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ATOPY PATCH TEST IN THE DIAGNOSIS OF FOOD ALLERGY IN CHILDREN WITH ATOPIC DERMATITIS

Miloš Jeseňák, Peter Bánovčin

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Summary: Atopic eczema/dermatitis syndrome (AEDS) is one of the most common chronic allergic diseases in children. Among the allergens found to be relevant in AEDS, aeroallergens and food allergens are the most important. The exposure of these patients to their relevant protein allergens can trigger an exacerbation or maintain the disease. AEDS is frequently associated with food allergy, which complicates the management in approximately 40% of these children. Atopy patch test (APT) can help in detecting food allergies in children with AEDS. The earliest publication on patch testing in eczema was described in 1937 by Rostenberg, but the first controlled clinical trial was provided by Mitchell in 1982. APT with food allergens were introduced into clinical use in 1996 by the group of Isolauri. APT test is performed epicutaneously with typical immediate-type allergens (aeroallergens or foods). As a number of apparently minor test modifications greatly influence the sensitivity, specificity, and reproducibility of the APT, the European Task Force on Atopic Dermatitis (ETFAD) has developed a standardized APT technique. APT has developed into a valuable additional tool in the diagnostic work-up of food allergy in infants and children with atopic dermatitis.

Key words: *Atopic eczema/dermatitis syndrome; Food allergy; Diagnosis; Atopy patch test; Children*

Atopic eczema/dermatitis syndrome (AEDS) is one of the most common chronic allergic diseases in children. It is characterized by recurrent intense pruritus and a typically age-related distribution and skin morphology (erythematous and pruritic lesions, excoriations, papules and lichenification), and affecting 10–20% of children, although the incidence is increasing (during the last 5 years it has increased twofold) (9). The prevalence among adults is about 1–3%. AEDS is multifactorial disease mediated by both intrinsic abnormalities (immunological and pharmacological) and extrinsic factors, such as food, airborne and contact allergens, and irritants (16). Among the allergens found to be relevant in AEDS, aeroallergens and food allergens are the most important (4). Food allergy has been demonstrated to play an important role in the pathogenesis of AEDS (relevant clinical hypersensitivity to food has been shown in 30–70% patients with mild to severe AEDS in double-blind, placebo-controlled oral food challenges) (3,7). Egg white, nuts, seafood and peanuts are the most frequently (80–90%) incriminated allergens; in children soy, cow's milk and wheat flour are also responsible. There is no specific marker for AEDS and the diagnosis is made by a combination of clinical features. Therapeutic consequences of diagnosis of an allergy are based upon avoidance strategies, which can be very expensive and distressing for the patient (4). While immediate-type clinical reactions to food can quite easily be identified by history or measurement of

specific IgE in combination with positive oral food challenge, the evaluation of food allergy in the absence of immediate clinical reactions (worsening of eczema) still presents diagnostic difficulties – particularly in polysensitized children with AEDS (13). Skin prick testing, measurement of specific IgE and food challenges are helpful in the IgE-mediated reactions, but diagnosing patients who have the non-IgE (cell mediated) or mixed (IgE and cell mediated) disorders remains challenging with our current diagnostic tools (18). Oral provocation test remains the “gold standard” for the food allergy diagnosis. The provocation is performed as double-blind placebo-controlled food challenge (DBPCFC), e.g. with “masked” (lyophilized) foods in colour and flavour neutral formulas after at least 2 week of corresponding elimination diet. For AEDS, early (within 1–2 h after provocation) and late reactions (2–24 h) can be distinguished (4).

Atopy patch test (APT) seems to be a valuable additional diagnostic tool in the diagnostic work-up of food allergy in children with AEDS, especially with regard to late-positive clinical reactions. APT involves the epicutaneous application of intact protein allergens (1st type of allergen) in a diagnostic patch test setting with an evaluation of the induced eczematous skin lesions after 24 to 72 hours. The APT reaction is initiated by binding of allergens to epidermal IgE⁺CD1a⁺ cells, which present allergen to allergen-specific T_{H2} cells in the dermis. Subsequent release of T_{H2}-cell-

derived cytokines will lead to an inflammatory reaction in which among others skin-infiltrating eosinophils are involved (2). Biopsy specimens of the positive patch test sites in patients with AEDS were found to have initial T_{H2} cell infiltration, followed by a predominance of T_{H1} cytokines and eosinophils (4). Similar biopsy findings have been observed in the skin of atopic dermatitis patients during acute and chronic lesions (9). The close microscopic and macroscopic similarities between the specimens from APT sites and lesional skin of patients with AEDS indicate that the

Tab. 1: The European Task Force on Atopic Dermatitis (ETFAD) protocol for atopy patch testing (20).

The ETFAD protocol for APT	
• Test area: uninvolved skin of upper back	
• No tape-stripping, scalpel abrasion or pre-treatment (acetone...)	
• Large Finn Chambers (12 mm) on Scanpor tape	
• Purified allergen in petrolatum as test substance with standardized allergen concentration (in biologic units or µg/mL major allergen content)*	
• Occlusion time of 48 hours	
• Reading at 48 hours (20 minutes after removing of the set) and 72 hours	
Exclusion criteria for APT:	
- test site free of topical steroids for 7 days	
- test site without ultraviolet treatment for 4 weeks	
- patients free of oral steroids, cyclosporine A or tacrolimus	
- avoidance of antihistamins for 5 days	
- non-pregnant	
* Whereas the availability of standardized food allergens is poor and many foods contain more than one protein which can cause allergic reaction, fresh native foods or dried foods dissolved in saline or water can be used for APT (20).	

Tab. 2: Revised European Task Force on Atopic Dermatitis (ETFAD) key for atopy patch test reading (8, 20).

Key for atopy patch test reading		
-	negative	negative
?	only erythema, questionable	
+	erythema, infiltration	positive
++	erythema, few papules (< 3)	
+++	erythema, many or spreading papules (>4)	
++++	erythema, papules and vesicles	

Tab. 3: Distinguishing among allergic and irritant reactions (12).

Allergic reactions	Irritant reactions
Slow, crescendo	Rapid, decrescendo
Persistent	Short duration
Unsharp margin	Sharp margin
Marked erythema	Mild (brownish) erythema
Infiltration, papules	Bulla, necrosis

APT is a valid model to study allergic inflammation in AEDS (22).

As a number of apparently minor test modifications greatly influence the sensitivity, specificity, and reproducibility of the APT, the European Task Force on Atopic Dermatitis (ETFAD) has developed a standardized APT technique (Table 1), although some conditions still remain controversial especially the poor availability of standardized food allergen mixtures (the situation for aeroallergens is different, because the standardized testing material is already existing) (8). In literature we can find different methods of preparing the test materials and these differences among the authors cause controversial results (20). Most of authors recommend the use of native food allergens (e.g. fresh cow's milk containing 3.5% fat, native whisked hen's egg, wheat- powder dissolved in saline or water - 1 g/10 ml, soy milk), however, the main allergen concentration is not sure. Some standardization in food APT testing bring new so-called "Ready-to-use" APT with already integrated allergens of known, standardized concentration (Diallertest® and E-patch® for cow's milk, Rapid patch set® for 10 food allergens) (5,17). The use of fresh native food is advantageous, because then this testing material could be used also by skin prick testing or food challenges and the results of these three tests are better comparable (12). Until validation data are available, fresh foods should be preferred for testing over commercial extracts (20). In the future, we should expect the use of recombinant proteins (for some aeroallergens they are already existing, e.g. *Malassezia furfur*) (20). Most of the authors use aluminium chambers with diameter of 12 mm (Finn Chambers) placed on hypoallergenic tape (Scanpor Tape). There are only a few reports on the suitability of alternative materials such as rectangular plastic cups, now available on the market (12). The application site (uninvolved area of upper back), according to some authors, should be checked 15 minutes after the beginning of the testing for eventual presence of immediate skin reaction (12), although this is possible especially by using "Ready-to-use" APT with transparent membrane, which allows easy evaluation of eventual immediate response without removal of testing set. In other cases it is possible to remove the set and then re-stick it again on the back of tested subject.

The crucial moment in food APT testing is the "reading" of skin response what requires wide clinical experience with classic standard epicutaneous and also epicutaneous atopy patch tests (6). The APT should result in a clear "yes" or "no" answer. Positive reaction may be classified using + for erythema and infiltration, ++ for erythema and less than 3 papules, +++ for erythema and four or more or spreading papules, ++++ erythema, papules and vesicles (Table 2). Irritant reactions (sharply defined brownish erythema, decrescendo phenomenon, blistering, lack of clear infiltration) are not positive (Table 3). Erythema without palpable infiltration is considered as questionable, finally negative reaction (12) (Table 2).

Any food can be assessed with patch testing, but cow's milk, hen's egg, wheat and soy have been studied most extensively (12–14,18,19). Side effect are uncommon, although reports of contact urticaria in small number patients (13) or irritation reactions (10,11,21) have been reported. The combination of positive APT results together with defined levels of specific IgE makes DBPCFCs in some cases superfluous (14). APT helps to prevent unnecessary restrictive diets which may be the consequence of misjudging late reactions by clinical assessment alone (13).

According to our experience, APT is simple, cheap and informative diagnostic method, especially when determining delayed type of allergic reactions in children with suspected food allergy. We prefer the use of fresh food allergens or wheat dissolved in saline placed in rectangular plastic cups on hypoallergenic tape. This method could help in the assessment of suspected food before following oral exposure test.

APT with food allergens may increase the identification of food allergy in patients with ADES especially in these cases: suspicion of food allergy without predictive specific IgE levels or a positive skin prick tests; severe and/or persistent AEDS with trigger factors of unknown origin; multiple IgE sensitizations without proven clinical relevance in patients with AEDS (20). It is suitable to perform skin patch test among infants and pre-school children, when total IgE amount in blood is normal and skin prick test are negative (15). Food challenges are still necessary for the appropriate diagnosis of food allergy in patients with AEDS. Elimination diets based solely on *in vitro* or skin tests are inadequate, if the history is not convincing. A negative open challenge may confirm the absence of food allergy, in positive cases; a DBPCFC is recommended (13).

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PAR INDEX IN THE EVALUATION OF THE STABILITY OF THE ORTHODONTIC TREATMENT RESULTS. A REVIEW

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Summary: The evaluation of the treatment results is normally done to estimate the nature and quality of work, so that justice can be done to the work that we do and also that the patients will be satisfied. The primary motive of every orthodontist should be to treat the patient effectively and successfully with long lasting results. Thus the patients are to be assessed, using an appropriate method. PAR index was developed in the recent years to evaluate the treatment results and it is considered as a simple, objective and a reliable manner for evaluating the stability after orthodontic treatment. The index can be applied to different components of the dentition and scores are applied to each component after which the individual scores are multiplied with their respective weightings to balance the impact of the individual components of the overall result. They are then summed up to establish an overall total. In this manner, the method was carried out for the study casts of the three different phases of the treatment i.e. before the onset of the treatment, immediately after treatment and 2 years after treatment for assessing the stability after orthodontic treatment.

Key words: Orthodontic treatment; Stability; Index

Introduction

Assessing the stability after orthodontic treatment is a subject of interest as it helps to evaluate the post treatment results and thereby the quality of the work. Evaluating these post treatment results and assessing the orthodontically treated long-term post treatment results has been a topic of interest from several decades (13). Stability refers to a state, which does not change but instead remains constant in nature. Long-term post treatment stability is an issue of great concern to all orthodontists, as stability at the end of the result is one of prime objectives of orthodontic treatment, for without stability neither proper function nor esthetics can be maintained.

Stability of the orthodontic treatment results

Although we know that the stability after orthodontic treatment is essential for maintaining function and esthetics of an individual, it seems that a large variability in orthodontic treatment outcome exists for different individuals. The variabilities could be a result of various factors like treatment approach, co-operation of patient, growth and adaptability of the hard and soft tissues. Also, many additional factors are known to influence stability after orthodontic treatment. These additional factors include the type,

duration and the timing of the retention appliance (11, 15). Also, many studies have been found that have described the long-term stability of specific type of malocclusions (7, 13, 14, 16). Few other studies showed the stability of orthodontic treatment outcome longitudinally for specific occlusal traits such as open bite, overjet, overbite, cross bite, intercanine and intermolar distance and lower anterior crowding (5). Thus a lot of factors are to be taken into account to establish stability after orthodontic treatment and in order to maintain and improve the stability after establishing the results, it is advisable to assess the stability after treatment.

Further studies have shown that the comparison of pre-treatment, immediate post treatment and post treatment after few years can be done to assess the stability (19, 20). The study casts of pre treatment, immediate post treatment and post treatment after few years can be used for this purpose and it can be assessed to improve the quality of future treatments (10, 11) and occlusal indices have been developed to assess treatment standards and to determine the success of the treatment (4, 8, 10). While using an index, usually the scores are applied to the dental features of a certain malocclusion and the sum of these scores ranks the malocclusion, to which a weighting is then added (6). Many indices were developed over the years and each of it has its own advantages and disadvantages.

PAR index and its use

In order to overcome the difficulties created by using indices inappropriately, two indices were developed. One to measure the orthodontic treatment need called the Index of treatment need (IOTN) and the other to assess the standard of treatment called the Peer Assessment Rating index (PAR index).

PAR index was introduced by S. Richmond and it was mainly developed to record the malocclusion in the mixed and permanent dentition. The index was formulated over a series of six meetings in 1987 with a group of 10 experienced

orthodontists (18). The index is considered as a simple, objective and a reliable manner (19) for evaluating the stability after orthodontic treatment. Several studies have been conducted which demonstrate the method for assessing the stability after orthodontic treatment using the PAR index (1, 2, 9, 12, 17, 21, 22). According to the rules of the PAR index, each of the components of the dentition i.e. anterior segment (upper and lower), buccal segment relationships on both sides, overjet, overbite and midline discrepancies can be scored, which is then multiplied with their respective weightings after which the overall score is established. In this way each component is to be assessed for the three different phases of treatment i.e. the pre treatment, post treatment and 2 years post treatment study casts for assessing the stability.

Tables 1-5 show scoring system for each component of the dentition by PAR index. According to the PAR system, there exists a PAR index ruler, which is designed to facilitate an easy measurement for recording the malocclusion of the dentition. The ruler has certain information's in it that it is capable of summarizing the complete details and thereby is known to facilitate a quick assessment of the study.

There are basically three methods of assessing improvement using the PAR index (9, 20).

Tab. 1: Contact point displacements. Scores and their respective displacements to be used while assessing the alignment of anterior segment of the dentition.

Score	Displacements
0	0 to 1 mm
1	1.1 to 2 mm
2	2.1 to 4 mm
3	4.1 to 8 mm
4	Greater than 8 mm
5	Impacted teeth

Tab. 2: Scores in all three planes of space to be used while assessing the buccal segment relationship.

Antero posterior relation	Vertical relation	Transverse relation
Score	Score	Score
0 - Good interdigitation (Class I, II, III)	0 - No open bite	0 - No cross bite
1 - Less than half from full interdigitation	1 - Lateral open bite on at least two teeth greater than 2 mm	1 - Cross bite tendency
2 - Half a unit (cusp to cusp)		2 - Single tooth in cross bite
		3 - More than one tooth in cross bite
		4 - More than one tooth in scissor bite

Tab. 3: Scores to be used while assessing the overjet.

Overjet	Anterior cross bites
Score	Score
0 - 0 to 3mm	0 - No cross bite
1 - 3.1 to 5mm	1 - One or more teeth edge to edge
2 - 5.1 to 7mm	2 - One single tooth in cross bite
3 - 7.1 to 9mm	3 - Two teeth in cross bite
4 - Greater than 9mm	4 - More than two teeth in cross bite

Tab. 5: Scores for recording the midline that are needed to assess the midline discrepancies.

Centerline
Score
0 - Coincident and upto one quarter lower incisor width
1 - One quarter to one half lower incisor width
2 - Greater than one half lower incisor width

Tab. 4: Scores to be used while assessing the overbite.

Open bite	Overbite
Score	Score
0 - No open bite	0 - Less than or equal to one third coverage of the lower incisor
1 - Open bite less than or equal to 1mm	1 - Greater than one third but less than two thirds coverage of the lower Incisor
2 - Open bite 1.1 to 2 mm	2 - Greater than two thirds coverage of the lower incisor
3 - Open bite 2.1 to 4 mm	3 - Greater than or equal to full tooth coverage
4 - Open bite greater than 4 mm	

a) Absolute reduction in the weighted PAR score

The absolute or the exact reduction in the weighted PAR score reading means the PAR score that can be obtained by deducting of the weightings of pre treatment scores with the weightings of the immediate post treatment scores. This helps in assessing the improvement or deterioration of the results. Also on deducting the weightings of the immediate post treatment score with the weightings of the post treatment scores after few years. It is possible to determine the persistence or deterioration of stability after treatment.

b) Assessing using the nomogram

During the study, if the nomogram is used to assess the improvement of the PAR index, then a nomogram is plotted using the pre treatment weighted PAR score on the horizontal 'x' axis and the post treatment weighted PAR score on the vertical 'y' axis, i.e. the pre and the post treatment scores are read on their respective axis. Where the intercept falls, it indicates the degree of improvement that only provides three broad bands of treatment change (9, 10, 11, 12, 13):

- worse - no different,
- improved,
- greatly improved.

Similarly the immediate post treatment scores are plotted on the horizontal 'x' axis and post treatment scores after few years on the vertical 'y' axis to assess the stability. After knowing the result it is possible to categorize the results obtained. For example, a high standard of treatment is achieved when the proportion of cases falling into "worse or no different" category of an individuals case is negligible and the mean percentage reduction in weighted PAR score is high, e.g. greater than 70 percent (7, 12).

