literature, the histology of the primary breast cancer was invasive lobular carcinoma. Symptoms of colorectal metastases from breast cancer are not specific. In this case subjective problems dominated: flatulence, belching, and diarrhea, without spasms, pain, or weight loss. Symptoms usually mimic those of a second primary colorectal carcinoma or inflammatory bowel disease (6). Differential diagnoses include diverticulitis, inflammatory bowel disease and ischemic colitis (4). There is no consensus for management of colorectal metastases from breast cancer due to the small number of described cases (6). Chemotherapy and hormonal therapy are usually recommended, and in the case of solitary metastasis, also surgery (2, 11).

Conclusions

This case report is a very rare situation. Metastases to gastrointestinal or gynaecological tracts are more likely in invasive lobular carcinoma than invasive ductal cancer. The pathologist should determine whether or not they check estrogen and progesterone receptor status not simply by signet ring cell morphology but also by consideration of clinic-pathological correlation of the patient, such as the presence of a past history of breast cancer, or the colorectal localization of poorly differentiated carcinoma, which may occur less frequently than in the stomach. Always consider possibility of invasive lobular carcinoma metastasis if patient has a past history of breast cancer, particularly invasive lobular carcinoma, even if many years before. Also consider if CT scan appearances of stomach mimic linitus plastica i.e. gastric thickening.

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CASE REPORT

EFFICACY OF LOW-DOSE TOCILIZUMAB ON RELAPSING ADULT-ONSET STILL'S DISEASE

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Summary: Still's disease is an inflammatory disorder of unknown etiology. First-line therapy is based on corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs) but the frequency of relapses and corticosteroid-induced adverse events are limiting factors. The efficacy of intravenous tocilizumab (TCZ) has been shown at a dose of 8 mg/kg but the corticosteroid-sparing effect of intravenous low-dose TCZ followed by subcutaneous (SC) injection in the course of the disease has been poorly investigated. We report the case of a 28-year old Caucasian woman presenting a relapse of Still's disease eleven months after diagnosis under treatment with 6 mg of methylprednisolone. TCZ at a dose of 4 mg/kg every 2 weeks was combined with 32 mg of methylprednisolone, followed by 162 mg SC every 3 weeks. Evolution was rapidly favourable with a decrease in corticosteroid doses. We reviewed previously published cases.

Keywords: *Still's disease; Tocilizumab; Relapse*

Introduction

Still's disease is a systemic inflammatory disorder of unknown etiology. The term "Still's disease" has been first used in 1971 to describe a series of adult patients with clinical features similar to those found in children with systemic juvenile idiopathic arthritis but who did not meet the criteria for rheumatoid arthritis (1). Clinical manifestations include fever, skin rash, arthralgia and arthritis, sore throat, lymphadenopathy, pericarditis and pleural effusion (2). Laboratory findings include leukocytosis, increase in c-reactive protein (CRP) level and sedimentation rate, and hyperferritinemia (2). The first-line therapy is based on corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs) but the frequency of relapses and corticosteroid-induced adverse events are limiting factors. High levels of proinflammatory cytokines such as interleukin-1 (IL1), IL6, tumour necrosis factor-alpha (TNFα) or interferon-gamma (IFNγ) support the use of biotherapies, including anakinra (3) and tocilizumab (4).

The efficacy of intravenous tocilizumab has been shown at a dose of 8 mg/kg/month (5). However, the corticosteroid-sparing effect of low-dose tocilizumab in the course of the disease is not well established.

We report the case of a patient with relapsing adult-onset Still's disease (AOSD) who showed a favourable evolution first under low-dose tocilizumab then under subcutaneous injections (162 mg every 3 weeks) corresponding of a cumulative dose of 3.6 mg/kg/month. We also reviewed the literature to identify similar cases.

Case report

A 28-year old Caucasian woman without relevant past medical history was diagnosed with AOSD in May 2014 based on the presence of fever, evanescent rash, arthralgia, sore throat and myalgia. She had leukocytosis (14,620 cells/mm^3, 12,590 PMN/mm^3) with elevated CRP (243 mg/L) and erythrocyte sedimentation rate (49 mm/h). The ferritin level was 2,372 µg/L. The diagnosis of AOSD was made based on the Yamaguchi's criteria.

She received methylprednisolone at a dose of 0.5 mg/kg and NSAIDs. The evolution was favourable with a rapid decrease in inflammatory parameters and clinical improvement. The corticosteroid dose was progressively tapered. Eleven months after diagnosis, while receiving 6 mg of methylprednisolone, the patient relapsed with fever, arthralgia, myalgia, sore throat and elevation in inflammatory parameters (leukocytosis: 27,470 cells/mm^3, 26,130 PMN/mm^3, CRP: 126 mg/dL, ferritin level: 738 µg/L). She then received 32 mg of methylprednisolone but to prevent corticosteroid-induced adverse events (fluid retention and Cushingoid side effects), tocilizumab was administered at a dose of 4 mg/kg every two weeks with a total of four intravenous doses.

