MEDICAL UNIVERSITY

„Prof. Dr Paraskev Stoyanov“ – Varna

MEDICAL FACUTLY

DEPARTMENT „ INFECTIOUS DISEASES, PARASITOLOGY AND DERMATOVENEREOLOGY “

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INFANTILE HAEMANGIOMAS – EPIDEMIOLOGICAL CHARACTERISTICS AND MANAGEMENT APPROACH

SUMMARY

OF A THESIS FOR THE EDUCATIONAL AND SCIENTIFIC DEGREE „DOCTOR OF PHILOSOPHY“

Medical Speciality: Dermatology and Venereology

Professional domain: 7.1. Medicine

Higher education: 7. Health care and sports

Scientific supervisors:

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Varna, 2021 г.

This dissertation was approved at a Departmental meeting of the Department for Infectious diseases, Parasitology and Dermatovenereology of Medical University – Varna (Protocol № 4/23.05.2021 г.) and was administered for a public viva/defence with a Rector's Order № P-109-224/19.05.2021 г.

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The thesis contains *131 pages, illustrated* with *9 tables, 27 figures* and *3 appendices.*

ABBREVIATIONS

CI	*confidentiality interval*
ERK	*extracellular signal-related kinases*
FGF	*fibroblast growth factor*
FGFR4	*receptor 4 for fibroblast growth factor*
GLUT-1	*glucose-transporter protein 1*
HAS	*Haemangioma Activity Score*
HASI	*Haemangioma Activity and Severity Index*
HDCS	*Haemangioma Dynamic Complication Scale*
HemEPCs	*Haemangioma endothelial progenitor cells*
HemSCs	*stem cells derived from haemangioma*
HFB	*Haemangioma Family Burden*
HSS	*Haemangioma Severity Scale*
ICC	*intraclass correlation coefficient*
ICSI	*intracytoplasmic sperm injection*
IFN	*interferon*
IGF2	*insulin-like growth factor-2*
ISSVA	*international society for the research of vascular abnormalities*
MAPK	*mitogen-activated protein kinase*
MMP2, MMP9	*matrix metalloproteinases 2 and 9*
Pref-1	*Preadipose factor-1*
SD	*standard deviation*
TLR 7	*toll-like receptor 7*
VAS	*visual analogue scale*
VEGF	*vascular endothelial growth factor*
VEGFR2 *u* ***VEGFR3***	*receptors for vascular endothelial growth factor*
ДЧХ	*Liver infantile haemangiomas*

I. INTRODUCTION

Haemangiomas are the most common benign tumors in children under 1 year of age, affecting approximately 4-5 to 10% of those born at or around term and up to 30% of premature infants. They are characterized by three well-defined phases of development with different clinical characteristics: rapid proliferative phase, steady-state phase and slow spontaneous regression. The different morphological characteristics of the lesion at different stages require a specific approach to treatment according to the phase. Haemangiomas usually appear in the first days or weeks after birth with spontaneous regression by age 9 in about 90% of patients. Despite their benign nature and the expected regression, 40-50% of patients with haemangiomas develop telangiectasias, discoloration, fibrous fatty tissue, skin atrophy or scars. In some of the patients, the active haemangiomas are associated with functional and / or life-threatening complications.

The clinical presentation of haemangiomas depends on their location and depth. Haemangiomas in the upper dermis are bright red, raised plaques or nodules, while haemangiomas in the deep reticular dermis and subcutaneous fat tissue are indurated lesions with less well-defined borders, a bluish tinge, and no surface change. Superficial haemangiomas are localized or segmental. Segmental lesions affect one or more anatomical areas and carry a higher risk of life-threatening complications and association with structural abnormalities. In some cases, infantile haemangiomas may be part of clinical syndromes, which complicates diagnostic clarification and requires a complex therapeutic approach. Such syndromes are haemangiomatosis and PHACES and PELVIS / SACRAL / LUMBAR syndromes.

The etiopathogenesis of haemangiomas is poorly understood. It is believed that a number of factors may influence intrauterine development and trigger haemangioma development. There are various hypotheses about the pathogenesis of infantile haemangiomas - placental origin; genetic defect or somatic mutation of endothelial cells; influence of external factors that create a favorable environment for cellular proliferation. During the proliferative phase, there is increased secretion of some angiogenic markers such as major fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), insulin-like growth factor-2 (IGF2), as well as increased numbers of receptors for these factors such as VEGFR2, VEGFR . Effective treatment and spontaneous involution are associated with

decreased VEGF and bFGF, both in the haemangioma itself and in the patient's blood. The factors that lead to this disrupted regulation are not fully understood. Various studies point to hypoxia during pregnancy and the perinatal period as a major factor

Treatment of childhood haemangiomas has progressed significantly with the introduction of β-blockers as first-line therapy. Propranolol and its analogues are the preferred method of treatment for haemangiomas associated with a functional disorder or with potential life-threatening complications. For smaller haemangiomas, topical treatment may be recommended.