It is possible to understand from the above nomogram (Fig. 1) that the 'x' axis is a presentation of scores before treatment with the scores starting from 0 to 60 with a difference of 5 points between the scores. The 'y' axis is a presentation of scores after treatment with the scores starting from 0 to 45, also with a difference of 5 points between the points. The lines in the nomogram represents the demarcation between the worse or no different cases, improved and the greatly improved bands of treatment change. For example: The point in the no different band of treatment change indicates that the treatment was without improvement.

Similarly, the point in the second category of treatment change shows that the treatment was with good improvement. The second category of treatment change represents PAR points of less than 22. This category shows the significant difference after treatment. It helps a clinician to evaluate the quality of work, by helping to estimate whether there was really some significant improvement or not after treatment. For example, the point in the improved category indicating that the treatment results were with significant improvement (Fig. 2).

On the other hand the point in the third category of treatment change shows that the treatment was with improve-

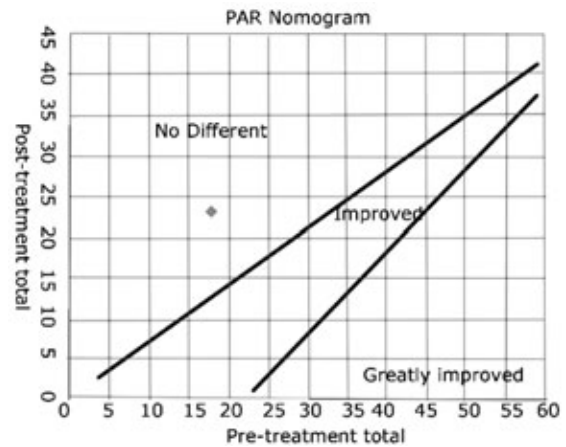


Fig. 1: The presence of the point in the *No different* category of the nomogram indicates that the treatment was without improvement.

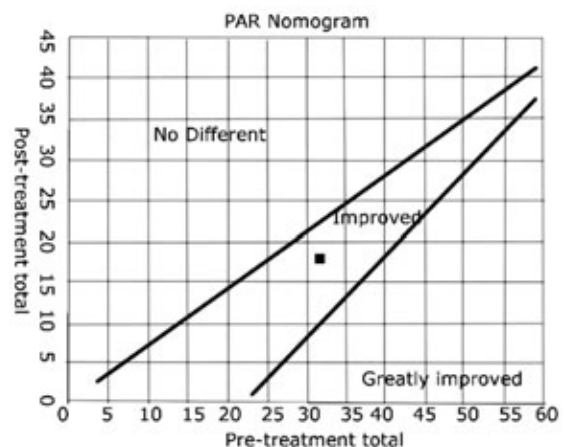


Fig. 2: The presence of the point in the *Improved* category of the nomogram indicates that the treatment was with good improvement.

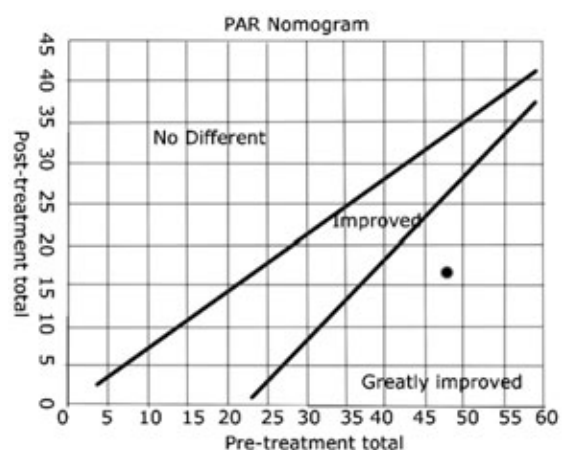


Fig. 3: The presence of the point in the *Greatly improved* category of the nomogram indicates that the treatment was with excellent improvement.

ment to a larger extent. The third category of treatment change represents PAR points of greater than 22. This category also shows the significant difference after treatment. It helps a clinician to evaluate the quality of work, by helping to estimate whether there was really greater improvement or not after treatment. For example, the point in the greatly improved category indicating that the treatment results were with greater improvement (Fig. 3).

c) Percentage reduction in the weighted PAR score

The percentage reduction method of detecting the improvement of orthodontic treatment is known to reflect the change relative to the pre treatment score. For example, a case in which the score is from 4 to 10 would represent 76.9% improvement. Accordingly a high standard of treatment was considered as being achieved when the percentage reduction in weighted PAR score was high.

After assessing the scores obtained by any of the three methods of assessment, the result obtained can be further evaluated. A high standard of treatment is known to be achieved:

- when the proportion of cases falling into "Worse or no different" category is negligible,
- when the mean percentage reduction in weighted PAR score is high.

Also, when a percent of greater than 30 % suggests that the practitioner is treating a large number of cases to a comparatively good standard. Thus in this way, the quality of work of an orthodontist can be obtained. The stages of improvement can be classified as represented by Tab. 6.

Tab. 6: The values for each stages of improvement (taken from Spidlen, M. et al.: Efektivita ortodontické léčby, 2003).

Improvement	Nomogram classification	PAR score change (Percentual reduction)
Worse or no different	< 30 %	< 30 %
Improved	>= 30 %, < 22 PAR	30 -70 %
Greatly improved	>= 22 PAR	> 70 %

Discussion

The availability of a definition for normal occlusion (3) set a standard for comparison of treatment outcomes. The six keys of occlusion that contribute to normal occlusion were introduced by Andrews during the 1970's. According to Andrews the presence of these features were essential to achieve an optimal occlusion. The six keys to normal occlusion are molar inter-arch relationship, mesio-distal crown angulation, labio-lingual crown inclination, absence of rotation, tight contacts and curve of Spee. Thus in order to detect the clear definition of what constitutes a deviation from normal occlusion, it became necessary to increase the subjectivity of assessing results.

Besides this the grading of orthodontic treatment results at study group meetings has been practised for many years. The concept of individuals grading their own treatment results can be a self-teaching device and may improve the quality of future treatment. In the field of research the need for accurate measure is even more critical.

One of the possible ways to assess the treatment results and to compare it with the normal occlusion is by the way of assessing it with the indices. Several orthodontic indices have been developed to assess the treatment need and outcome.

But many of the indices have their own limitations. Basic requirement for use of an index is to be valid, reliable easy to use and to amend for modification. PAR index is one of them and is known to satisfy all the required objectives. It measures occlusal characteristics and has been used for assessment over the years after its introduction into the field of orthodontics. The PAR index is a weighted combination of seven occlusal traits, upper and lower anterior alignment, right and left buccal occlusion, overjet, overbite and centreline. Later the weightings for the separate components were derived from the validation studies that were done due to the panel assessment study.

Conclusions

The experience of the PAR index is still at an early stage, although several practical uses are described that the PAR index has an excellent validity that has been demonstrated and has also been tested in a series of investigations. Intra examiner reliability was also excellent (6, 16, 18, 21). Thus finally according to the study of Dr. Richmond, it was said that it was possible to teach staff without dental qualification or training to use the weighted PAR index to a high level of reliability. Thus in this way, the PAR index can be used for assessing the stability after orthodontic treatment.

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EVALUATION OF THE STABILITY AFTER ORTHODONTIC TREATMENT USING PAR INDEX

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Summary: The aim of the present study is to establish the results using the scores obtained from the methodology that was applied for assessing the stability after 2 years post orthodontic treatment and stability in a sample of 69 individuals. After applying PAR index to the collected group of patients, a series of scores were obtained, which were then needed to be evaluated further to establish the results. The scores were evaluated using the nomogram. A comparison was done between the post treatment and the 2 years post treatment PAR weighting scores to determine if the results were stable after orthodontic treatment. The majority of the orthodontic patients were treated to a good standard and that the results appeared to be stable.

Key words: Orthodontic treatment; Stability; PAR index

Introduction

Stability acts as a good criterion, which is known to help in judging a successfully treated case. However the reality is found to be different that the treated cases relapse after a certain period of time. A question that is often raised regarding long-term stability results after orthodontic treatment in which length of period should give an appropriate estimate of stability. From the cost effectiveness point of view, a lifelong lasting effect of orthodontic therapy would be the preferred consequence. In some studies (3), it has been stated that expecting a high degree of stability after 20 years exceeds the usual expectations in the other fields of medicine and dentistry, other long term changes of the dentition due to growth, aging, periodontal diseases and caries as well as various types of dental restorations should be considered. Also studies have shown that an evaluation period of 10 years does justice to the nature of orthodontics (18, 19).

Although permanent retention is an option of achieving long-term stability, but as trained orthodontist it becomes duty to deal with the factors associated with the relapse and to aim in removing the relapse factors. These factors are to be taken into consideration and the retention phase of the patient should be planned so that the post treatment changes can be overcome to a large extent. The retention phase is defined as that phase of orthodontic treatment, which eliminates the factors causing relapse. It is defined by Moyers as “maintaining newly moved teeth in position

long enough to aid in stabilizing their correction”. The retention of teeth in ideal functional and esthetic position has always been a challenge to the orthodontist.

To determine the persistence of stability, the PAR index was used to assess the study casts of the collected group of 69 patients during the three different phases of the study. After which a series of scores were obtained, they were then evaluated using the nomogram. Studies have shown that the scores obtained can be plotted on the nomogram and accordingly results could be obtained (1, 2, 4, 5, 6, 8, 14, 15, 16), which in turn helps in the evaluation of the overall quality of orthodontic treatment.

Materials and Methods

The study was retrospective in nature as it was done with the help of the study casts for the measurements of the occlusal traits before treatment, after immediate treatment and during the 2 years post treatment phase. During the selection of the sample for the study, the following criteria were considered:

1. The patient's co-operation and consent was necessary and after which the impressions were made in the post treatment phase.
2. The patients were required to belong to the group of more than or equal to 2 years of post treatment phase.
3. The sample of the study casts collected involved group of patients, who were treated by fixed appliances or by a combination of removable and fixed appliances.

4. Further the study consisted of study casts, where treatment was done in both dental arches.
5. The study casts were considered to be intact without any broken margins.

Initially, the sample of patients were needed to be selected for assessing the stability after 2 or more years of orthodontic treatment from the Department of Dentistry in Hradec Králové. The study was done using the the PAR index. Approximately 90 patients were invited for the study, out of which 73 patients reported and agreed for the study. After completion of more than 2 years of orthodontic treatment, the decision was made for the impression of patients. The patients were between the age groups of 15–29 years. The male to female ratio was 18:55, indicating that a large number of patients were females, who received orthodontic treatment. The average time of completion of orthodontic treatment in these patients were more than two years as the treatment was completed in 2004 and they were in the period of retention after treatment during the year 2006. Further, the study got limited to 69 patients because only 69 patients met all the required criteria for carrying out the study. After the collection of the sample, the scores were applied to each component of the dentition, i.e., to anterior segment both upper and lower arch, buccal segments on both right and left side, overjet, overbite and to the centerline. The scores were applied to the collected group ac-

cording to the PAR index. Each score was known to depict the condition of the dentition. A score of zero indicates a good alignment, while higher score indicates a higher irregularity of the dentition. According to the PAR index, the difference between the score at the beginning and at the end of the treatment showed the degree of the improvement and the success of the orthodontic procedure.

The evaluation was done twice by the same clinician to reduce the risk of errors. The interval between the measurements was 6 weeks.

The pattern and sequence of assessment that was done for the different components of the dentition using the PAR index demonstrates Tab. 1. Then the individual scores were multiplied by the weightings derived for the five components of the PAR index. For example: The upper and the lower anterior segments were multiplied by 1, right and left buccal occlusion by 1, overjet with 6, overbite with 2 and centerline with 4 as shown in Tab. 2. This is done because of the fact that the PAR index components are weighted to balance the impact of the individual components of the overall result. After which the individual components with their weightings are summed up to establish the overall total as shown by Tab. 3.

Similarly, after applying the PAR index to the three phases of the study, i.e. to the pre PAR weighting score, post PAR score weighting score and the 2 years post treat-

Tab. 1: The scores for each components of the dentition of pre treatment casts obtained on applying the PAR index.

Patients	Upper anterior segment	Lower anterior segment	Right buccal occlusion	Left buccal occlusion	Overjet	Overbite	Centerline
1.	4	2	0	0	1	0	1
2.	5	2	2	3	1	2	2
3.	4	1	3	3	1	1	2
4.	1	1	2	2	1	0	0

Tab. 2: The scores with their respective weightings for all the 69 patients that are assessed before treatment.

Patients	Upper score with weightings	Lower score with weightings	Right buccal relation with weightings	Left buccal relation with weightings	Overjet	Overbite	Centerline
1.	4 X 1	2 X 1	0 X 1	0 X 1	1 X 6	0 X 2	1 X 4
2.	5 X 1	2 X 1	2 X 1	3 X 1	1 X 6	2 X 2	2 X 4
3.	4 X 1	1 X 1	3 X 1	3 X 1	1 X 6	1 X 2	2 X 4
4.	1 X 1	1 X 1	2 X 1	2 X 1	1 X 6	0 X 2	0 X 4

Tab. 3: The scores with their overall total that is obtained by adding each of the components that were multiplied with its respective weightings.

Patient	Overall total
1.	16
2.	30
3.	27
4.	12

Tab. 4: Total weightings in three phases of treatment.

Patient	Pre treatment PAR weightings	Post treatment PAR weightings	Post retention PAR weightings
1.	16	0	3
2.	30	3	4
3.	27	7	9
4.	12	0	0

ment PAR weighting score, results described in the Tab. 9 were obtained.

This way the PAR index was applied for assessing the stability in the collected sample of 69 individuals.

Results

The scores obtained after applying the PAR index were subjected for assessment using the nomogram. The nomogram is one of the possible ways of detecting the improvement or the deterioration of the treatment and thereby helps in assessing the stability and quality of work.

In the study of assessing the improvement with the help of nomogram, the pre-treatment weighted PAR score was given on the horizontal 'x' axis and the post-treatment weighted PAR score on the vertical 'y' axis. The pre-treatment and the post-treatment scores were read on their respective axis. Where the intercept falls, it indicates the degree of improvement which helps to provide three broad bands of treatment change, i.e.:

- worse - no different,
- improved,
- greatly improved.

Firstly the nomogram was plotted with the total PAR weightings of pretreatment on 'x' axis with the scores starting from 0 to 40 with a difference of 5 between each reading, similarly the total PAR weightings of post treatment were plotted on 'y' axis with the scores starting from 0 to 30 with a difference of 5 between each reading. The following is represented with the Fig. 1. The graph helps in detection of improvement of the cases, i.e. it helps to know if the treatment was worse without any significant improvement, which could be referred to as worse - no different, or if there was a significant improvement, which could be re-

ferred to as improved, or if there was a marked improvement, which could be referred as greatly improved, thus in this way the treatment results can be assessed using the nomogram.

The nomogram was obtained by placing the overall total of the patients that were obtained before and after treatment during the study. Thus we can see from the nomogram that:

1. No patients fell into the category of *No different* band of treatment change.
2. A large group of patients are in the range of 0 to 22 points, indicating that the cases fell into the category of *Improved* band of treatment change.
3. A fewer group of patients fell into the range of PAR score greater than 22 points, indicating that the cases fell into the category of *Greatly improved* band of treatment change.

The nomogram was plotted with the total PAR weightings after treatment on 'x' axis and post treatment of more than 2 years on 'y' axis starting from 0 with a difference of 5 points on each axis as shown in Fig. 2. The nomogram was obtained by placing the overall total of the patients that were obtained after and during 2 years of post treatment phase of the study. Thus we can see from the nomogram that

1. Most of the patients fell into the first category of treatment change, indicating that the cases showed no significant variation during the post treatment phase of more than 2 years.
2. On the other hand, one point was evident in the second category of the change in treatment, indicating that the case was slightly deviating from the achieved result.
3. No patients fell into the third range of the treatment change, indicating that no case exhibited such a great deviation after treatment.

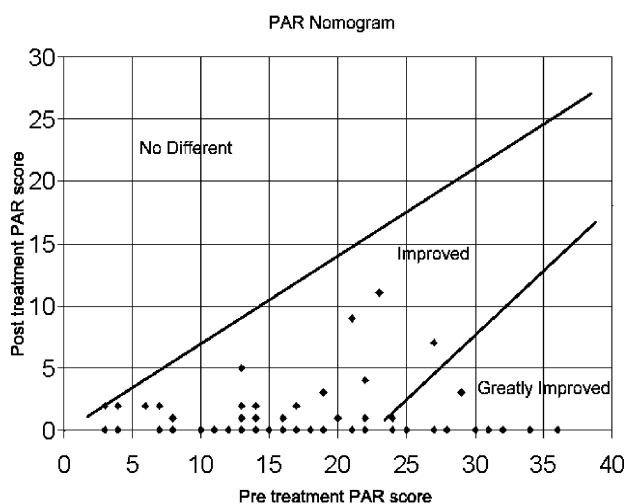


Fig. 1: Represents the PAR nomogram that has Pre treatment PAR score on the x axis and the Post treatment PAR score on the y axis.