Two months after the first administration of tocilizumab, the dose of methylprednisolone was reduced to 8 mg/day. At that time, the DAS28 CRP score was 2.7 (3.77 at the time of the first tocilizumab administration). No adverse event was induced by tocilizumab. She then received subcutaneously 162 mg of tocilizumab every 3 weeks. The evolution was favourable and the corticosteroid dose was tapered to

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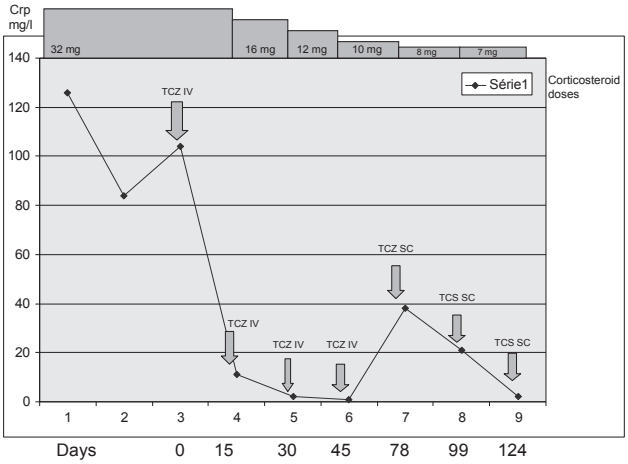

Fig. 1: Evolution of the c-reactive protein level according to the corticosteroid dose.

6 mg/day three months after the first administration of tocilizumab. The evolution of inflammatory parameters and corticosteroid doses is shown in figure 1. The ferritin levels rapidly decreased from 1,060 µg/L at the time of the first intravenous tocilizumab injection, to 82 µg/L at the time of the first subcutaneous tocilizumab injection and 60 µg/L on Day 124. On Day 124, the DAS28 CRP score was 2.76.

Discussion

AOSD is an inflammatory disorder characterized by high levels of proinflammatory cytokines such as IL1, IL6, IL18, TNFα and IFNγ (6). The first-line therapy is based on corticosteroids, and often long-term high doses are required with their well-known adverse events (7). In an attempt to provide a corticosteroid-sparing effect, disease-modifying antirheumatismal drugs (DMARDs) such as methotrexate have been tested (8). Other drugs, including cyclosporin A, leflunomide, azathioprine, hydroxychloroquine, D-penicillamine, tacrolimus, mycofenolate mofetyl and intravenous immunoglobulins have also been tested in small case series and case reports (9, 10, 11, 12).

The rationale for the use of the anti-IL6 receptor antibody, tocilizumab, in AOSD is based on the central role of IL6 in the inflammatory process (13). IL6 is a pleiotropic cytokine involved in immunoglobulin production, B-cell, cytotoxic T-cell and macrophage differentiation, T-cell proliferation, hematopoiesis modulation, and hepatocyte stimulation to produce acute-phase proteins such as CRP (5). High IL6 levels have been observed in the blood of AOSD patients and have been shown to correlate with the disease activity (14). Tocilizumab is a humanized antihuman monoclonal IL6 receptor antibody whose efficacy has been proven in patients with rheumatoid arthritis (15) and juvenile idiopathic arthritis. Its efficacy has also been shown in small series of AOSD patients (16–18) with various administration methods but 8 mg/kg were used in most cases. Cipriani et al (17) have shown a clinical and biological improvement in 11 AOSD patients treated with 8 mg/kg every 4 weeks and a corticosteroid-sparing effect while no adverse events have been reported.

Furthermore, in their series of 14 patients treated with 8 mg/kg every 4 weeks, Puéchal et al have shown that the DAS28 score dropped from 5.61 to 3.21 at 3 months and to 2.91 at 6 months (16). An improvement in all disease activity scores was observed. The systemic symptoms, including fever and rashes, resolved in 86% of patients and at 6 months, the mean prednisone dose was reduced to 10.3 mg/day. They have shown no correlation between the tocilizumab dose and the decrease in corticosteroid dose (some patients received 8 mg/kg every 4 weeks and others every 2 weeks). The following adverse events related to tocilizumab have been reported: necrotizing angiodermatitis in one patient, and chest pain and chills at the time of each administration in one patient leading to tocilizumab discontinuation.

Tocilizumab is commonly used in patients with refractory diseases previously treated with other immunosuppressant drugs. In the study by Puéchal et al (16), all patients had previously received methotrexate and anakinra and in the study by Elkayam (18), the mean number of previously used DMARDs was 3.6. Thus, it remains unknown whether lower doses are effective in mild forms without requiring the use of immunosuppressive drugs. The high cost of tocilizumab should also be noted and could be a rationale for considering the use of lower doses of tocilizumab, in particular when the cost of tocilizumab off-label use is not supported by national insurance reimbursements like in Belgium. Another Belgian specificity which contributed to the choice of tocilizumab is the unavailability of anakinra. Thus, the use of lower doses (i.e. 4 mg/kg) may be an option.