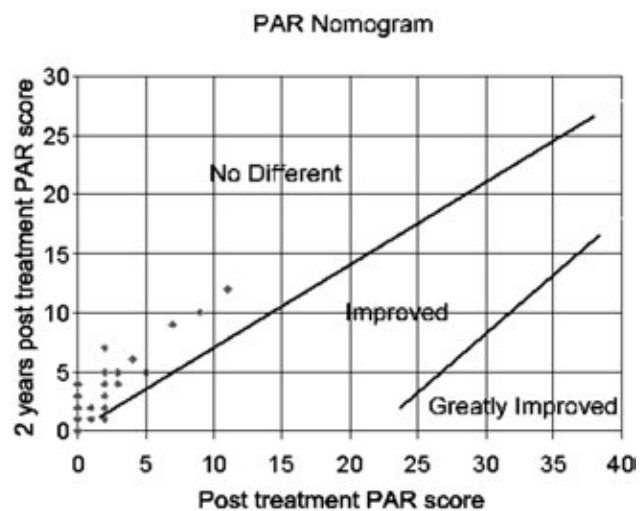


Fig. 2: Represents the PAR nomogram that has Post treatment PAR score on the x axis and 2 years post treatment PAR score on the y axis.

Discussion

PAR index was used for the study of assessing stability after orthodontic treatment in different phases i.e. before treatment, immediate after treatment and during post treatment phase of more than 2 years. No attempt was made to compare treatment results by different types of appliances. The study was mainly involved in a group of patients who were invited for the study and upon the consent of the patients and their parents. The study was started and it included patients with fixed appliance therapy or a combination of fixed and removable appliance therapy and the majority of the patients were females due to the fact that the ratio of male to female was 18:55 at the onset of the treatment, which gradually reduced to the ratio of 17:52, the reason being the inavailability of the criteria that was essential for the study. On assessing the study casts during each phase of the study, a set of scores were obtained that were then multiplied with their respective weightings and then the overall total was obtained. It was then plotted on the nomogram and assessed for the results. It was found that a large number of patients fell into the category of *Improved* and *Greatly Improved* range of improvement, which showed that the majority of the cases were treated to a very good level. Also when the post treatment and the post treatment phase of more than 2 years were compared, majority of the patients fell into the group of *No different* category of treatment indicating that the treatment results were stable and that it did not exhibit significant variations. For an institution as well as for a private practise such an analysis can contribute to quality assurance of treatment outcome over the years. The assessment also acts as an important tool in the process of total quality management of orthodontic care provision.

In this study, the improvement in PAR score at the post treatment stages can be explained to some extent by the treatment period, more recent the period the better quality was obtained in the results. Although it is known that the occlusal deterioration occurs in the period that follows orthodontic treatment, the dual arch fixed appliance treatment achieves and maintains the best post-treatment results (17). Also the stability of the results can be due to the fact that the patients were still in the retention phase of the treatment. PAR index helped in evaluating the study casts and thus is known to offer uniformity, objectivity and standardization in assessing the outcome of orthodontic treatment. However it needs to be revised in the light of new knowledge and the changing perceptions of standards and mainly to overcome the limitations that exist with the index. PAR index exhibits certain obvious limitations that must be considered when it is used in the evaluation of treatment outcomes, e.g., it cannot identify inappropriate arch expansion, inclination of incisors and improvement in appearance or psychosocial well being.

Conclusions

In this study, the relapse pattern was comparable for a group of patients treated orthodontically in the Department of Dentistry in Hradec Králové. When the study was made between the pre treatment and the post treatment phases, a majority of the patients of the group fell into the *Improved* or the *Greatly improved* range, indicating that the cases were treated to a good standard. On the other hand, when the study was compared for the stability of the result after more than 2 years of post treatment phase, it showed that the cases fell into the range of *No different* category indicating the cases appeared to be stable to a large extent. As a conclusion, it is possible to say that the majority of the cases were treated to a good standard and that the results appeared to be stable. However this can be supported by saying that the patients were in the retentive phase after treatment that helped in maintaining the results obtained. It is advisable to further carry out the study in order to detect the persistence of stability after few more years of treatment without the presence of retention appliance. More additional research in the field of indices becomes essential with time to provide the comparison of the results.

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OUR APPROACH TO THE SPASTIC HIP SUBLUXATION AND DISLOCATION IN CHILDREN WITH CEREBRAL PALSY

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Summary: The purpose of this study was to evaluate the effectiveness of our approach to the spastic hip subluxation and dislocation in children with cerebral palsy. We evaluated 56 hips in our consecutive patients who had been operated on at our department between January 2003 and December 2005. There were done soft-tissue release procedures in 42 hips, osseous reconstructive surgery in 11 hips and osseous palliative surgery in 3 hips. The duration of follow-ups was 1–3 years after surgery. We achieved good result in 15 hips after soft-tissue release, fifteen hips had a fair result, nine a poor result and three a failure. No redislocation was observed after osseous surgery in our patients. Two patients observed no pain after osseous palliative surgery, transient pain in the hip was in one case. In all hips the range of motion (abduction) was increased. The personal hygiene and possibilities of rehabilitation were improved. Childhood is the optimal time to intervene to maximize the function of the patient with cerebral palsy. The musculoskeletal treatment of the child prevents future problems with pain and deformity.

Key words: Cerebral palsy; Soft-tissue release; Osseous reconstructive surgery; Palliative osteotomy of the femur

Introduction

Different surgical procedures can be used in the management of spastic hip subluxation and dislocation in children with cerebral palsy. There are two important factors for the indication of the hip surgery. Severity of the hip migration can be evaluated according to Sharrard into four groups: 1. normal or stable hip (the femoral head completely covered by the acetabulum), 2. dysplastic hip (migration percentage <33), 3. subluxated hip (migration percentage 33–99), and 4. dislocated hip (complete loss of the acetabular contact) (12). Prognosis for ambulation is dependent on the geografic type of cerebral palsy (hemiplegia, diplegia, quadriplegia) and on the predicting of ultimate motoric ability according to Vojta (3, 14).

The purpose of this study was to evaluate the effectiveness of our approach to the spastic hip subluxation and dislocation in children with cerebral palsy.

Material and Methods

We evaluated 56 hips in our consecutive patients who had been operated on at our department between January 2003 and December 2005.

The soft-tissue releases were performed in 42 hips. The age of the fourteen spastic diplegia children (27 hips) and

eight spastic quadriplegia children (15 hips) at the time of surgery was 3–17 years (the average age 8.6 years). Eleven patients (21 hips) were able to walk and eleven patients (21 hips) were unable to walk. There were done these following soft-tissue procedures: open adductor tenotomy in 16, adductor and rectus femoris tenotomy in 18 hip joints, adductor, rectus femoris and iliopsoas tenotomy in 4 hips. Additional rectus femoris tenotomy was used in 4 hip joints.

The osseous reconstructive surgery was done in 11 hips. The age of the six spastic diplegia children (9 hips) and one spastic quadriplegia child (2 hips) at the time of surgery was 1.5–18 years (the average age 8 years). All these patients (11 hips) were able to walk. There were performed these different surgical procedure: femoral osteotomy in 6, Salter pelvic and femoral osteotomy in 2 hip joints, Steel pelvic osteotomy, open hip reduction with San Diego acetabuloplasty and femoral osteotomy, open hip reduction with femoral shortening and rotational osteotomy in 1 hip joint each.

The osseous palliative surgery was indicated in two spastic quadriplegia children (3 hips) unable to walk. The age at the time of surgery was 10 and 16 years (the average age 14 years).

The duration of follow-ups was 1–3 years after surgery. Each patient underwent a careful clinical examination, and radiografic evaluation at follow-up. Physical examination

included assessment of the range of the hip joint motion. X-rays included anteroposterior views of the pelvis to assess the severity of the hip migration. All hips after soft-tissue release procedure were classified according to the hip migration percentage into four surgical outcome groups: 1. good (migration percentage <25), 2. fair (migration percentage 25–39), 3. poor (migration percentage 40–60), and 4. failure (migration percentage >60).

Results

Fifteen hips (36%) after soft-tissue release procedure had a good result, fifteen (36%), a fair result (Fig. 1, 2), nine (21%), a poor result, and three (7%), a failure. The outcome was unsatisfactory (poor or failure) in older children referred to soft-tissue release procedure late (Table I).

No redislocation was observed after osseous reconstructive surgery in our patients (Fig. 3, 4). The spastic diplegia patients were able to walk, the spastic quadriplegia patient had a minimal ability to walk.

Two patients observed no pain after osseous palliative surgery, transient pain in the hip was in one case. In all hips the range of motion (abduction) was increased. The perineal care and possibilities of rehabilitation were improved (Fig. 5, 6, 7).

Discussion

The spastic hip subluxation develops in response to the muscle imbalance (spasticity and contracture of the adductors and flexors of the hip) and spontaneous improvement of the subluxated hip should be never expected. On the contrary a risk of a 10% increase in the migration percentage per year was reported in the subluxated hip joints (9). The preoperative migration percentage, age at surgery, level of neurological involvement, used surgical technique and one-year postoperative migration percentage were reported in the literature as predictors of final soft-tissue release procedure outcome (2, 4, 6, 8, 9, 13). The comparison of outcome results is due to differences among the series difficult, but the rate of satisfactory outcomes (good and fair results) in our patients after soft-tissue-release procedure (72%) is comparable with other series (5, 8).

Soft-tissue procedures alone are not adequate for severe acetabular dysplasia with established subluxation or dislocation of the hip in older child. Because of this limitation of isolated soft-tissue release, a combined soft-tissue release and reconstructive bony procedure have been used for the treatment of severe dysplasia of the hip secondary to neuromuscular disease (7, 10). Salter pelvic osteotomy is useful in children with cerebral palsy to 11 years of age only

Tab. 1: Results for 42 hips after soft-tissue release procedure according to the age at the time of the surgery.

Age at the time of the surgery	Surgical outcome group			
	Good migration percentage <25	Fair migration percentage 25–39	Poor migration percentage 40–60	Failure migration percentage >60
To 6 years	15	6	2	0
6 to 12 years	0	4	0	1
Older than 12 years	0	5	7	2

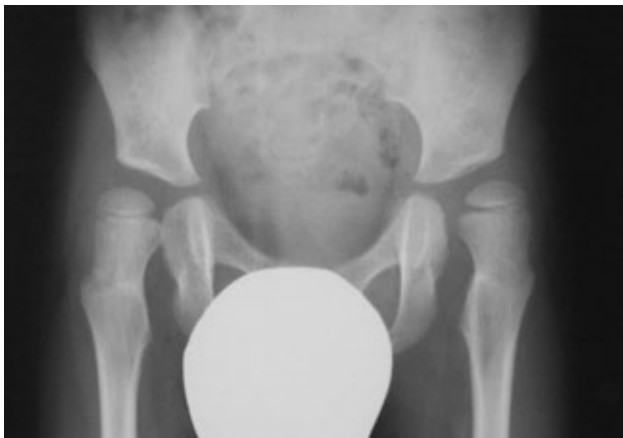


Fig. 1: Radiograph of a four and half-year-old boy with spastic diplegia. The migration percentage was 33% in the right hip and 38% in the left hip before the performance of soft-tissue release bilaterally.



Fig. 2: At one and half year postoperatively, the range of hip abduction was improved bilaterally, the migration percentage was 27% in the right hip and 33% in the left hip. Spontaneous increase in the migration percentage was prevented.

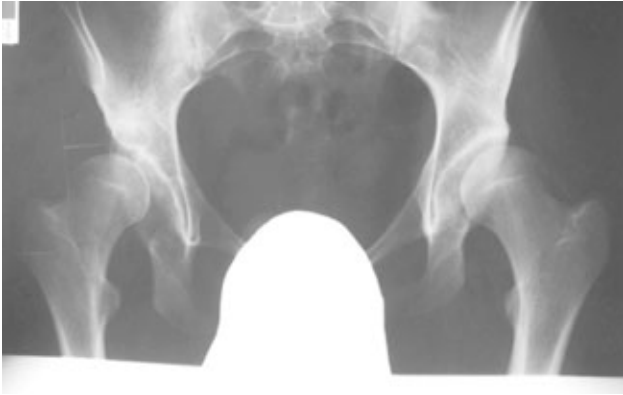


Fig. 3: Anteroposterior radiograph of a seventeen-year-old boy with spastic diplegia who was able to walk. Preoperatively, the right hip was subluxated and painful.



Fig. 4: After Steel triple osteotomy, the right femoral head coverage was much improved and the patient was pain-free.

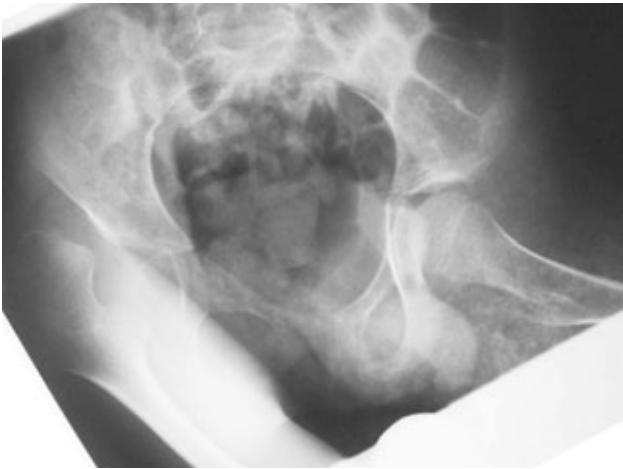


Fig. 5: Radiograph of a ten-year-old boy with spastic quadriplegia who was unable to walk. Preoperatively the right hip with windblown deformity was dislocated and painful.



Fig. 6: After the palliative Schanz abduction femoral osteotomy, the range of abduction was increased.



Fig. 7: At one year postoperatively, the personal hygiene and possibilities of rehabilitation were improved. The patient was pain-free.

when posterior acetabular deficiency is not present and acetabular index is < 35 degrees (1). Acetabuloplasty is recommended for patients with flexible triradiate cartilage (to 11 years of age) when posterior acetabular deficiency is present and acetabular index is > 35 degrees. Osteotomies that free the acetabulum (Steel, Ganz) are indicated in children with cerebral palsy older than 11 years of age only when the articular surfaces of the joint are congruous or become so once the acetabulum has been corrected. Chiari osteotomy is useful in patients with incongruous joints over 11 years old and the shelf procedure is appropriate for adolescents in which no other osteotomy will establish a congruous joint. The global acetabular deficiency, demonstrated by computerized tomographic studies, suggests that redirectional pelvic osteotomies may be unsuitable for such patients because these procedures have the potential to induce posterior hip instability (1, 15). We have not seen the posterior hip dislocation or subluxation in any our patient.

Reconstructive surgery has an uncertain outcome for dislocated hips in older patients with no potential for ambulation and a palliative procedure is frequently the only method of choice (11). In our series palliative Schanz osteotomy have provided a pain control, increased range of motion and improved personal hygiene.

Conclusions

A childhood is the optimal time to intervene to maximize the function of the patient with cerebral palsy. The musculoskeletal treatment of the child prevents future problems with pain and deformity. Hip subluxation develops in response to muscle imbalance (spasticity and contracture

of the adductors and flexors of the hip) and bony deformity develops secondary. Primary is correction of this muscle imbalance by soft-tissue release. In the setting of more significant hip subluxation the isolated soft-tissue release is inadequate and osseous surgery is indicated.

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PALEOPATHOLOGICAL FINDINGS OF DRY SKULLS WITH PLAGIOCEPHALY

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Summary: In literature, plagiocephaly is defined as abnormal asymmetrical head shape with unilateral flattening of the anterior and/or posterior part of the skull. It is classified as synostotic or deformational. Synostotic plagiocephaly is the result of premature unilateral fusion of neurocranium sutures. Positional plagiocephaly is caused by asymmetrical intrauterine and/or postnatal external forces. The authors report findings of both types of plagiocephaly in the dry skull collection from pertaining Broumov Ossuary (13th – 18th century), Czech Republic. In the paper, the incidence of paleopathological asymmetrical skull deformities is discussed in the context with recent clinical data.

Key words: *Plagiocephaly; Craniosynostosis; Synostotic plagiocephaly; Unicoronal synostosis; Unilambdoid synostosis; Deformational plagiocephaly; Paleopathology*

Introduction

Plagiocephaly is a general term for abnormal asymmetrical cranium shape with unilateral flattening of the anterior or posterior part of the skull. The term meaning “oblique head” (“lopsided skull”) derives from the Greek word roots “plagios” and “kefalê”. Pathogenically, plagiocephaly can be classified as synostotic (organic) or deformational (positional, functional, postural, nonsynostotic) (21). Synostotic plagiocephaly is caused by premature unilateral fusion of the coronal suture (frontal or anterior synostotic plagiocephaly), lambdoid suture (occipital or posterior synostotic plagiocephaly, true occipital plagiocephaly) or lateral skull sutures (1,4,17,21). In the literature, the term “hemicranial plagiocephaly” is sometimes used for deformities associated with multiple unilateral sutural fusions (1). Deformational occipital (posterior) plagiocephaly is caused by intrauterine and/or postnatal external forces on the one side parieto-occipital region of the developing skull (13).

Morphological characteristics of the frontal plagiocephaly (Figs. 1, 2) are following (5,10,18,19): asymmetrical “rhomboid” skull shape (in vertex view), asymmetry of the skull base (predominantly of the anterior cranial fossa), ipsilateral flattening of the forehead and ipsilateral compensatory occipital bossing, contralateral compensatory bossing of the forehead (asymmetrical “pointed” forehead), asymmetry of the nose root (it is deviated towards fused coronal suture), asymmetry of the orbits (ipsilateral orbit is enlarged and elevated), and asymmetrical position of the ears (the ear of the affected side is situated anterior and superior). Three forms of frontal plagiocephaly are defined: mild form

(with minimal frontal flattening, symmetrical orbits, and a midline nose), moderate form (with ipsilateral flattening, compensatory contralateral bossing, and nasal root deviation), and severe form (with vertical orbital dystopia, severe ipsilateral flattening, contralateral bossing, and deviation of the nose root) (19). Occipital synostotic plagiocephaly (Fig. 1) is characterized by the asymmetrical “trapezoid” skull shape (in vertex view), ipsilateral flattening of the forehead and bulging of the mastoid process (it is pathognomonic for unilambdoid synostose), compensatory contralateral bossing of the forehead and occiput: “pointed” occiput, asymmetry of the ears (ipsilateral ear is situated posterior); facial asymmetry is not mostly severe (12,16,21). Occipital positional plagiocephaly (Fig. 2) is associated with asymmetrical “parallelogram” skull shape, unilateral parieto-occipital flattening, ipsilateral frontal bossing, contralateral frontal flattening and occipital bossing, asymmetry of external nose (nasal root is in a midline position but nasal bones and piriform aperture are deviated on the contralateral side), asymmetry of the orbits (contralateral orbit is situated low then ipsilateral orbit), asymmetry of the ear position (ipsilateral ear is situated anterior and superior) (9,11,13,16,21). Positional plagiocephaly can be classified as mild (without facial asymmetry) or severe (with facial asymmetry) (3).

Material and Methods

The incidence of the synostotic and/or deformational plagiocephaly was investigated in the collection of 745 dry skulls (647 skulls of adults of both sexes, 98 skulls of children) from “Broumov Ossuary” (13th–18th century), Czech

Republic. All skulls were minutely investigated by cranioscopy and craniometry. Positive findings of plagiocephaly were documented by the digital camera Camedia 3030 ZOOM, Olympus. In the part of them, X-ray and/or computed tomography (CT) examination was performed.

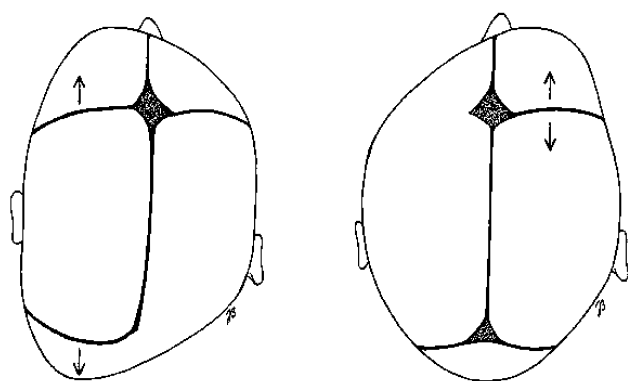


Fig. 1: Schematic picture of the skull shape deformities in the occipital (A) and frontal (B) synostotic plagiocephaly; arrows: compensational growth of the cranium.

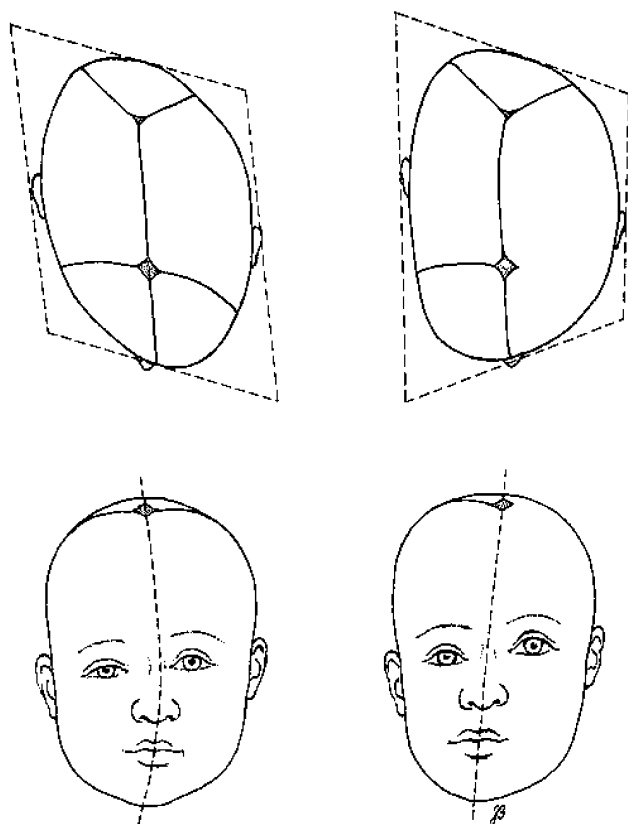


Fig. 2: Schematic drawing of morphological characterizations of the occipital positional plagiocephaly (A) and frontal synostotic plagiocephaly (B). Upper: skull shape (vertex view), lower: face asymmetry.

Results

Of the 745 examined dry skulls, 106 skulls (14.22 % – 87 adult skulls, 19 children's skulls) had evidence of plagiocephaly. From the total number of skulls, synostotic plagiocephaly (Figs. 3–7) was noticed in 13 (1.74 %) skulls (8 adult skulls, 5 children's skulls), occipital deformational plagiocephaly (Figs. 8–10) in 93 (12.48 %) skulls (79 adult skulls, 14 children's skulls). Frontal synostotic plagiocephaly with unicoronal fusion was seen in 2 (0.27 %) skulls (1 adult skull, 1 child's skull) (Fig. 3, 4). Occipital unilambdoid synostotic plagiocephaly was diagnosed in 1 (0.13 %) skull of adult individual. Synostotic plagiocephaly caused by asymmetrical obliterations of some skull sutures was observed in 7 (0.94 %) skulls (5 adult skulls, 2 children's skulls) (Fig. 5). Premature fusions of multiple cranium sutures associated with synostotic plagiocephaly and another form of synostotic skull deformity (scaphocephaly or brachycephaly) was found in 3 (0.40 %) skulls (1 adult skull, 2 children's skulls) (Figs. 6, 7).

Discussion

Presented paleopathological study is focused on the incidence of synostotic and/or deformational plagiocephaly in the dry skull collection from the Broumov Ossuary (13th– 18th century), Czech Republic. Although asymmetrical skull deformities are quite common today, not many cases have been described in the paleopathological literature (1).

In the literature, the craniosynostosis is defined as a premature fusion of one or more of cranial sutures (8). It can occur as an isolated defect (nonsyndromic) or as a part of the syndrome (e.g. Crouzon, Apert, Pfeifer, Cole-Carpenter). It may affect the coronal, sagittal, metopic, lambdoid and/or lateral sutures in isolation or in combinations (14). The skull growth is restricted predominantly in a direction perpendicular to the premature fused suture. In response to the closed suture, the remaining sutures undergo compensatory growth. Uncorrected craniosynostosis may be associated with deformity of the craniofacial skeleton and it can also cause serious constriction of the developing brain (craniostenosis), eyes (orbitostenosis), and facial viscera (faciostenosis) (14). Craniosynostosis is classified into two main categories: "simple" (or unisutural) when only one suture is involved (Fig. 11), and "complex" (compound) when two or more sutures are involved. It is usually more severe when more than one suture closes too early. The occurrence of the various types of simple craniosynostosis is approximately as follows: sagittal 50–55%, coronal 20–29%, metopic 4–10%, and lambdoid 2–4% (8).

Although the etiology of craniosynostosis is currently unknown, animal experiments and a recent interest in molecular biology point toward interplay between the dura mater and the underlying brain (14). The cranial suture fate is programmed by the dura derived paracrine signals. The dura

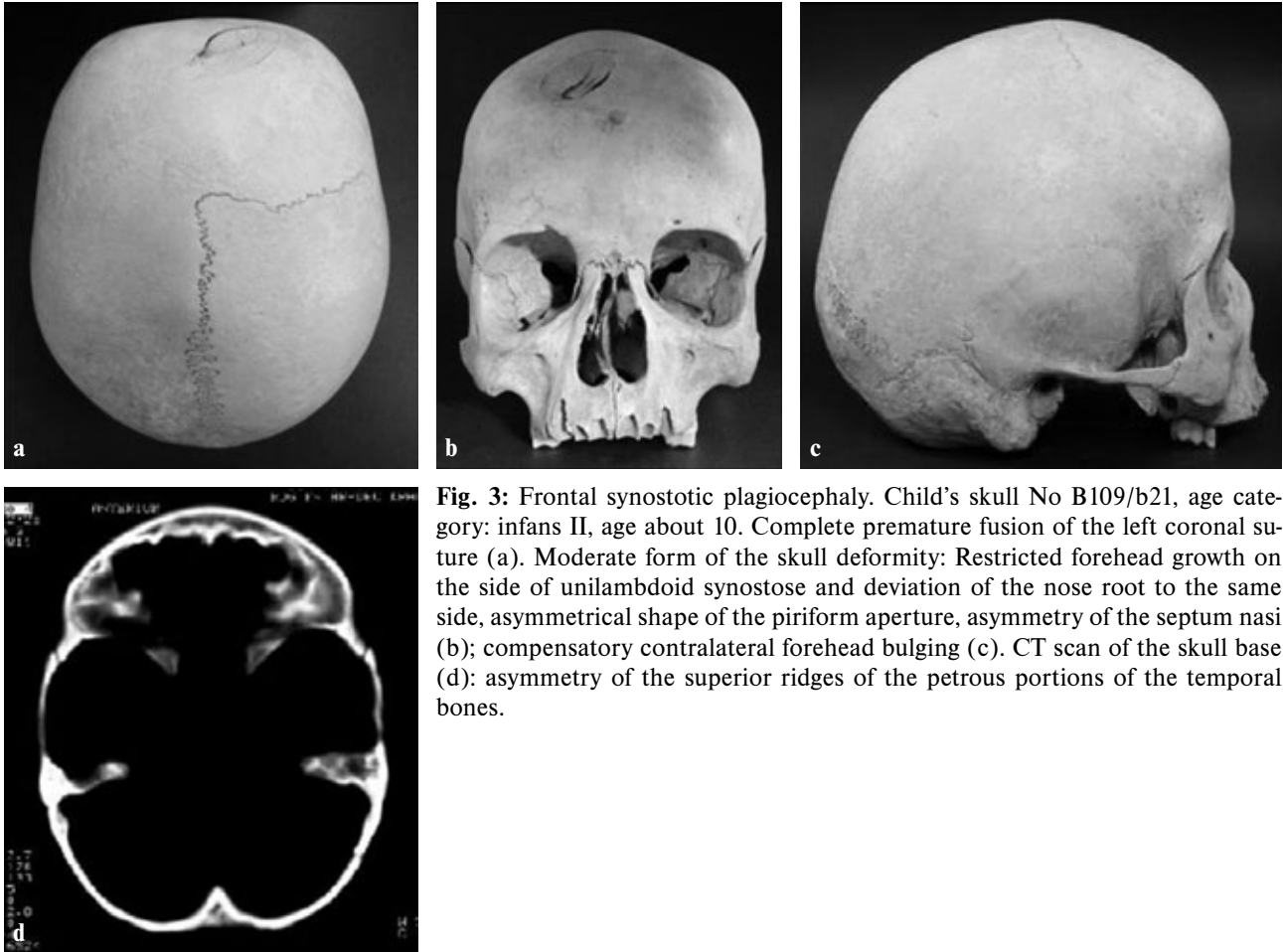


Fig. 3: Frontal synostotic plagiocephaly. Child's skull No B109/b21, age category: infans II, age about 10. Complete premature fusion of the left coronal suture (a). Moderate form of the skull deformity: Restricted forehead growth on the side of unilambdoid synostose and deviation of the nose root to the same side, asymmetrical shape of the piriform aperture, asymmetry of the septum nasi (b); compensatory contralateral forehead bulging (c). CT scan of the skull base (d): asymmetry of the superior ridges of the petrous portions of the temporal bones.

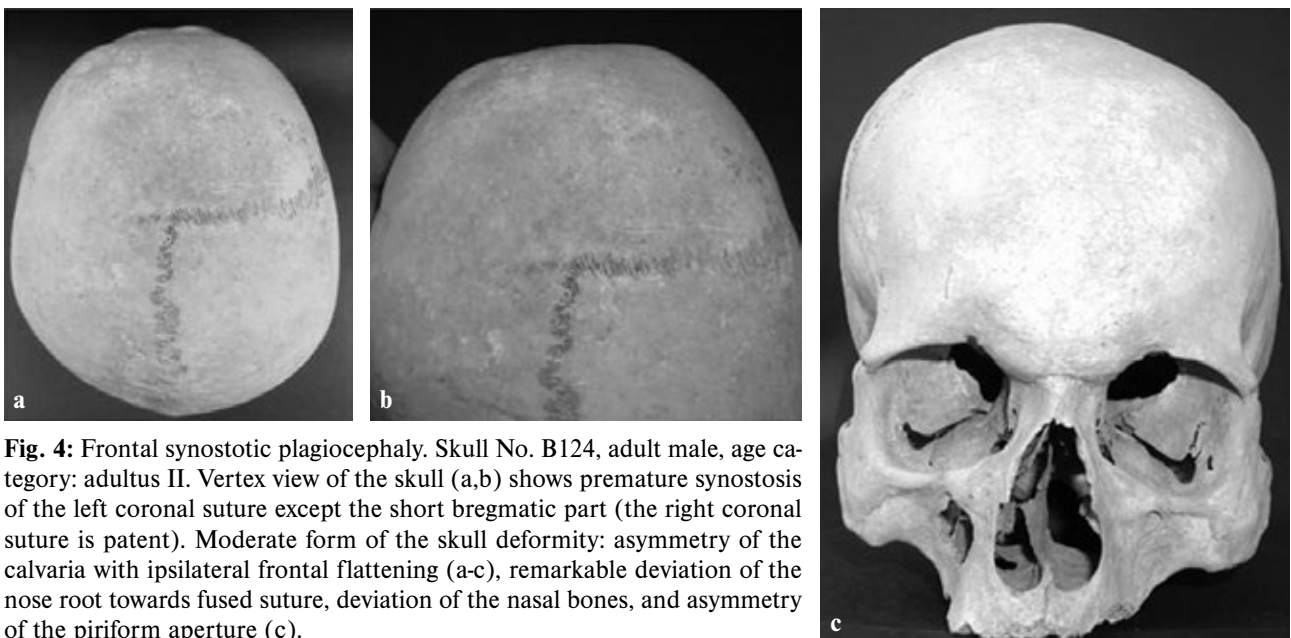


Fig. 4: Frontal synostotic plagiocephaly. Skull No. B124, adult male, age category: adultus II. Vertex view of the skull (a,b) shows premature synostosis of the left coronal suture except the short bregmatic part (the right coronal suture is patent). Moderate form of the skull deformity: asymmetry of the calvaria with ipsilateral frontal flattening (a-c), remarkable deviation of the nose root towards fused suture, deviation of the nasal bones, and asymmetry of the piriform aperture (c).

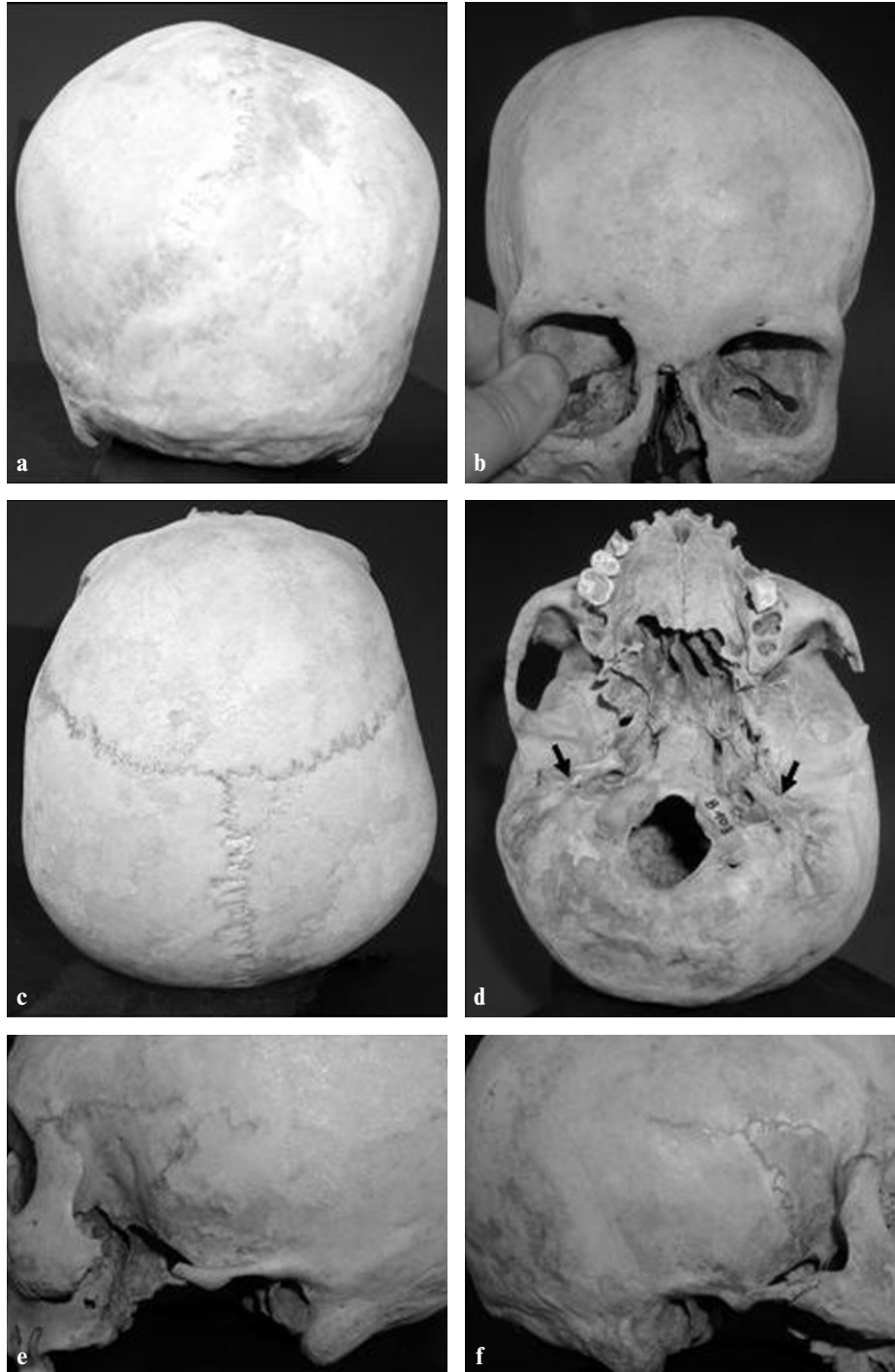


Fig. 5: Synostotic plagiocephaly associated with multiple premature sutural fusions. Skull No B103, adult male, age category: adultus II. Premature fusion of the right lambdoid suture (a), bilateral symmetrical fusion of the occipitomastoid sutures (d,e,f), bilateral partial and asymmetrical fusion of the squamous suture (e,f). Parietooccipital flattening at the side of obliterated lambdoid suture (a-d). Asymmetrical course of the sagittal suture (c) with deviation towards the affected side. Scoliosis of the external cranial base, asymmetrical position of the external acoustic meatus (d, arrows). Asymmetry of temporal fossae (e,f): on the right side, the surface of the greater wing of the sphenoid bone is shorter. Remarkable face asymmetry: asymmetrical shape of the orbits, deviation of the nose root to the side of the lambdoid synostose, asymmetrical shape of the piriform aperture (b).