We identified four other cases of AOSD patients who received low-dose tocilizumab (4 mg/kg) in the literature (Table 1). Nakahara et al have reported the case of a 24-year old man with refractory disease (19). All DMARDs and immunosuppressive drugs had been discontinued before tocilizumab injection. They have shown that the CRP level normalized one week after administration of a single dose. They have then increased the dose to 6 mg/kg then to 8 mg/kg. After injection of the 15th dose of tocilizumab, the patient experienced deep venous thrombosis and massive hematochezia. At that time, the prednisone dose was of 12.5 mg/day. Iwamoto et al (20) have reported the case of a 23-year old AOSD woman whose flares recurred when the prednisolone dose was reduced to 9–20 mg/day. They have shown a dramatic improvement in both systemic and joint symptoms without any serious adverse event. Ortiz et al (17) have reported 2 cases who had received a treatment regimen of 4 mg/kg every 4 weeks. Both patients had previously received methotrexate and other immunosuppressive drugs (cyclosporin A, anakinra). They have observed a clinical response and a reduction in glucocorticosteroid doses in both cases.

Tab 1: Patients with Still's disease treated with 4 mg/kg of Tocilizumab identified in the literature compared to our case.

	Ortiz-Sanjuan (17)	**Nakahara (20)**	**Iwamoto (21)**	**Present case report**
N	2	1	1	1
Age		35	23	28
Dose interval	4 weeks	1 week	2 weeks	2 weeks
Interval between diagnosis and TCZ initiation	1.4 years 0.4 years	11 years	25 months	11 months
Previously cumulated prednisolone dose	?	?	?	4.5 g
Other previous immunosuppressive drugs	Case 1: MTX, CyA, Anakinra Case 2: MTX	MTX, bucillamine, gold salts, sulfasalazine, AZT, CyA	MTX, gold salts, MTX, CyA, plasmapheresis	None
Concomitant drugs	Case 1: GC Case 2: MTX	GC	GC	GC
Outcome	Clinical response and reduction in GC in both cases	Clinical response and GC discontinuation	Clinical response and reduction in GC	Clinical and biological response. Reduction in GC
Relapse	No	21 months after TCZ discontinuation	–	–
Adverse events	None	Deep vein thrombosis Massive hematochezia	None	None
Number of IV doses		1 dose of 4 mg/kg followed by 13 doses of 6 mg/kg and 8 mg/kg thereafter followed by a 21-month remission. Readministration of 8 mg/kg thereafter	13	4

Abbreviations: AZT: azathioprine; CyA: cyclosporin A; GC: glucocorticosteroids; IV: intravenous; MTX: methotrexate; TCZ: tocilizumab

Our case presented several specific features: it was the first case in which no concomitant DMARDs were used, showing that the early use of low-dose tocilizumab was effective to achieve remission. Moreover, the third dose was very low (3.5 mg/kg) and appeared as effective as higher doses and just before the SC administration, the CRP level was slightly elevated with a rapid decrease after SC TCZ injection which suggests that SC TCZ could not only maintain a stable disease activity but also suppress active disease. Tocilizumab induced a rapid clinical and biological response and the administration of subcutaneous injections every 3 weeks (162 mg) appeared effective.

In conclusion, this case report supports the concept that AOSD may be managed with tocilizumab doses lower than the 8 mg/kg/month classically used in previously published refractory cases. It should however be noted that in our patient, tocilizumab was used "early" for its steroid-sparing effect and not as a third- or fourth-line therapy in a refractory case.

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CASE REPORT

FIVE ROOTS PATTERN OF MEDIAN NERVE FORMATION

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Summary: An unusual combination of median nerve's variations has been encountered in a male cadaver during routine educational dissection. In particular, the median nerve was formed by five roots; three roots originated from the lateral cord of the brachial plexus joined individually the median nerve's medial root. The latter (fourth) root was united with the lateral (fifth) root of the median nerve forming the median nerve distally in the upper arm and not the axilla as usually. In addition, the median nerve was situated medial to the brachial artery. We review comprehensively the relevant variants, their embryologic development and their potential clinical applications.

Keywords*: Median nerve; Formation; Five roots; Brachial plexus*

The median nerve (MN) as it is widely known is formed by the union of the lateral root coming from the lateral cord and the medial root arising from the medial cord of the brachial plexus. These two roots embrace the third portion of the axillary artery (AA) joining each other anterior or lateral to it (21). MN's variability has been well documented in the literature and concerns the number of its roots, the level of its formation and its positioning with respect to the AA. Its normal pattern of formation by two roots has been mentioned varying between 48–88.3% (9, 13), whereas its formation by three or very rarely four roots has been described as well (14, 17). In addition, low MN's distal to the axilla has been found in an incidence of 3.5% to 40% of the specimens (7, 14), whilst the MN's anterior or medial positioning in regards to AA has been prescribed in the literature (13).