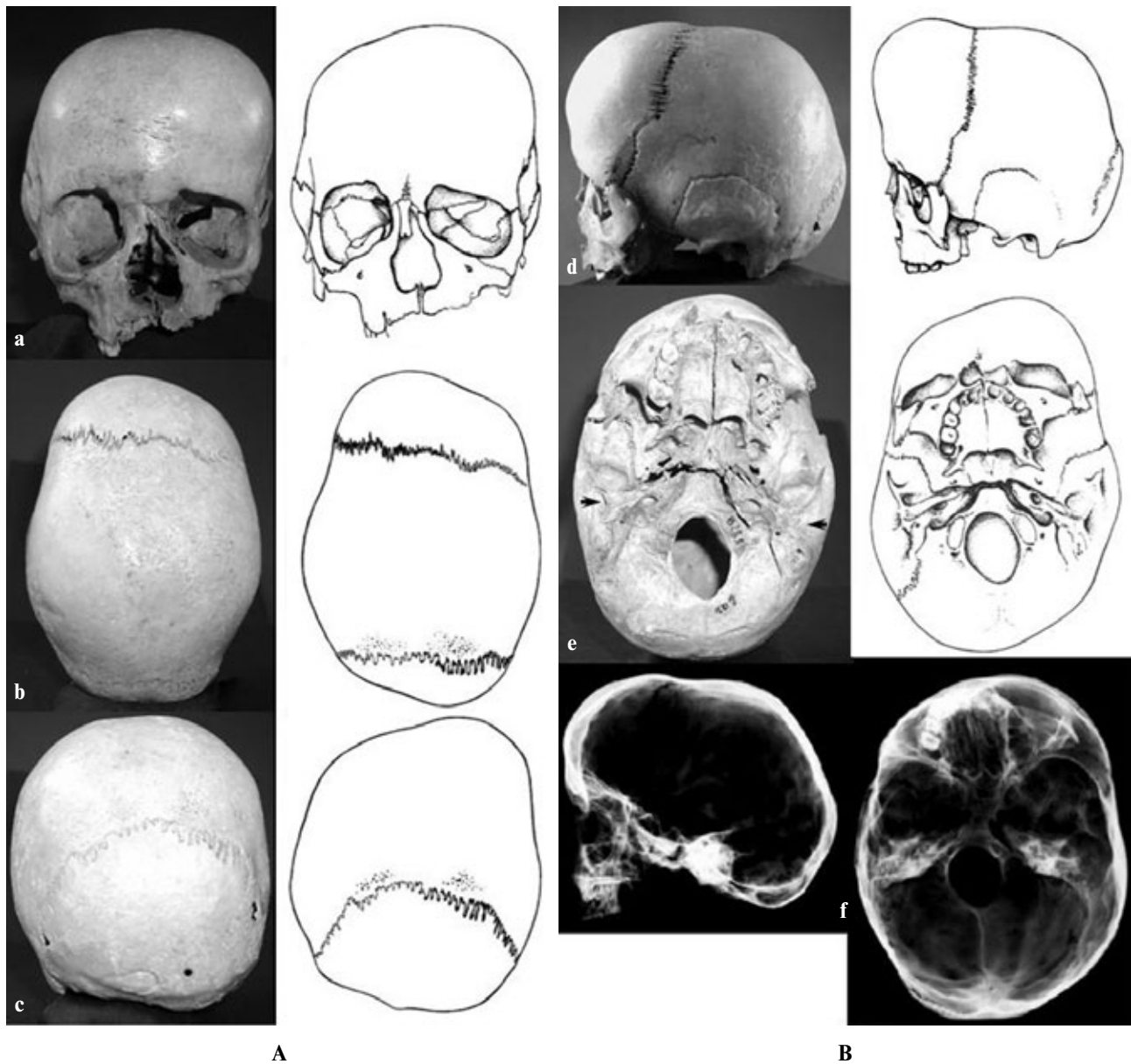
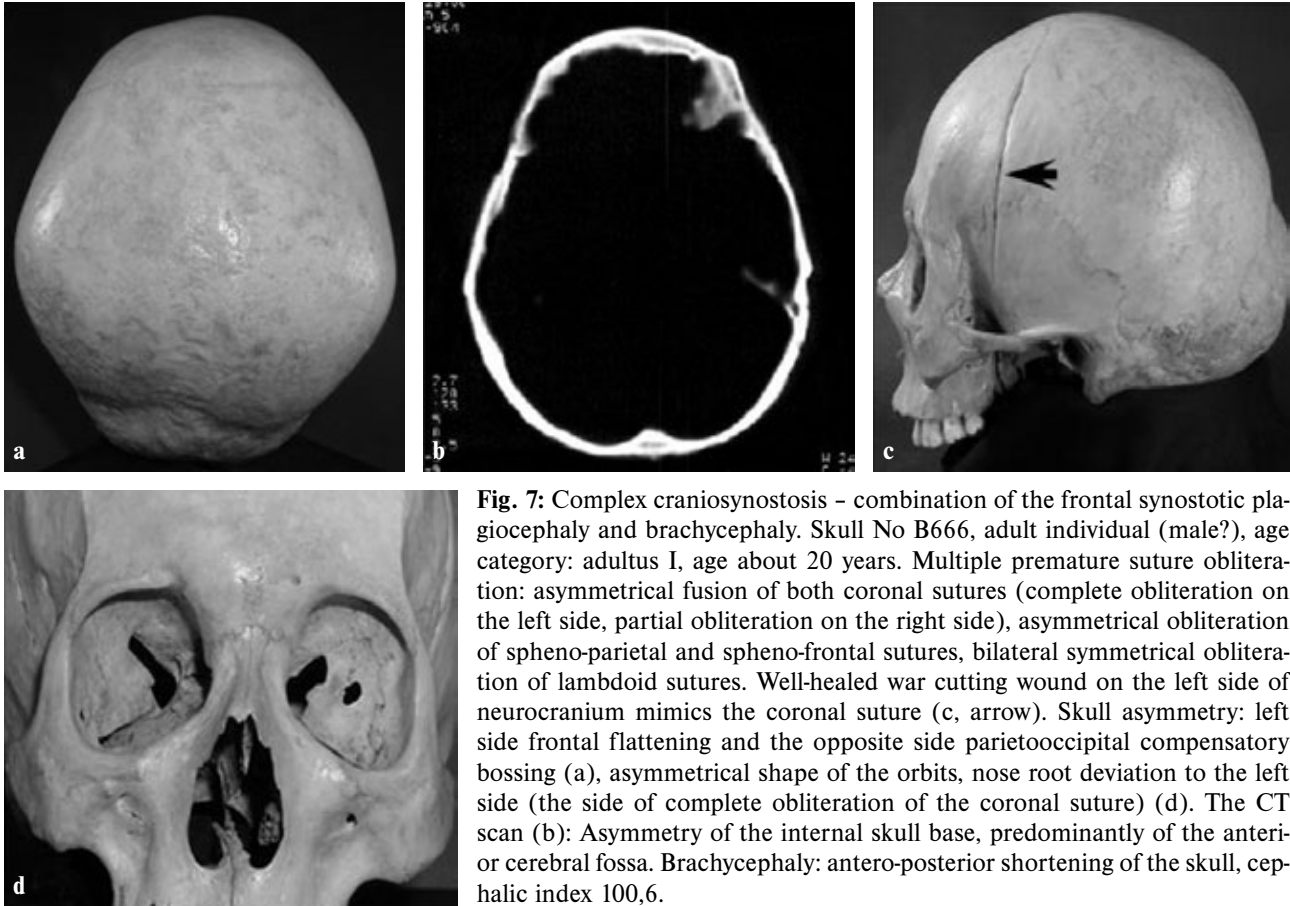


Fig. 6 A,B: Complex craniosynostosis with severe skull deformity: combination of the synostotic plagiocephaly with scaphocephaly Child's skull No B255/b92, age category: infans I, age about 5. Multiple premature suture synostosis: Partial obliteration of the right side coronal suture (A/b); complete obliteration of the sagittal suture (A/a), the left side sphenoparietal suture (B/d) and occipitomastoid suture (B/d,e); obliteration of the posterior part of the left side squamous suture (B/d). Asymmetrical deformity: Frontal flattening (A/a,b, B/e) and occipital bossing (B/e) on the side of fused coronal suture, compensatory frontal bossing on the opposite side (A/a,b, B/d,e), occipital flattening on the side of obliterated occipitomastoid and squamous sutures (A/c), asymmetry of the external acoustic meatus (B/e-arrows), asymmetrical shape of the foramen magnum (B/e); remarkably asymmetrical orbits – on the side of coronal synostosis, the orbit is enlarged and its upper margin is deviated upward (A/a); deviation of the nose root to the side of coronal fusion, asymmetry of the piriform aperture (A/a). Scaphocephaly: hyperdolichocephaly (A/b), cephalic index 74. Radiograph (B/f): long and narrow skull, asymmetry of the skull base. Distinctive impressioes digitatae are suspect from intracranial hypertension.



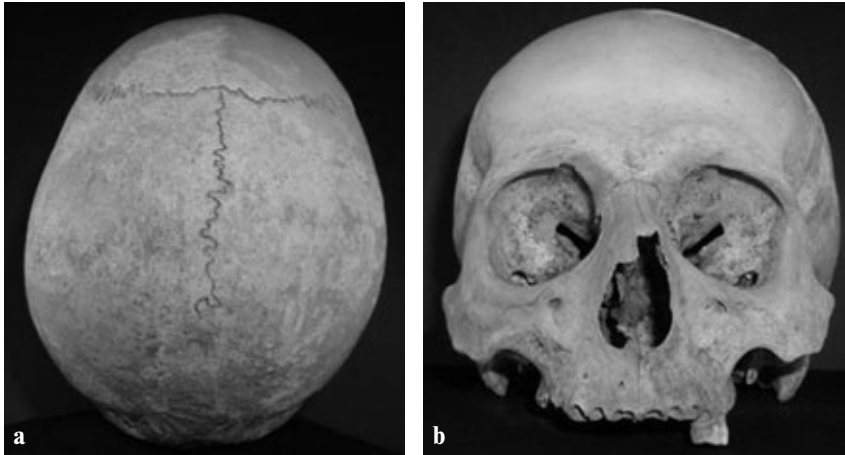


Fig. 9: Occipital positional plagiocephaly. Skull No B120, adult male, age category: adultus II. Parietooccipital skull flattening on the right side and ipsilateral compensatory frontal skull bossing. "Parallelogram" skull shape (a). Midline position of the nose root, deviation of the ossa nasalia to the left side (b).

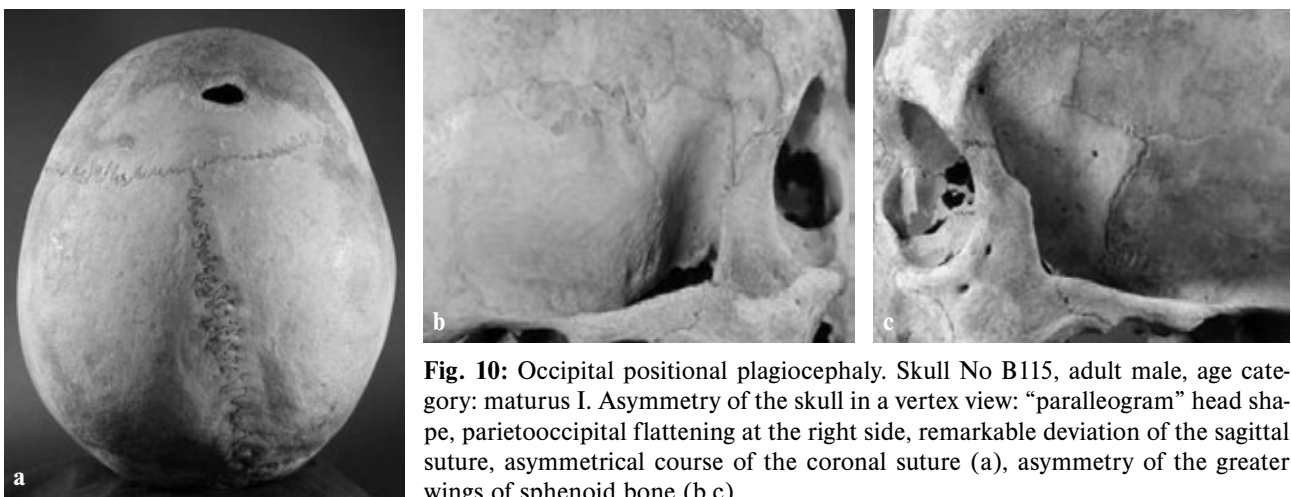


Fig. 10: Occipital positional plagiocephaly. Skull No B115, adult male, age category: maturus I. Asymmetry of the skull in a vertex view: "parallelogram" head shape, parietooccipital flattening at the right side, remarkable deviation of the sagittal suture, asymmetrical course of the coronal suture (a), asymmetry of the greater wings of sphenoid bone (b,c).

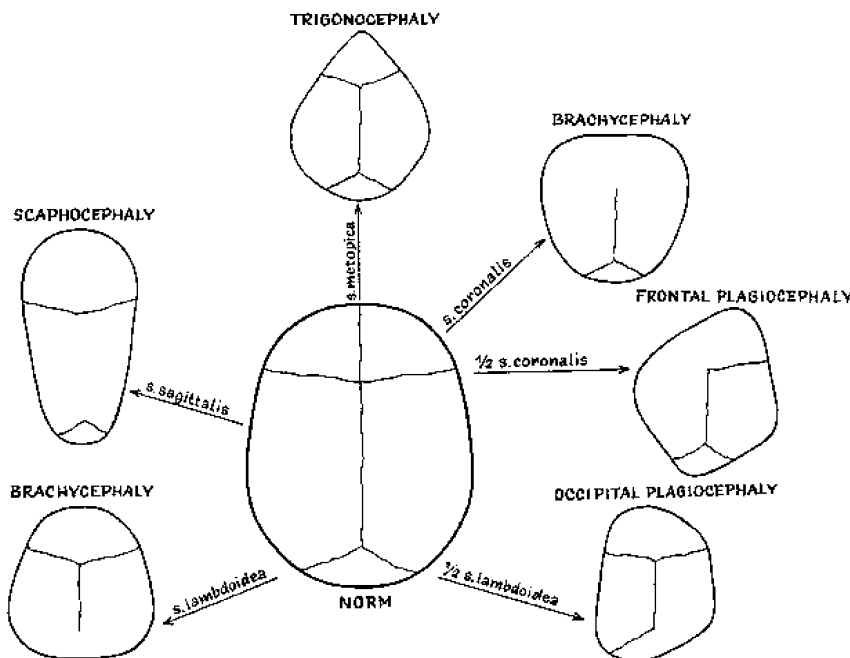


Fig. 11: Schematic drawing of simplex craniosynostosis: typical deformities of the skull.

acts as a regionally specialized endogenous tissue engineering and provides the osteogenic factors (e.g. fibroblast growth factors). These factors regulate fetal osteogenic growth and possibly influence fetal suture patency (20). Mutations in the gene coding for fibroblast growth factors cause some syndromes with craniosynostosis (2).

In the contemporaneous population, craniosynostosis has been reported to occur approximately once per 2,000 live births (8). Unicoronal craniosynostosis – scarce form of craniosynostosis – happens in approximately one out of every 2,500 live birth (2), rare unilambdoid craniosynostosis happens in approximately three out of every 100,000 births (6). In contradistinction to recent clinical studies, in Broumov historical populations dating to the 13th–18th centuries, the incidence of synostotic plagiocephaly was rather high (1.74 %): unicoronal craniosynostosis was present in 0.27 % skulls, unilambdoid craniosynostosis in 0.13 % skulls, synostotic plagiocephaly with asymmetrical fusions of some skull sutures in 0.94 % skulls, and combination of synostotic plagiocephaly with another form of synostotic skull deformity in 0.40 % skulls. Kabbani and Raghuvver (8) assume that the true incidence of craniosynostosis in population is higher than clinical studies assert. The present report supports the assumption of these authors.

Positional plagiocephaly is the most common cause of plagiocephaly (15). It results from asymmetrical cranial growth without craniosynostosis. The practice of artificial skull deformation by pressure to an infant's head dates back to 2000 BC when several ancient civilizations have used head-molding to produce desired skull shape. Positional occipital plagiocephaly is caused by constant external pressure on the one side parieto-occipital region of the developing skull (3). Risk factors associated with the positional head deformity are prolonged exposure to traditional “back” sleeping position, premature birth, congenital muscular torticollis, and intrauterine constraint (e. g. in multiple gestation or oligohydramnion) (13). Following the American Academy of Pediatrics recommendation (in 1992) that babies sleep in supine position to reduce the risk of Sudden Infant Death Syndrome, the incidence of deformational plagiocephaly has increased dramatically. Before 1992, the incidence was one of every 300 healthy born infants. Nowadays, the condition occurs in one of every 60 live birth (7). High positional plagiocephaly occurrence (12.48%) in skulls of Broumov historical populations was probably pre-

dominately caused by traditional back sleeping position of newborns and suckling infants in past centuries.

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LITHIUM MICRODIALYSIS AND ITS USE FOR MONITORING OF STOMACH AND COLON SUBMUCOSAL BLOOD PERFUSION – A PILOT STUDY USING ISCHEMIC PRECONDITIONING IN RATS

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Summary: During shock, exposure of gut to ischemia determines patient's survival. Ischemic preconditioning (ISP) elevates nitric oxide and blood perfusion, whereby it protects organs against subsequent severe ischemia/reperfusion. Using appropriate flow marker, microdialysis may serve to monitor interstitial microcirculation. Hence, our aim was to test the reliability of lithium as a flow marker (lithium microdialysis, LM) on an ISP model. Rats were divided into three groups. Two (ischemic and preconditioned) groups underwent 30 min celiac artery occlusion (CAO) with 2.5 h reperfusion. 25 min before CAO, the latter experienced 5 min ischemia. Sham-operated animals served as controls. LM in stomach and colon submucosa, serum nitric oxide, hepatic and pancreatic enzymes were measured. In stomach, LM indicated a decrease in blood perfusion evoked by CAO ($p < 0.01$) in both experimental groups. During reperfusion, the ischemic animals showed a restoration of microcirculation, unlike the preconditioned ones, whose blood perfusion failed to regenerate ($p < 0.001$). For any group, LM showed no microcirculation modification in colon. Serum analytes remained unchanged. We conclude that LM appears to be a potentially suitable indicator of gastrointestinal interstitial microcirculation. However, we failed to demonstrate any beneficial effect of ISP on pancreas, systemic nitric oxide and local/remote microcirculation within studied organs.

Key words: Microdialysis; Microcirculation; Gastrointestinal tract; Ischemic preconditioning

Introduction

It is well recognized that in critical situations, the prognosis of patients depends largely on maintenance of adequate blood perfusion in order to preserve the barrier integrity and metabolic functions of gastrointestinal tract and its adjacent organs. Recent evidence has shown that the viability of gut exposed to ischemic insult can be enhanced by ischemic preconditioning (ISP) taking place prior to sustained ischemia (1, 3). Because of deleterious impact ischemia in splanchnic organs may exert on the entire organism via bacterial- and toxin translocation and cytokine production, ISP offers an interesting therapeutic approach for clinical practice (14).

ISP refers to a process by which a (series of) brief ischemic episode(s) confers protection against subsequent prolonged ischemia/reperfusion. Regarding the mechanism, a number of neurohumoral mediator pathways have been proposed, where elevated nitric oxide followed by enhanced blood perfusion plays a central role (14). ISP can be examined with respect to one specific organ, or considering

other organs at a distance. The former differentiates between local and regional ISP, whereas the latter defines an inter-organ or remote ISP (rISP). With respect to abdominal organs, ISP has been exemplified in the liver (14), pancreas (3), small intestine (10) or stomach (1). However, rISP in colon has not attracted much interest, yet.

In many organs including the gut, *in vivo* microdialysis is a well-established miniinvasive means of local tissue metabolism monitoring (2). Using appropriate flow marker (also called tracer), this method offers opportunity to assess tissue microcirculation based on an assumption that the washout of tracer from the probe is related to extracellular fluid kinetics, which depends primarily on microcirculatory conditions. However, the most frequent tracer molecule (ethanol) is volatile, its precise analytical determination may require sophisticated instruments (like mass spectrometer), or needs to be labeled radioactively (15). Recently, a novel, apyrogenic, non-radioactive and freely diffusible flow marker with well-defined toxicity in humans – lithium, has successfully been applied to describe blood perfusion modifications in rat liver, kidney and muscle

interstitium (7). Nevertheless, further information on the usefulness of this promising tracer are needed.

Hence, the aim of this study was twofold: first, to verify the hypothesis that lithium could be used for monitoring of microcirculation in the alimentary tube and second, to evaluate the influence of celiac ISP on celiac artery occlusion (CAO)-induced microcirculatory changes in stomach and descending colon. Both hypotheses were tested on a rat model of gastric ischemia and ISP performed at the level of celiac artery trunk (1). Furthermore, the systemic effects of ISP and CAO were assessed using selected serum enzyme activities and nitric oxide concentration.

Material and Methods

Materials. Flame photometer serum-standard solution (Eppendorf, Hamburg, Germany) with Li^+ concentration of 2.00 mmol l^{-1} was used as microdialysis perfusion medium.

Animals. Adult male Wistar rats weighing 260 to 330 g, were used. The animals were housed in the animal quarters under controlled environmental conditions. They had free access to standard rat chow except 16–18 hours before experiments, when they were fasted. Tap water was provided *ad libitum*. The rats received care in accordance with the guidelines set by the Institutional Animal Use and Care Committee of the Charles University in Prague, Czech Republic. All experimental procedures were approved by local ethics committee.

Experimental protocol. The animals were randomly assigned to three groups denoted as S, IS, and ISP ($n = 6$ –10 in each group). The first group (S) was sham-operated. The second group (IS) underwent a 30 min period of complete CAO with subsequent 2.5 h of reperfusion. The rats in the third group (ISP) were preconditioned by exposure to a short ischemia/reperfusion period (5 and 25 min, respectively), followed by prolonged ischemia/reperfusion (30 min and 2.5 h, respectively). During all procedures, the animals were under general i.p. pentobarbital anesthesia (50 mg kg^{-1} followed by $15 \text{ mg kg}^{-1} \text{ h}^{-1}$) and operated in supine position. The rectal temperature was monitored and maintained at 38.5°C with a heating lamp. The trachea was cannulated to ensure patent airways. After midline laparotomy, the celiac artery was disclosed and underlaid by smooth rubber tubing to assist later clamping. Ischemia/reperfusion was accomplished by placement/removal of a micro-bulldog clamp at the level of celiac artery origination from abdominal aorta. The success of each intervention was verified visually (assessment of blood flow distal from the site of occlusion).

Gastric and colon submucosal microdialysis technique. After laparotomy, stomach was exteriorized and moistened continuously. Avoiding blood vessels, a 6 mm long tunnel was made from serosal aspect in the submucosal layer of its glandular part from greater to lesser curvature by means of

a 26 G needle. For the descending colon, similar procedure was followed along with its long axis at a distance of 5 cm from the anus. Into the tunnels, microdialysis probes (CMA/20, active length 4 mm, outer diameter 0.5 mm, cut-off 20 kDa, CMA/Microdialysis, Solna, Sweden) were carefully inserted and fixed in place with sutures. After each experiment, probes were tested for leakage and their former positions were verified by histology (Fig. 1). After surgery, the abdominal opening was closed to avoid fluid losses. Microdialysis catheters were perfused at $1.2 \mu\text{l min}^{-1}$ using a perfusion pump. An initial 60 min stabilisation period was followed by 5 h of experimental procedure (see above) with continuous dialysate sampling in 30 min intervals into microvials. Samples were stored at -20°C until lithium determination. At the end of experiment, arterial blood was withdrawn and the removed serum was stored at -20°C until analysis.

Blood perfusion measurement. The extent of submucosal blood perfusion was expressed as lithium inflow – outflow concentration ($\text{Li}_{\text{in-out}}$) difference, i.e. Li efflux as reported previously (7). Dialysate Li^+ was determined using EFOX 5053 flame photometer (Eppendorf, Hamburg, Germany) according to manufacturer's instructions.

Determination of nitric oxide in serum. Nitric oxide was measured indirectly (and expressed) as nitrate and nitrite (the stable nitric oxide metabolites) using HPLC with UV-VIS detection and fluorimetry methods described elsewhere (8).

Determination of serum enzyme activities. Hitachi 917 autoanalyser (Boehringer, Mannheim, Germany) with commercially available reagent kits (Roche Diagnostics GmbH, Mannheim, Germany) were utilized. For the study of the extent of liver injury, alaninaminotransferase (ALT), aspartataminotransferase (AST), lactatdehydrogenase (LDH) and cholinesterase (CHE) serum activities were investigated. To evaluate the level of pancreatic damage, lipase (LIP) and amylase (AMYL) activities were determined. All analyses were performed in accordance with manufacturer's instructions.

Histology. The removed tissues were fixed in 10% formaline, embedded in paraffin and further treated according to procedures standard for hematoxylin-eosine stain.

Statistics. Data are expressed as means $\pm$ standard error of mean (SEM). For statistical evaluation, Analysis Of Variance with Fisher's LSD multiple comparison *post hoc* test (programs NCSS 2004 and Statistica) were used. The chosen level of significance was $\alpha=0.05$.

Results

Blood perfusion estimation. Fig. 2 summarizes the time course of $\text{Li}_{\text{in-out}}$ differences in all groups in the submucosa of stomach (a) and colon (b). The zero interval of microdialysate collection (obtained within 60–90 min after commencement) provided basal values, where no

statistically significant difference was found between the stomach and colon. Likewise, between-group comparisons showed comparable baseline values in stomach as well as in colon.

Blood perfusion in stomach (Fig. 2a). In the S group, absolute values of Li_{in-out} difference showed no variation in time. Brief (5 min) ischemia in the ISP group caused a significant drop in Li_{in-out} difference from its baseline ($p<0.05$). Within the IS and ISP groups, 30 min interval of CAO produced significant depressions in Li_{in-out} difference when compared with corresponding basal levels ($p<0.01$). Immediately after onset of reperfusion, the values of Li_{in-out} difference raised in both groups, reaching levels not significantly different from their baselines. In the next 2 hours, Li_{in-out} difference within the IS group continued to elevate slowly, while the opposite holds true for ISP group, where we observed a steadily falling trend ($p<0.001$). However, none of the changes observed within the ligated groups was

sufficient to statistically manifest also in between-group comparisons with controls.

Blood perfusion in descending colon (Fig. 2b). Similar to gastric perfusion, the S group provided constant values of Li_{in-out} difference over the entire experiment. Neither within-group, nor between-group comparisons in parallel time intervals disclosed differences reaching the level of significance.

Serum nitric oxide and enzymatic activities. The extent of potential organ impairment and modulation of the body's metabolism was evaluated by determination of selected serum analytes. Fig. 3 depicts the grade of nitric oxide production modulation by ischemia and ISP. Even though the mean serum concentration of nitric oxide in IS group was 165% of that in control group (S), the difference was only marginally significant ($p=0.06$). The ISP group gave results similar to controls. Likewise, the activities of serum enzymes in question were not statistically different (Fig. 4).

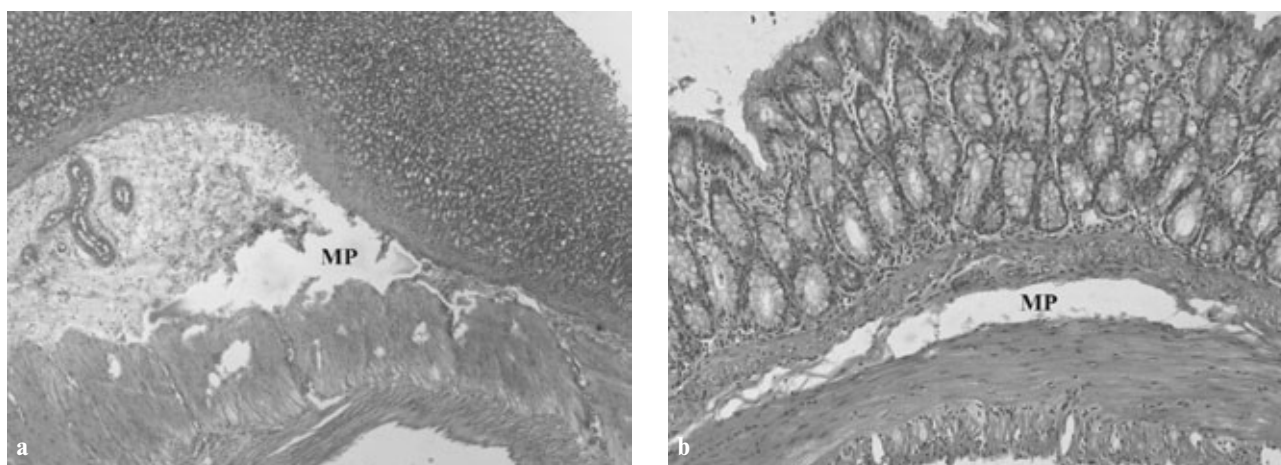


Fig. 1: Histological appearance of the wall of stomach (panel a) and descending colon (panel b) – a tangential section to previous probe positions marked MP (“microdialysis probe”). Sham-operated group, haematoxylin-eosine stain.

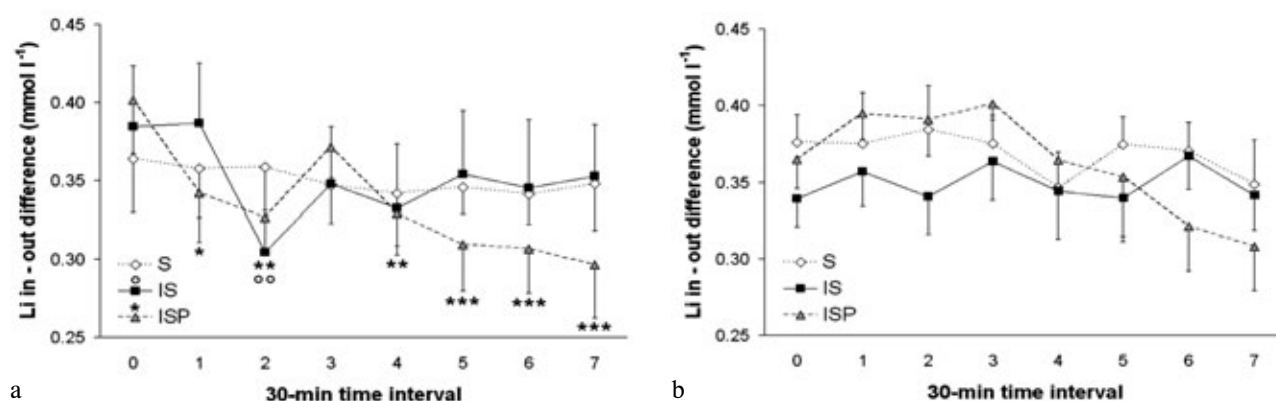


Fig. 2: A comparison of submucosal stomach (a) and colon (b) blood perfusion among the three groups (S, IS, ISP) followed in time and expressed as Li_{in-out} difference. Within the groups, markings *, °, **, °° and ***, °°° denote $p<0.05$; $p<0.01$ and $p<0.001$, respectively as compared to corresponding reference (baseline) levels. Results are mean $\pm$ SEM of 6–8 rats (2 animals excluded due to hemorrhage around the probe).

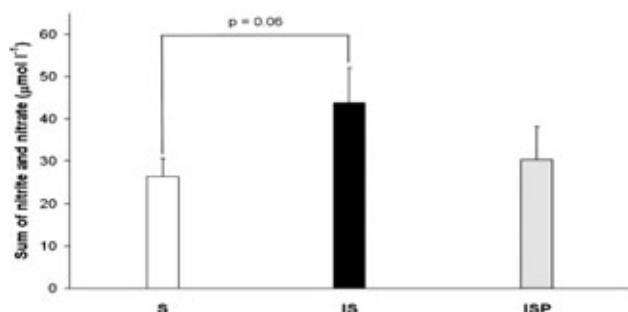


Fig. 3: Effect of ischemia (IS) and ischemic preconditioning (ISP) on serum nitric oxide (shown as the sum of nitrite and nitrate and compared with sham-operated group, S). Results are mean + SEM of 6–10 rats.

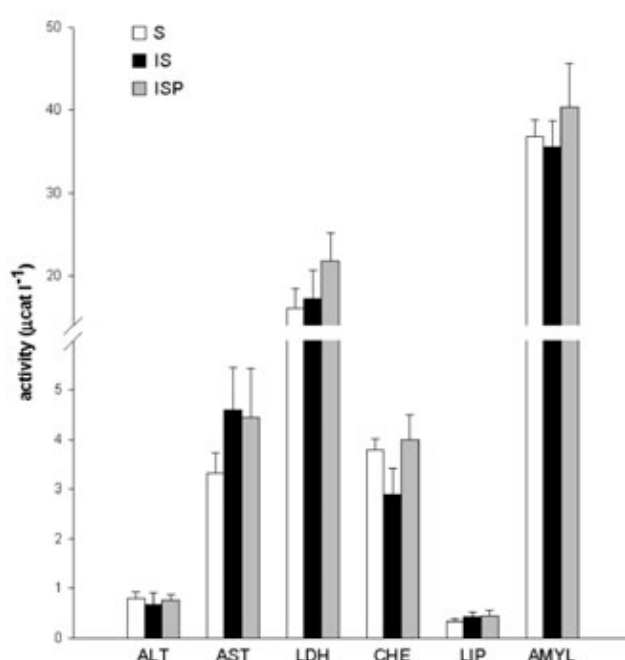


Fig. 4: Effects of ischemia (IS) and ischemic preconditioning (ISP) on serum enzyme activities (expressed as comparisons with sham-operated group, S). Results are mean + SEM of 6–10 rats. Level of significance was not reached in any of the studied parameters.