Such variations of the MN regarding its formation can potentially confuse the surgeon intervening in the axilla and arm region, as well as the anesthetist during axillary block anesthesia. Moreover, lesion of MN's additional roots can alter the expected clinical manifestations. In the current study, we display a very rarely detected in the literature combination of a MN with five roots, placed medially to the AA and brachial artery and formed in the arm region.

Case report

During routine educational dissection on the left cervical, axilla and arm region of a 78-year-old male cadaver, we came across an unusual combination of variations concerning the ipsilateral MN. After meticulous dissection of the left axilla including removal of the left clavicle, resection of the major as well as the minor pectoral muscles and careful dissection of the connective and adipose tissue of the axillary cavity, we detected the precise pattern of MN's formation, the level of its formation as well as the relationship of MN to the adjacent AA and brachial artery. It was found that the left MN was formed by five roots instead of two, as is expected normally. Specifically, three roots originated from the lateral cord of the brachial plexus approximately at the level of the coracoid process coursing obliquely anterior to the AA and joining individually the medial root of the MN to form immediately the so-called main medial root of the MN, that constitute the fourth MN's root. The trunk of the MN was formed by the union of the aforementioned main medial root and the lateral (fourth) root of MN at the level of the upper third of the arm. The lateral root of MN travelled in front of the AA, directing ultimately medial to the AA. The trunk of MN was situated medial to the brachial artery (Fig. 1). The cause of our specimen's death was unrelated to the current study, whereas no other variants, pathologies or evidence of previous surgical procedures on the relevant regions were present. Moreover, the contralateral brachial plexus and median nerve did not display any variation as regards their formation and topography. Our findings were repeatedly documented by photographs.

Discussion

The MN has two roots from the lateral and medial cords of the brachial plexus, which embrace the third part of the AA, uniting anterior or lateral to it (21). The medial root crosses in front of the AA. Ultimately, MN trunk is formed at the lower border of the axilla (12). Apart from the abovementioned usual detected MN's pattern of formation, MN can be formed by the union of three roots, two lateral and one medial (10, 17). Such an incidence has been noticed in 11.7% (13), 14% (8), 26% (7) or as high as 52% (9). Occasionally, the third additional lateral root was derived

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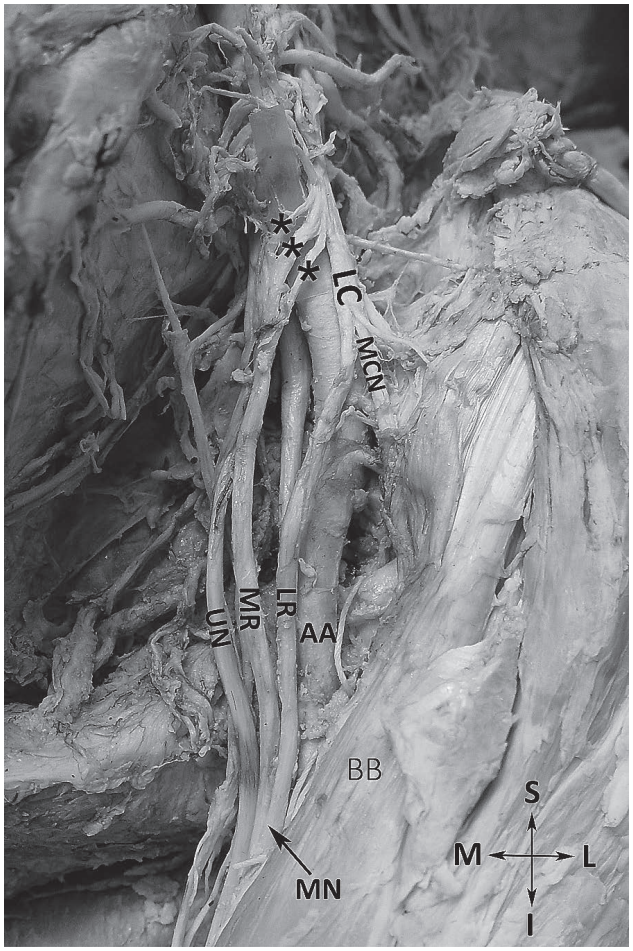

Fig. 1: The left median nerve (MN) is shown forming by the junction of the lateral root (LR) and medial root (MR) of the MN that are derived from the lateral (LC) and medial cord of the brachial plexus, respectively. The medial root receives three additional roots (asterisks). (AA: axillary artery, MCN: musculocutaneous nerve, UN: ulnar nerve, BB: biceps brachii muscle, S: superior, I: inferior, M: medial, L: lateral).