Discussion

This pilot work presents a new modification of blood perfusion measurement using microdialysis in two distinct parts of rat alimentary tube. Our most important findings were twofold: first, the anticipated stability of Li_{in-out} difference in the S group and its response to ischemic periods in the experimental groups and second, the unexpected decrease in Li_{in-out} difference observed during reperfusion in the ISP group as well as statistically invariable serum levels of nitric oxide and enzyme activities.

In sham-operated animals, the stability of Li_{in-out} difference indicates a steady state of microcirculation throughout the experiment. The decreases seen in stomach during ischemia and/or ISP in respective groups correspond to the changes induced by CAO. In the course of reperfusion, the flow marker was indicating either a predictable tendency of stomach microcirculation to return to the initial pre-ischemic levels (IS group), or after a short-term rise displayed an unexpected deterioration of tissue microcirculation till the end of experiment (ISP group). This is contrary to studies which, using other techniques, recognized preservation of postischemic microcirculation as one of the main mechanisms of protection elicited by ISP (1, 10). In addition, applying this method, there was no detectable change in tissue perfusion of descending colon suggesting practically no effect of remote (stomach) ischemia and/or ISP in this part of alimentary tract. Such finding is interesting since beneficial relations due to ISP were found to exist in many distant organs with virtually no difference between local and remote effects as to potency (4, 1).

In general terms, the mechanism of local/remote effects of ISP is likely to be dependent on signalling pathways including systemic elevation of nitric oxide production, whereas pure sustained ischemia is associated with a decline in nitric oxide level (9). We did not observe the proclaimed hemodynamic effects of ISP, but concurrently neither did we detect a significant rise in serum nitric oxide metabolites in the ISP group. This might to some extent be explained by the adenosine/xanthine theory, which would suggest an inadequate reperfusion phase during preconditioning in the third group. Be that the case, instead of blood perfusion improvement, ISP would have resulted in longer and more severe (1 h) ischemia with accumulation of disproportional amounts of xanthine leading to production of excessive superoxide anion able to remove the generated nitric oxide (13).

With regard to the time window for ISP, it was documented, that one 5 min episode of arterial occlusion effectuated 30 min in advance of prolonged ischemia was sufficient to reduce lesion area in rat stomach (12). In small bowel, Hotter et al. (6) produced comparable effects (nitric oxide generation, organ protection) with 10 min ischemia only 5 min before prolonged ischemic period. As to the duration of injurious ischemia, marked changes in organs like stomach, pancreas or liver are achieved after 30 to 120 min (1, 3, 9, 11). Even though our protocol met all the reported criteria for the induction of ischemia and ISP in stomach, CAO was found to lack the ability to produce (and thus also modify) significant liver or pancreatic damage demonstrated by others (9, 5, 11, 3).

Conclusions

This pilot study demonstrates a new alternative of gastrointestinal interstitial blood perfusion measurement by LM. Under given experimental conditions, the technique

allowed a detection of selective microcirculation modulation in rat stomach, but failed to detect reported protective potency of local ISP in this organ. Lack of other splanchnic or systemic effects as indicated by absence of change in LM and selected serum biochemical parameters requires further studies.

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REACTIVATION POTENCY OF NEW GROUP OF ACETYLCHOLINESTERASE REACTIVATORS AND THEIR COMPARISON WITH CURRENTLY AVAILABLE OXIMES

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Summary: In this work, in vitro potency of novel serie of monoquaternary pyridinium oximes to reactivate cyclosarin-inhibited acetylcholinesterase (AChE) was tested. Currently available oximes (pralidoxime, obidoxime, trimedoxime, HI-6 and BI-6) were used as oximes for comparison. As resulted, none of tested new reactivators was able to reactivate AChE inhibited by cyclosarin. Also pralidoxime, obidoxime and trimedoxime did not reach good reactivation results. Only oximes HI-6 and BI-6 achieved sufficient reactivation potency. From obtained results, it can be deduced, that only reactivators with oxime group in position two are able to reactivate cyclosarin-inhibited AChE.

Key words: Oxime; Reactivator; Acetylcholinesterase; Cyclosarin; Nerve agent; In vitro

Introduction

Currently used antidotes against nerve agent intoxications consist of anticholinergics (atropine mainly) and acetylcholinesterase (AChE; EC 3.1.1.7) reactivators (pralidoxime, obidoxime and HI-6) (4). Unfortunately, abilities of these antidotes are limited. For example, soman inhibited AChE is after several minutes not treatable due to the aging (dealkylation) (5,6). Also AChE inhibited by tabun and cyclosarin is difficult to reactivate due to the lone electron pair at the amide group (tabun) or presence of bulky cyclohexyl group (cyclosarin), respectively (1,7). Currently the most promising AChE reactivator – oxime HI-6 – is not able to reactivate tabun or pesticide-inhibited AChE (1,14).

Due to this fact, many laboratories throughout the world are interested in the synthesis of new AChE reactivators with better reactivation potency (2,8,9–15). There were synthesized new reactivators differing in the number of quaternary pyridinium rings, in the length of the connection chain between pyridinium rings, presence of other heteroarene rings, using of different functional groups or in the presence and position of oxime groups. There are also several works aimed to elucidate the relationship between structure of AChE reactivators and their biological activities (3,10).

Unfortunately, till today, there was not synthesized one broad-spectrum AChE reactivator able to satisfactorily reactivate AChE inhibited by all kinds of nerve agents (broad

spectrum reactivator) (4,10). In 2005, we have prepared new group of potential AChE reactivators with oxime groups in position four at the pyridinium rings. According to our former results, pKa of some of these reactivators were promising and comparable with currently available oximes (13).

In this work, we wanted to test if also reactivation potency of newly developed oxime is promising as their pKa. For this purpose, in vitro reactivation potency of nine new potential AChE reactivators was tested. Their reactivation potency was compared with currently available oximes (pralidoxime, obidoxime, trimedoxime, HI-6 and BI-6). Cyclosarin was used as the appropriate member of the nerve agent family.

Material and Methods

All tested AChE reactivators were synthesized earlier at our department (13). Their purities were tested by ¹H-NMR (Varian Gemini 300; 300 MHz) and TLC using DC-Alufolien Cellulose F (Merck, Germany) and elution BuOH-CH₃COOH-H₂O 5 : 1 : 2, detection by solution of Dragendorff's agent (solution containing 10 ml CH₃COOH, 50 ml H₂O and 5 ml of basic solution prepared by mixing of two fractions – fraction I.: 0.85 g Bi(NO₃)₃, 40 ml H₂O, 10 ml CH₃COOH; fraction II.: 8 g KI, 20 ml H₂O). Nerve agent cyclosarin (O-cyclohexylmethylfluorophosphonate) was obtained from the Military Technical Institute (Brno,

Czech Republic) and was of 95% purity. All other chemicals used in this experiment were purchased from Fluka and Aldrich (Czech Republic) and used without further purification.

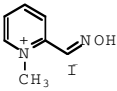
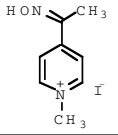
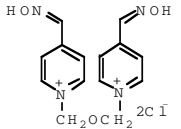
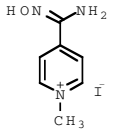
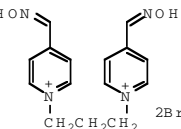
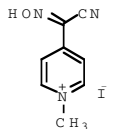
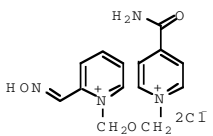
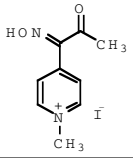
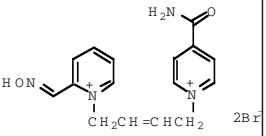
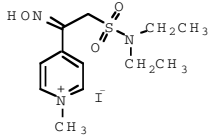
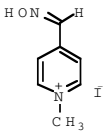
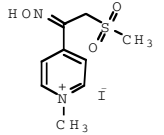
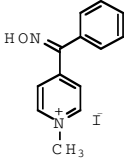
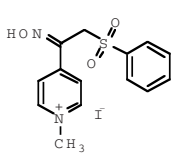
In vitro testing of reactivators involved a standard collection of experimental procedures. The whole method is in detail described in the work of Kuca and Cabal (10). Their efficacy was evaluated in 10% rat brain homogenate that was incubated with cyclosarin for 30 minutes and then, the tested oxime of appropriate concentration (10^{-4} and 10^{-2} M) was added for 10 minutes. Afterwards, the activity of brain AChE was determined using potentiostatic method with the use of automatic titrator RTS 822 (Radiometer, Den-

mark). The data about initial rate of enzyme reaction with substrate were transformed to the percentage of increase in the activity of reactivated enzyme in the reaction mixture.

Results and Discussion

All results obtained are shown in Table 1. As it can be clearly seen, all newly developed reactivators were not able to reactivate cyclosarin-inhibited AChE. Also three oximes used for comparison were poor reactivators. Only two oximes (HI-6 and BI-6) were potent reactivators of cyclosarin-inhibited AChE. Moreover, these oximes seem to be very promising because of their sufficient reactivation potency

Tab. 1: Structures and reactivation activity of tested AChE reactivators.

No	Name	Structures	Reactivation potency [%] [*]	Reactivation potency [%] [#]	No	Name	Structures	Reactivation potency [%] [*]	Reactivation potency [%] [#]
1	Pralidoxime		4	0	8	K119		0	0
2	Obidoxime		4	2	9	K121		0	0
3	Trimedoxime		0	0	10	K122		1	0
4	HI-6		52	28	11	K123		0	0
5	BI-6		57	60	12	K124		0	0
6	4-PAM		0	0	13	K125		0	0
7	K120		0	0	14	K126		0	0

* concentration of tested reactivators 0.001 M; # concentration of tested reactivators 0.00001 M

at lower concentration (10^{-5} M). If both oximes are compared, BI-6 seems to be the best reactivator tested in this work.

As described in introduction, searching for new broad-spectrum reactivators still continues. Synthesis of many AChE reactivators of different chemical structures demonstrates this fact (2,8,9–15). Unfortunately, till today, there exists no single reactivator able to satisfactory reactivate AChE inhibited by all kinds of nerve agents (4,9).

According to our results, unfortunately, none of the newly developed reactivators was able to reactivate cyclosarin-inhibited AChE. From the oximes tested as compounds for comparison, only two oximes (HI-6 and BI-6) were potent reactivators of cyclosarin-inhibited AChE. Trimedoxime, obidoxime, pralidoxime and 4-PAM were poor reactivators of cyclosarin inhibited AChE. Obtained results confirm that bisquaternary oximes (BI-6 and HI-6) are more potent reactivators compared to monoquaternary ones (pralidoxime, 4-PAM, K119–K126) (9). Moreover, it is clear from the results that reactivators with oxime group at the position two are the most potent reactivators of cyclosarin-inhibited AChE. These results are in good agreement with our previous results (9,11).

Main reason for the insufficient reactivation potency of novel reactivators seems to be the presence of the oxime group in position four, which is considered to be favourable in the case of tabun and pesticide inhibitions (1). Due to this fact, in former studies, the whole serie of new oximes should to be tested in this manner.

Thanks to our present knowledge of structural requirements of AChE reactivators, at this place, we could predict structure of probably the most promising AChE reactivators, which should be synthesized in future. This reactivator should be bisquaternary compound having in its structure two oxime groups both at separate pyridinium rings. Position of the oxime group should be two at the first pyridinium ring and four at the second pyridinium ring. Ideal length of the connection chain should be three or four membered.

Conclusion

Unfortunately, none from the newly developed monoquaternary pyridinium oximes was able to reactivate cyclosarin-inhibited AChE. As shown from the results, bisquaternary AChE reactivators seem to be more potent oximes compared to monoquaternary ones. However, also obidoxi-

me and trimedoxime did not reach good reactivation results. Only oximes HI-6 and BI-6 achieved sufficient reactivation potency. From the results obtained, it can be concluded, that only bisquaternary reactivators with oxime group in position two are able to reactivate cyclosarin-inhibited AChE.

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FREQUENCY OF NEONATAL HYPOGLYCEMIA IN LARGE FOR GESTATIONAL AGE INFANTS OF NON-DIABETIC MOTHERS IN A COMMUNITY MATERNITY HOSPITAL

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Summary: Large for gestational age (LGA) infants are at increased risk for hypoglycemia. The aim of the study was to determine the frequency of neonatal hypoglycemia in LGA infants of non-diabetic mothers in a Community Maternity Hospital in Gaziantep, Turkey. Hospital records of 5229 infants of non-diabetic mothers were examined retrospectively. Newborns with birth weight more than 4000 g were defined as LGA. The control group consisted of 100 appropriate for gestational age (AGA) newborns. Capillary blood glucose was measured at the second hour of life. Glucose values lower than 40 mg/dL (2.2 mmol/L) were defined as hypoglycemia. Ninety-six (1.8%) of the 5229 infants were found to be LGA. The mean capillary glucose levels of the LGA newborns were significantly lower than those of the AGA newborns (54 mg/dL (3.0 mmol/L) vs. 95 mg/dL (5.2 mmol/L), $p < 0.0001$). Neonatal hypoglycemia was established in 16 of 96 LGA infants (16.7%). In the control group hypoglycemia was absent. The rate of hypoglycemia in LGA infants was significantly higher than the rate in the AGA infants ($p = 0.0000$). As hypoglycemia is not rare in LGA infants and can have serious consequences, blood glucose levels should be screened routinely in LGA infants.

Key words: Neonatal hypoglycemia; Large-for-gestational age

Introduction

Hypoglycemia is one of the major metabolic abnormalities of the newborn and is usually defined as a plasma glucose concentration less than 40 mg/dl (2.2 mmol/L) (7). It is well known that large for gestational age infants (LGA) are at risk for transient hypoglycemia, though the mechanism is not clear. It has been suggested that hyperinsulinism may be responsible for the hypoglycemia (1). Fetal hyperinsulinism may cause excessive intrauterine growth. If undiagnosed and untreated, hypoglycemia may cause serious complications, such as brain damage (4).

The aim of the study was to determine the frequency of neonatal hypoglycemia in LGA infants of non-diabetic mothers in a Community Maternity Hospital in South East Turkey. The most higher prevalence of diabetes mellitus (9.4%) was in Turkey is Gaziantep city, where the study was performed. In addition to, Gaziantep is the second city for the higher prevalence of obesity (27.2%) (9). Maternity Hospital of Gaziantep serves to the population of town and rural. Patients with low social-economic status of both populations are referred to this hospital.

Material and Methods

The study population consisted of infants of non-diabetic mothers who were born at the Community Maternity Hospital of Gaziantep, between the years 2003–2004. Data was extracted from hospital records of 5337 infants. Mothers of 61 infant had known diabetes mellitus and gestational diabetes mellitus was diagnosed in 47 mothers. 108 infants of these diabetic mothers were not included in the study and the frequency of LGA was evaluated in 5229 infants of non-diabetic mothers.

Newborns with birth weight more than 4000 g were defined as LGA and their blood glucose level were measured and recorded in their charts. An oral glucose tolerance test (OGTT) was not performed routinely in mothers of LGA infants to exclude GDM. Mothers' diabetes status was based mainly on verbal maternal history and in a few patient (5%) on OGTT as recorded in the charts.

The control group consisted of 100 newborns whose weight was between 3000–3500 g and who were defined as appropriate for gestational age (AGA). Glucose levels of all infants (LGA and AGA) were measured via capillary blood

by glucometer (Glucometer Elite, Bayer Diagnostics, Germany). All readings were obtained at the second hour of life by the neonatal care unit nurse. Glucose values lower than 40 mg/dL (2.2 mmol/L) were defined as hypoglycemia.

Results

A total of 5229 infants of non-diabetic mothers were born during the study period. Among them, ninety-six (1.8%) were found to be LGA. Neonatal hypoglycemia was established in 16 of 96 neonates with LGA (16.7%). In the control group (n=100) with normal weight, hypoglycemia was absent. The percentage of infants with hypoglycemia in the LGA group was significantly higher than the percentage in the AGA infant group ($p=0.0000$).

The mean capillary glucose levels of the LGA newborns were significantly lower than those of the control group with normal weight for the gestational age (54 mg/dL (3.0 mmol/L) vs. 95 mg/dL (5.2 mmol/L), $p<0.0001$). The clinical characteristics of the infants with LGA and AGA are shown in Table 1.

Tab. 1: Clinical characteristics of the infants with LGA and AGA.

	LGA	AGA	p
Mean blood glucose (mg/dL)	54±19	95±21	<0.0001
Mean blood glucose (mmol/L)	3.0±1.1	5.2±1.2	<0.0001
Mean birth weight (g)	4347±265	3351±278	<0.0001
Mean age of the mother (years)	29±7	28±6	NS

LGA: Large for gestational age; AGA: Appropriate for gestational age; NS: Non significant

Discussion

Neonatal hypoglycemia is a common problem in LGA infants (8). It is well known that neonatal hypoglycemia occurs more frequently in LGA infants than AGA infants (12). The incidence of neonatal hypoglycemia in LGA infants is reported as having a wide range (8.1–33%) (5,6, 10–12). This wide range may be explained by the differing definitions of hypoglycemia in the various studies, and by the non-uniform time of capillary sampling in the different studies.