from the anterior division of the middle trunk of the brachial plexus (3.08%) as noted in a material of infants by Uzun et al. (19). It must be emphasized that many investigators consider as the additional lateral root of MN the connecting branch from musculocutaneous nerve to MN. The formation of MN from one lateral root and two medial roots from the medial cord of brachial plexus has been mentioned as well (15). Very rarely the MN has been found to be formed by the union of four roots. Budhiraja et al. observed the formation of MN from four roots in 3.57% of their material. They noted that the two additional lateral roots took their origin, the one from the lateral cord and the other from the musculocultaneous nerve (6). The two unusual lateral roots in a case report of Uzun et al. were noted to take their origin from the musculocutaneous nerve and joined the MN (20), whereas Pandey et al. (14), Satyanarayana et al. (18) and Kolagi et al. (11) observed three roots from the lateral cord of the brachial plexus to be passing obliquely anterior to the AA and joining separately with the medial root of the MN. Our case report constitutes to the best of our knowledge a unique, presumably the first described in the literature, case of MN's formation from five roots combined with the distal site of MN's formation in the region of arm and the medial location of MN in relation to the brachial artery.

In some instances there are additional roots of MN derived from the lateral cord of the brachial plexus or the MN's lateral root communicating either with the medial cord of the brachial plexus or with the MN's medial root that have been termed "communicating branches" (10, 13, 14), or "interconnecting branches" (2) or "intercordal neural communications" (4). Badawoud et al. noticed the presence of two such interconnecting branches uniting the two roots of the MN in 2.1% and the presence of one interconnecting branch joining the MN's roots in 6.3% of their specimens (2). Pandey et al. mentioned the existence of one or two communicating branches from the lateral cord to the medial root of MN in 2.3% of their cases (14). Interestingly, Goyal et al. observed the presence of a unique communicating branch displaying a recurrent course (10). Apparently, these communicating branches in most cases represent additional roots of the MN. Such communicating branches or additional MN's roots could be prone to lesion during radical neck dissections and surgical procedures undertaken into the area of axilla and the upper arm (19). Such branches could explain unusual sensory loss after injury or surgical intervention in the axilla region (9, 10) and their knowledge is crucial during interpretation of neurophysiologic examination of the upper limb. Moreover, these anastomosing branches or additional MN's roots may result in AA's compression reducing the blood supply to the upper limb (2, 3).

The site of union between the lateral and the medial root of the MN is quite variable and has been found as far down as the elbow (5). Pandey et al. found low fusion of two roots of the MN in 3.5% of the cadavers (14), whereas Fazan et al. observed the MN's formation in the region of the arm in a high prevalence of 7% (9). Similarly, Nasr mentioned that MN began at the level of the coracobrachialis muscle insertion in 6.7% of the subjects (13). However, Budhiraja et al. reported that such low origin of MN is detected in higher incidence, thus 17.3% (6). Similarly, Channabasanagouda et al. observed the MN's formation in the arm's region with an extremely high incidence of 40%. In particular, such MN's formation was located in the upper third of the arm in 20%, in the middle third in 12% and in the lower third of the arm in 4% of the studied specimens (7). The abovementioned authors stated that such ectopic MN's formation might confuse the operative surgeon during surgical intervention in the arm or the anesthetist while performing block anesthesia.

The AA is clasped between the medial and lateral roots of brachial plexus, the medial one crossing in front of the vessel. The commencement of the MN is thus lateral to the artery (12). Otherwise, the two MN's roots embrace the third part

of AA uniting anterior or lateral to it (21). MN is formed on the anterior surface of AA in 1.53% (6) or 8.3% (13) or 10% (7). Such anterior relationship of the MN and AA possesses clinical value, since MN roots potentially compress the AA including ischemic symptomatology. Occasionally, MN has been noted forming posterior to the AA (5, 13). Furthermore, as it occurs in our case, the MN can course across the medial surface of the AA and the initial portion of the brachial artery, a relationship not so infrequently detected, since it can be noted in an incidence of 4.7% (14) or 6% (7) or 13.3% (13).

As regards the embryologic MN's development the ventral root fiber can be seen growing out toward the end of the fourth week, whereas slightly later in the fifth week the ganglia and dorsal root fiber anlages are developed. At the tenth millimeters stage a typical spinal nerve with its ventral and dorsal ramus become evident. The nerves supplying the arm are clearly indicated in embryos of five weeks. The trunks of the last four cervical, together with the first thoracic nerves unite into the primitive brachial plexus (1). The contact between these nerves and the differentiating mesodermal condensations is mandatory for accomplishing the functional differentiation. Any disturbance of coordination of these processes may result in variations in morphology of brachial plexus (4, 16).

Conclusively, such a combination of MN's variants should be kept in mind on behalf of the surgeons and the anesthetists dealing with the region of axilla and arm. In particular, the additional roots of MN could induce AA's compression resulting in ischemic disturbances of the ipsilateral upper limb, whereas its accidental laceration could lead in unexpected clinical signs and symptoms. Furthermore, the awareness of the atypical formation and location of MN is critical for the anesthetist performing brachial plexus block, as well as for the plastic, vascular, orthopedic surgeon, traumatologist and neurosurgeon operating in the region of the axilla.

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CASE REPORT

LARYNGEAL ELECTROMYOGRAPHY IS HELPFUL FOR CARDIOVOCAL SYNDROME

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Summary: Laryngeal electromyography is used in the evaluation of vocal cord paralysis to confirm the diagnosis, to guide the diagnostic work-up for etiology, to provide prognostic information and to help choose the correct treatment for the patient. Cardiovocal syndrome is characterised by vocal cord paralysis due to a cardiovascular disease. A wide spectrum of conditions can result in this syndrome. Here we present a case of cardiovocal syndrome in association with primary pulmonary hypertension. Laryngeal electromyography was used to guide the work-up of differential diagnosis and also for further intervention with respect to vocal cord paralysis in this patient.

Keywords: *Laryngeal electromyography; Vocal cord paralysis; Cardiovocal syndrome; Ortner's syndrome; Hoarseness*

Introduction

Vocal cord paralysis represents a common neurolaryngological problem. It results in a breathy dysphonia with immobility of the ipsilateral vocal cord. Laryngeal electromyography (LEMG) is used in the evaluation of vocal cord paralysis. Information gathered from LEMG allows clinicians not only to confirm the diagnosis, but also to make the office work-up easier for clarifying the etiology (1, 2). In addition, it is well documented that LEMG can help to determine the prognosis of vocal cord paralysis (1, 3, 4).

Cardiovocal syndrome, also known as Ortner's syndrome, is a rare condition in which cardiovascular pathology is the underlying cause of hoarseness (5, 6). Although it was initially associated with mitral stenosis, various clinical conditions such as pulmonary hypertension, enlarged pulmonary artery, thoracic aortic aneurysms and aberrant subclavian artery can result in recurrent laryngeal nerve (RLN) paralysis (7). Additionally, similar compression mechanisms can be detected on aorta causing dysphagia aortica and on subclavian artery causing dysphagia lusoria (8, 9).

Here we present a case of cardiovocal syndrome in association with primary pulmonary hypertension. LEMG was used to guide the work-up of differential diagnosis and also for further intervention regarding vocal cord paralysis in this patient.

Case report

A 34-year-old woman presented to the Department of ENT and Head & Neck Surgery with a 2 months history of hoarseness that developed gradually. She also reported shortness of breath and dyspnea during exercise for the last couple of years. She did not mention any problem with swallowing but she aspirated occasionally. She was never a smoker. Voice Handicap Index-10 score was 32 (This is a patient-based self assessment voice symptom index on a scale from 0 to 40 (10)). Endoscopic laryngeal examination demonstrated left vocal cord paralysis and supraglottic compression (Figure 1a and b). Maximum phonation time was 8 seconds.

LEMG revealed a large amount of positive sharp waves and fibrillation potentials with markedly reduced recruitment of polyphasic motor unit action potentials at the left thyroarytenoid muscle (Figure 2a and b). However, the right thyroarytenoid muscle and both cricothyroid muscles were evaluated as normal with normal waveforms and a full interference pattern with an absence of any spontaneous activity. These findings were consistent with subacute partial axonal loss (palsy) of the left RLN.

On the basis of this data, investigation for etiology of the left RLN paralysis focused on the lower neck and upper chest. Ultrasonography of her neck was negative for any mass or lymphadenopathy. She consulted firstly an internist and then a cardiologist. Echocardiography revealed dilated right atrium and ventricle, distended pulmonary arteries