The frequency of neonatal hypoglycemia in LGA infants was found to be 16.7% in our study. Additionally, the mean blood glucose levels were significantly lower in LGA infants than in the AGA infants. These findings are consistent with the findings of one of the largest reports, but hypoglycemia was defined in that study as a capillary glu-

cose level lower than 30 mg/dl (10). Schafer-Graf et al. reported a 16% neonatal hypoglycemia rate in LGA infants, and found the results of the 1-hour maternal oral glucose tolerance test as the only predictor of neonatal hypoglycemia in the newborn (10).

In our study, the frequency of neonatal hypoglycemia was examined in LGA infants of non-diabetic mothers. Maternal glucose status was determined mainly based on documentation obtained from hospital charts, where mothers were asked about their diabetes history. Performing OGTT only in a small minority (5%) of the mothers is a limitation of the study. Nevertheless, our results are consistent with previous reports. This low ratio was related with low level of social-economic status of the pregnant women, because they were only admitted to the hospital for the time of the delivery.

The mean blood glucose levels were low in LGA infants, and they may be predisposed to hypoglycemia. Since hypoglycemia is an important metabolic disorder, with potentially serious consequences (including neurological complications), blood glucose levels should be screened routinely in all LGA infants.

Despite these, and previous findings, no consensus exists among medical professionals regarding the benefits of testing LGA infants of non-diabetic mothers for hypoglycemia on a routine basis (2,3,10).

In conclusion, hypoglycemia in LGA infants is not rare. Further studies are needed to evaluate the maternal and neonatal risk factors for hypoglycemia in the newborn, as well as the frequency of hypoglycemia in LGA infants.

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ASYMPTOMATIC ANEURYSMAL PORTOSYSTEMIC VENOUS SHUNT: A CASE REPORT AND REVIEW OF THE LITERATURE

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Summary: Spontaneous intrahepatic portosystemic venous shunt (SIPSVS) is relatively rare and not well recognized. Herein, we report 75-year-old female of an aneurysmal portosystemic venous shunt detected by colour Doppler ultrasound in check-up examination. A direct vascular communication between left portal vein and middle hepatic vein was confirmed by CT-angiography. The cause of intrahepatic portosystemic venous shunt is disputed. This abnormality, mainly described in cirrhotic liver and rarely in healthy liver, is usually revealed by hepatic encephalopathy or glycoregulation disorders. However, with improvements in imaging the number of reports of SIPSVS identified incidentally in patients without definite symptoms increasing.

Key words: Intrahepatic portosystemic shunt; CT-angiography; Aneurysm

Introduction

Macroscopic intrahepatic portosystemic venous shunts is defined as communications between the portal and the systemic venous circulation, measuring more than 1 mm in diameter, and at least partially located inside the liver. Portosystemic shunts are expected to be associated with portal hypertension, trauma, surgical intervention or liver biopsy, but incidentally seen otherwise normal individuals. These spontaneous intrahepatic portosystemic shunts (SIPSVS), except for dilated paraumbilical veins caused by portal hypertension, have generally been considered to be rare even though modern imaging modalities have successfully demonstrated these abnormalities (5,10,12,14,18,19,21, 23,27,28). We describe a case with a SIPSVS incidentally diagnosed in check-up investigations.

Case Report

A 74-year-old female admitted to clinic for health screening. She had no history of trauma, liver biopsy and surgical operation. There had been no episode of hepatic encephalopathy or hypoglycemic symptom. Laboratory data showed normal blood counts, liver and renal function tests, glucose and lipid levels. Ultrasonography, an examination for screening, delineated a snail-like anechoic area in the liver, its connection with the portal and hepatic veins, and marked dilatation of the veins connecting with the shunt in an area with 7 cm diameter in the left lobe of the

liver. Parenchymal echogenicity of the rest of the liver was normal and there was no focal parenchymal lesion. The main portal vein was patent and the course of the right and left portal veins was normal. Colour Doppler imaging showed turbulent flow in the portosystemic venous shunt. CT-angiography demonstrated an aneurysmatic SIPSVS of 23 mm diameter, between the left hepatic vein and the left portal vein in left lobe. Adjacent to that lesion, a couple of small (3–5 mm) SIPSVS were also seen (Figure 1). She was informed and reassured about that vascular abnormality. But, no intervention was performed because she did not have any symptom or finding.

One year later, the sonographic examination was repeated and no difference was found from the previous one. Then, she was undertaken follow-up.

Discussion

Although recent advances in USG and CT have detected asymptomatic SIPSVS in an increasing number of patients, SIPSVSs are rarely seen disorder. Park et al. categorized them into four different morphologic types (27). The most common type is a single large tube of constant diameter that connects the right portal vein to inferior vena cava. Type I includes patent paraumbilical veins, located in the liver. Shunts of this type are considered to be collateral pathways which develop in the hepatic parenchyma as a result of portal hypertension. The second type is localised peripheral shunt in which single or multiple communications



Fig. 1: CT-angiography appearances of portosystemic shunt in the patient.

are found between peripheral branches of portal vein and hepatic veins in one hepatic segment. The third type is aneurysmal with peripheral portal and hepatic veins being connected through an aneurysm. The fourth type has multiple communications between peripheral portal and hepatic veins diffusely in both lobes. According to this classification, our patient had an aneurysmatic type 3 connection between left hepatic vein and left portal vein. Chevallier et al. did another classifications as follows: Type I includes patent paraumbilical veins, located in the liver, and commonly encountered in portal hypertension. Types II, III include shunts, unique or multiple, between the portal branch and the hepatic vein, located either in two adjacent liver segments (type II) or in non-adjacent liver segments (type III). Type IV corresponds to any tubular communication developed between the right portal branch and the inferior vena cava. The exceptional patent ductus venosus or a patent umbilical vein should not be considered as IPSVS since their course is strictly extrahepatic (4).

The diagnosis of intrahepatic portosystemic venous shunts can be established by color Doppler, CT-angiography, MRI or conventional angiography (8,10,25,27,28). Color Doppler imaging demonstrate a direct communication of color flow signals between the portal vein and hepatic vein, in addition to the characterization of the Doppler spectrum at each sampling point from a continuous waveform signal (portal vein) to a turbulent signal (aneurysmal cavity), and finally, to a biphasic waveform signal (hepatic vein). Color Doppler imaging is useful in the diagnosis of an intrahepatic portosystemic hepatic venous shunt, and the measurement of shunt ratio may be useful in the follow-up and determining the therapeutic option (19). Magnetic resonance imaging also can clearly demonstrate the portal-hepatic venous shunt due to "flow void". Multiple diffuse shunts or a solitary shunt can be visualized. The solitary shunt can be either tubular, focally dilated or racemose in configuration (2,14,25,28). Color Doppler imaging and CT angiography are relatively non-invasive in diagnosis of SIPSVS.

The vascular malformation we present is believed to be a congenital anomaly, as no signs of cirrhosis or trauma were found. But, there are still many arguments about it. Even though certain embryological events remain obscure, certain developmental aberrations may cause this vascular connection. Some authors postulates a persistent venous anastomosis such as ductus venosus and right vitelline vein. Others advocate an acquired cause from rupture of a portal venous aneurysm into the hepatic vein or from a dilated hepatic vein communicating with the inferior vena cava via inferior phrenic and suprarenal vein (5,10,14,19,21,28,29). Persistent ductus venosus could be a remote possibility in our case. Rupture of the portal vein aneurysm is more probable mechanism. Another case was reported of a newborn with a shunt with spontaneous resolve in first year of age (8,13). SIPSVS may be demonstrated in any age of life between 20-day-old and late seventies (3,5,12,14,18,19,20,22,24,26,31).

Wide range distribution in case reports may make us speculate role of different mechanism in development of SIPSVS.

Awareness of intrahepatic communications is important because they can cause encephalopathy, and most of these shunts have been reported to be completely cured by transcatheter embolization. Potential complications of coil embolization may include evolution of portal hypertension caused by abrupt changes in portal hemodynamics and dislodgement of coils into the systemic circulation. Retrograde transcaval obliteration is the least invasive technique and is recommended as the first choice for treatment of portosystemic venous shunt except in patients with multiple shunts. Surgical intervention such as shunt ligation and hepatic resection, creation of an alternative portosystemic shunt are additional therapeutic approach described in literature (6, 17, 21, 26, 31).

Intrahepatic shunts may cause neurocognitive abnormality. A 23-year-old female patient with complaint of fatigue was reported to have right portal vein aneurysmal communication in otherwise healthy woman. Neuropsychological testing, imaging, and MR spectroscopy revealed changes similar to that described in patients with liver cirrhosis and subclinical hepatic encephalopathy. T1-weighted MRI showed a hyperintense globus pallidus, a feature seen in subjects with and without portal-encephalopathy. Portal systemic shunting in the absence of parenchymal liver disease reproduces neurological features described in cirrhosis (6). Because, she did not accept, we have not performed cerebral investigations in our patient.

This vascular abnormality accounts for the blood glucose problems: the portocaval shunt explains the early hyperglycemia by defective liver uptake of glucose and secondary hyperinsulinism occurs because of the reduced hepatic degradation of the insulin secreted in normal quantity. The late hyperinsulinism then leads to secondary hypoglycemia. These cases may benefit dietetic treatment (7, 9, 12, 18).

The size of SIPSVS has been reported to probably increase in time. However, in a year we have not seen a size change in the lesion of our patient. But, this issue remains to be defined with prospective studies.

In the literature, there are several reports of distinctive association with SIPSVS such as congenital biliary atresia, polycystic ovary syndrome, huge pelvic myoma, coronary artery fistulas, leptospirosis, hemangiomas and membranoproliferative glomerulonephritis. There are cases of SIPSVS associated with focal nodular hyperplasia or hereditary hemorrhagic telangiectasia (HHT) (1, 15, 16, 30, 32). HHT is an autosomal dominant disorder characterized by telangiectasias and arteriovenous malformations of potentially every organ (3). Hepatic involvement occurs in 8–31% of cases. Hepatic involvement is shown by examination of biopsy, sonography, Doppler sonography, CT, and MR imaging. This pictorial essay illustrates the broad spectrum of abnormalities of hepatic vessels and collaterals in HHT that are detectable by imaging techniques even in the early or clinically silent stages of the diseases. The association between

the hepatic vascular lesions and HHT is varied, ranging from telangiectasias to large shunts between three vascular channels. In an advanced stage of involvement, large portovenous shunts may be present. In another case, development of pulmonary hypertension was accepted to be due to SIPSVS. Because, it was assumed that vasoconstrictive agents which should be metabolized by the liver in normal subjects, passing through the intrahepatic shunt into the lung (11). Our patient had no abnormality that have been demonstrated. She had no complaint. Our patient underwent no treatment for the portosystemic shunt because she did not present with hepatic encephalopathy. That's why she was undertaken follow-up. One year later, there was nothing changed. Then we planned to follow her with intervals of two years.

In conclusion, clinicians should not be confused in incidental diagnosis of SIPSVS. However, when intrahepatic portosystemic shunt does present with hepatic encephalopathy, the correct diagnosis is required prior to appropriate treatment.

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NITRIC OXIDE AND SYSTEMIC SCLEROSIS

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Vascular endothelial dysfunction is a central event in the pathogenesis of a variety of human diseases, including systemic sclerosis. Systemic sclerosis (SSc) is a complex connective tissue disease that involves vascular dysfunction, immunological activation and fibrosis. In its elusive etiopathogenesis, nitric oxide (NO) is supposed to be involved. NO accounts for the activities originally ascribed to “endothelium-derived relaxing factor.” It is produced by all mammalian cells and provides pleiotropic properties. NO is produced by three nitric oxide synthase (NOS) isoforms: two are constitutive (endothelial eNOS and neuronal nNOS) and one is inducible (iNOS). Constitutive forms are Ca²⁺- and calmodulin-dependent enzymes and release, femtomolar or picomolar concentrations of NO in reaction to receptor stimulation. Inducible NOS is regulated at a pretranslational level and can be induced by several proinflammatory cytokines, such as tumor necrosis factor- α (TNF- α), interferon- γ (IFN- γ), and interleukin-1 (IL-1). iNOS releases large quantities (nanomolar concentrations) of NO several hours after exposure. Constitutive forms are active under physiological conditions, whilst the latter participates in tissue pathology (e.g. during inflammatory autoimmune disease as a contributor to tissue destruction). Hence, the molecule may act both as a regulatory mediator and as a potent cytotoxic agent (Kolb, 1998). This „nitric oxide paradox“ has been found to exist also in SSc, where NO may play a positive (vasodilating) as well as negative (cytotoxic, causing tissue dysfunction and injury) role (Ceric, 2002). However, the opposing effects of NO in SSc seem not to be simply explainable. This is supported by the fact, that literature is inconsistent even regarding the nature of change (increase or decrease) in NO level in SSc patients compared to healthy subjects. Furthermore, the controversial issue gains in complexity as the levels of NO are shown to vary depending on the location, analyzed material, disease activity and progression:

1) Biopsies of microvessels

In biopsies from patients with SSc, superficial microvessels showed a peak of eNOS expression (detected by im-

munochemical staining) in indurated skin which fell as the changes of the skin increased. By contrast, iNOS staining increased with the grade of skin damage (Cotton, 1999). Analogous to these results Romero described reduced expression of eNOS mRNA by dermal microvascular endothelial cells from patients with SSc (Romero, 2000). Described switch from eNOS to iNOS as the disease progresses may demonstrate both roles of NO – first positive and then cytotoxic.

2) Serum

Yamamoto observed significantly higher levels of serum NO metabolite nitrite in patients with SSc (Yamamoto, 1998), a finding later confirmed by other researchers (Sud, 2000; Takagi, 2003). Sud showed an association between worsening of lung functions (assessed by spirometry) and a trend towards higher serum NO levels (resp. nitrite and citrulline), even though no difference in NO levels among disease subgroups could be found (corrected for the extent of disease, disease duration, age and systemic involvement) (Sud, 2000). Nevertheless, some clinical features of SSc (the extent of skin fibrosis, short disease duration and fibrosing alveolitis) seem to give positive correlations with serum levels of NO metabolites (Takagi, 2003).

3) Peripheral blood mononuclear cells

Although not reaching statistical significance, the amounts of spontaneously released NO from blood mononuclear cells of patients with SSc exceed those of control subjects' (Yamamoto, 1998). In contrast, a more recent study showed that the concentrations of NO metabolites are decreased and the induction of iNOS is delayed in cultured peripheral blood mononuclear cells in patients with SSc (Allanore, 2001).

4) Exhaled air

The pulmonary production of NO may be elevated (Fajac, 1998; Kharitonov, 1997; Rolla, 2000) as lung macrophages express higher amounts of iNOS (Failli, 2002) in SSc patients compared to healthy controls. On the other hand, some found NO levels to be decreased in patients suffering from SSc with pulmonary hypertension (Kharitonov,

Tab. 1.

Author, year	Nitric oxide	No. of SSc patients	Method	Analysed material
Rolla, 2000	↑ (↓ in PAH)	47 (16 with PAH)	chemiluminiscence	Exhaled air
Kharitonov, 1997	↑ (↓ in PAH)	23 (6 with PAH)	chemiluminiscence	Exhaled air
Fajac, 1998	↑	14	chemiluminiscence	Exhaled air
Failli, 2002	↑	32	colorimetry	Lung macrophages
Yamamoto, 1998a	↑	14	colorimetry	PBMC
Allanore, 2001	↓	18	spectrophotometry	PBMC
Yamamoto, 1998b	↑	?	colorimetry	Serum
Takagi, 2003	↑	45	colorimetry	Serum
Sud, 2000	↑	34	colorimetry	Serum

PAH = pulmonary arterial hypertension; PBMC = peripheral blood mononuclear cells

1997; Rolla, 2000). Moreover, an inverse correlation between pulmonary hypertension and exhaled NO seems to exist (Rolla, 2000). The impairment of NO production in pulmonary arterial hypertension due to SSc may result from endothelial dysfunction characteristic for pulmonary arterial hypertension.

Above cited papers refer in good accords to elevated production of NO in serum and exhaled air. These changes reflect the situation in the whole body, where NO may act cytotoxic on vessels and cause their inflammation and damage. However, there is only little evidence about the situation directly in the skin and subcutis, which are affected by immunopathological process in the course of the disease.

Studying the relationship between NO and SSc brings a few problems. The results may vary depending on the used analytical methods, analyzed material (Table 1), patients' diet etc. In addition, the amount of NO produced may also differ with progression and activity of the disease, a fact that only a few authors took into account. For example, Cotton demonstrated a shift from eNOS to iNOS with progression of SSc (Cotton, 1999) and Yamamoto pointed out a correlation of total circulating NO with markers of endothelial damage and disease activity (Yamamoto, 1998). Moreover, the numbers of SSc patients included in the studies are rather low (Table 1).

Thus, further and larger studies are necessary to clarify the role NO plays in the development of SSc. The question of treating SSc patients with NO supplementation/inhibition at a particular stage and activity of the disease remains to be fully answered. The measurement of total NO in serum is unlikely to be representative for local contribution of this mediator to the pathogenesis of SSc. Therefore, a serial determination of NO production in situ respecting the pro-

gression of the disease is suggested as an optimal alternative.

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