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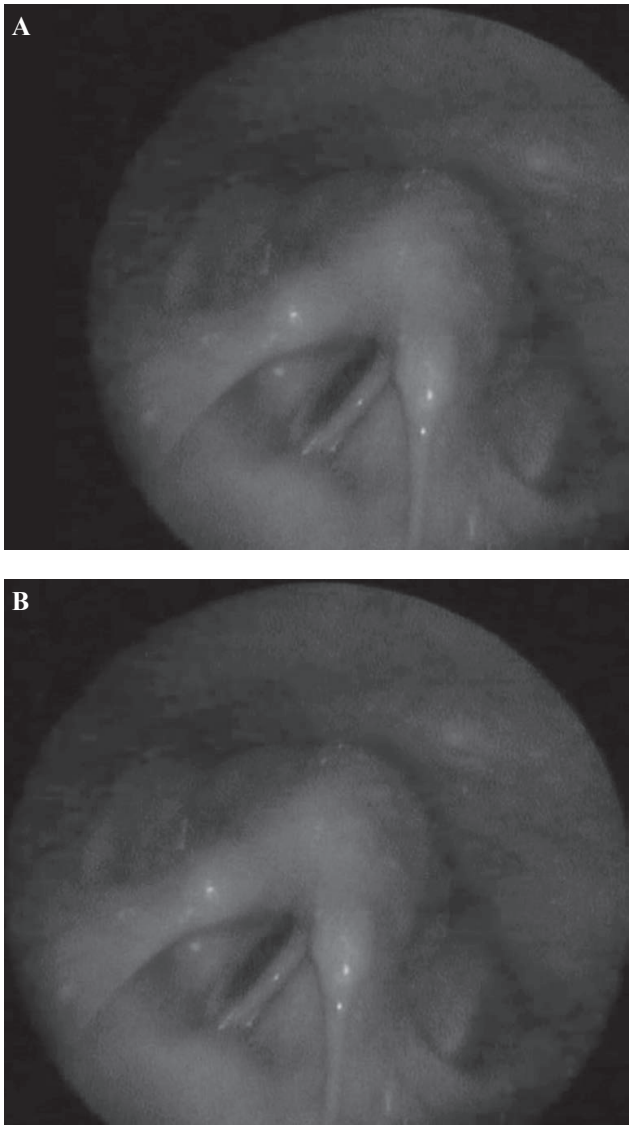

Fig. 1: Rigid endoscopic laryngeal examination showing left vocal cord paralysis. (a) During deep inspiration. (b) During phonation of /*i*/ vowel.

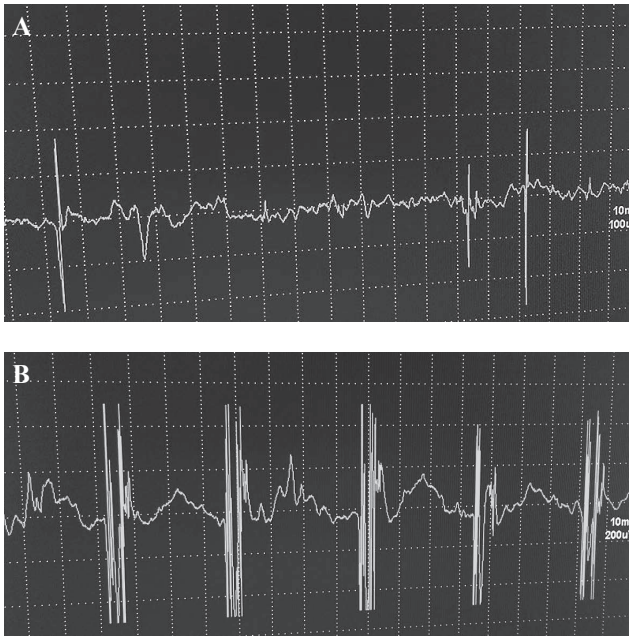

Fig. 2: (a) Fibrillation potentials on laryngeal electromyography. (Recorded from left thyroarytenoid muscle) (Sweep speed 10 ms/div, sensitivity 100 μV/div). (b) Markedly decreased recruitment pattern (single-fiber oscillation) on laryngeal electromyography (Recorded from left thyroarytenoid muscle) (Sweep speed 10 ms/div, sensitivity 200 μV/div).

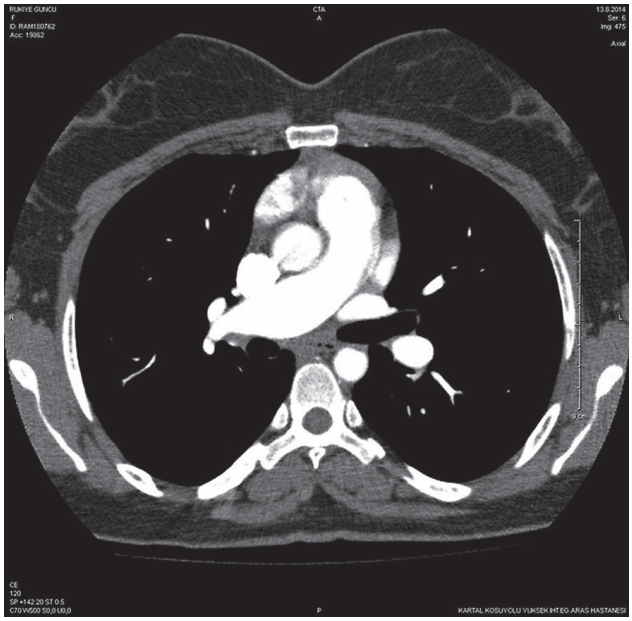

Fig. 3: CT pulmonary arteriography, axial view showing distended pulmonary arteries compressing the arch of aorta.

(main pulmonary trunk 30.8 mm, right pulmonary artery 23.4 mm and the left pulmonary artery 22 mm) with severe pulmonary hypertension (pulmonary artery systolic pressure 103 mm Hg). Mild pulmonary insufficiency and moderate tricuspid insufficiency were also noted. Signs of bilateral small airway disease and atelectasis of left upper lobe were noted in computer tomography (CT) scan images. CT pulmonary arteriography demonstrated distended pulmonary arteries compressing the arch of aorta (Figure 3).

The patient was diagnosed with primary pulmonary hypertension. Paralysis of the left RLN resulted from the distended pulmonary artery. In-office Radiesse® Voice (Merz Pharma GmbH & *Co*. KGaA, USA) injection was done to the left vocal cord of the patient through the cricothyroid membrane. Complete glottic closure was achieved after the injection. Maximum phonation time was 18 seconds and Voice Handicap Index-10 score was 15 at a postoperative 4 weeks-follow-up visit.

Discussion

Vocal cord paralysis must be investigated systematically. After taking a detailed history, an endoscopic laryngeal examination must be done with a flexible or rigid laryngoscope. There is no pathognomonic laryngeal position that indicates where the vagal nerve is injured in patients with vocal cord paralysis. Investigation to identify the precise cause should be done through detailed chest, neck, skull base and brain examinations.

LEMG helps to differentiate between superior laryngeal nerve palsy, RLN palsy and high vagal nerve palsy in vocal cord paralysis. The RLN controls all the muscles of the larynx except the cricothyroid. LEMG findings consistent with denervation or denervation and reinnervation in the thyroarytenoid muscle with normal LEMG findings in the cricothyroid muscle would indicate RLN involvement alone. This should direct the diagnostic work-up in the first place towards the chest and neck sites (1). In suspected patients, transthoracic echocardiography is performed to eliminate a cardiovascular cause. As a matter of fact, in our patient LEMG findings of denervation and reinnervation in the left thyroarytenoid muscle demonstrated isolated left RLN paralysis which later was shown to have resulted from a cardiovascular pathology.

The left RLN is more prone to injury by compression or traction because of its lengthy course, especially around the aortic arch and also its close relationship with major blood vessels in the mediastinum. The association of left RLN paralysis with a cardiovascular pathology was termed as cardiovocal syndrome (5, 6, 11, 12). It was first described in a series of three cases of mitral stenosis suffering from hoarseness by Ortner in 1897 (5). A wide variety of diseases, including congenital or adult cardiac conditions (such as mitral valve disorders, aneurysms of aorta and pulmonary artery, pulmonary hypertension, and tumors of left atrium and ventricles) and iatrogenic conditions (such as cardiovascular surgery and cardiac interventions) can result in hoarseness through RLN paralysis by either compressing or stretching effects (6, 11, 12). It was hypothesized that palsy results from compression of the left RLN between the pulmonary artery and either the aorta or aortic ligament due to cardiopulmonary pathology (6). In the present case, a dilated pulmonary artery due to primary pulmonary hypertension was the reason for the RLN paralysis.

LEMG is also used to determine the neurophysiological state of laryngeal paralysis, to provide prognostic information, and consequently to decide the correct treatment (1, 2, 4). The current evidence indicates that LEMG has a high specificity and positive predictive value, making it a reliable examination when abnormal findings are obtained. Abnormal findings, such as fibrillation potentials, positive sharp waves, and absent or reduced recruitment, predict a poor functional prognosis in vocal cord paralysis (4). However, when normal results are obtained using LEMG, it should be remembered that the sensitivity and negative predictive value are low for this test (3). In other words, poor prognosis is determined more accurately by LEMG, while one should be cautious when LEMG suggests good prognosis.

Treatment alternatives for vocal cord paralysis include observation, voice therapy and surgery such as injection laryngoplasty (temporary or permanent), medialization thyroplasty and reinnervation procedures (13). The decision regarding treatment is guided by the symptoms of the patient and expectations for recovery from the paralysis. Prognostic information provided with LEMG will help in this. It is recommended that patients with a clearly denervated larynx, i.e. those with spontaneous irritable muscles with fibrillation potentials, positive sharp waves, and complex repetitive discharges, will be good candidates for permanent procedures such as medialization laryngoplasty and, if necessary arytenoid adduction (1).

As our patient was severely hoarse and LEMG showed abnormal findings predicting a poor prognosis, a permanent injection laryngoplasty was performed in the office under local anesthesia.

Conclusion

Vocal cord paralysis is a common clinical condition; laryngeal electromyography (LEMG) is an extremely helpful test not only for guiding the diagnostic work-up but also for providing prognostic information in these patients. Cardiovocal syndrome is characterised by vocal cord paralysis due to a cardiovascular disease and can result from a variety of pathologies. Although rare, this syndrome should be kept in mind in differential diagnosis of vocal cord paralysis.

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