The revolutionary progress of the understanding of the pathological cascade of infantile haemangiomas and the introduction of an innovative highly effective and safe therapy, as well as the lack of data on haemangiomas in the Bulgarian population, motivated a series of studies on the etiology and therapy of haemangiomas in infants and children. The results of our studies, subject of this dissertation, help to clarify the role of risk factors for the development of haemangiomas, to establish the effect of treatment with topical β-blockers and to determine the impact of infantile haemangiomas on the life of the family.

II. PURPOSE AND AIMS

PURPOSE

The aim of this thesis is to establish the role of peri- and prenatal factors for the development of infantile haemangiomas; to study a new topical method for their treatment and to propose a working algorithm for diagnostic and therapeutic approach.

AIMS:

1. To investigate the epidemiological factors that may lead to the development of haemangiomas in infancy, including:

- Prenatal: assisted reproduction, medications during pregnancy, episodes of hypoxia during pregnancy, placental position, placental abnormalities, interventions during pregnancy, chronic diseases of the mother, age of mother and father, smoking and others.

- Perinatal: hypoxia at birth, method of delivery, medications during childbirth, need for interventions at delivery, birth weight and gestational age.

2. To assess the frequency of etiological factors, comparing the results with the available literature data and establishing the specificities for the Bulgarian population.

3. To prepare a form for standardized registration of children with haemangiomas in infancy and childhood, which will facilitate the creation of a database for the Republic of Bulgaria.

4. To create a scale for assessing the severity of haemangiomas, which could be used to determine the choice of treatment and to monitor the effectiveness of the chosen method of treatment and the need to change treatment.

5. To prepare a diagnostic algorithm for different types of haemangiomas according to their size, number and anatomical location.

6. To study the therapeutic effect and safety profile of a new drug for topical treatment of haemangiomas in infants and children - timolol maleate.

7. To optimize the timolol maleate regimen for adequate dosing and duration of treatment.

8. To identify the side effects of topically applied timolol maleate and to compare these with the expected side effects corresponding to the pharmacokinetics and pharmacodynamics of the drug.

9. To investigate the efficacy and safety of a newly formulated preparation with timolol maleate at a concentration of 1% in the form of a gel-emulsion in children with haemangiomas indicated for topical treatment.

10. To prepare criteria for indications for the use of topical treatment for haemangiomas in infants and children.

11. To study and evaluate the perception of parents about the impact of their child’s haemangioma on the quality of life of the family.

III. MATERIALS AND METHODS

3.1. MATERIAL

- The study group consists of patients diagnosed at the Clinic for Dermatology and Venereology at the University Hospital "Alexandrovska" EAD, Sofia; Euroderma Clinic, Sofia; university clinics and private dermatological practices in Sofia and the country for the period 2010 - 2020.

- The different studies included a total of 304 children of both sexes, selected for an 11-year period (2010-2020), aged between 2 and 48 months. The gender distribution was f:m = 2: 1. There were 206 girls (67.8%) and 98 boys (32.2%).

- The main criterion for inclusion was the presence of haemangiomas in infancy or childhood, regardless of previous treatment. Children without prior treatment were included in the group to study the effectiveness of timolol 1% cream and timolol maleate 0.5% solution and gel-forming solution.

- The main criterion for exclusion from the study for active treatment was haemangioma in the late stages of spontaneous regression

3.1.1. Epidemiological studies

- Study of the etiological factors, prenatal and perinatal, related to the development of haemangiomas in infancy in the Republic of Bulgaria in 256 children.

- Assessment of the established etiological factors in infants and children with haemangiomas, compared to a population group without haemangiomas corresponding to gender and age. The studied and the control group included 256 children.

- Characteristics of the etiological factors in patients with haemangiomas in the Republic of Bulgaria and their comparison with the etiological factors in other countries according to literature data.

3.1.2. Clinico-morphological studies

- Cover 256 patients with different subtypes and anatomical localizations of haemangiomas. The analysis is based on clinical examination and available retrospective photographic material.

- Study of the clinical and morphological characteristics of infantile haemangiomas in 256 infants and children.

3.1.3. Clinico-therapeutic studies

- Development of an instrument for evaluation of therapeutic efficacy.

- Development of an instrument to assess the severity of the haemangioma at the initial examination and to assess the most appropriate intervention.

- Development of a diagnostic and therapeutic algorithm for management of different types of haemangiomas according to their size, number and anatomical location

- Study of the therapeutic efficacy and safety profile of a newly formulated drug for topical treatment of infantile haemangiomas - timolol maleate, in 186 infants and children with a total of 207 haemangiomas.

- Optimization of the timolol maleate application regimen in terms of adequate dosage and duration of treatment.

- Identification of side effects of topical timolol maleate.

- Study of the therapeutic effect and safety profile of a newly formulated preparation with timolol maleate in a concentration of 1% in the form of a cream in children with haemangiomas that are indicated for topical treatment.

- Establishment of criteria and indications for the use of topical treatment for haemangiomas in infants and children.

- Establishment of criteria and indications for the use of systemic treatment for haemangiomas in infants and children.

4.2. CLINICAL METHODS

4.2.1. Method for analysis of data for clinical history and clinical presentation

A form has been developed that includes the needed information, one part to be filled in by the doctor and the other by the parents. (Appendix 1). Parents provide detailed information on the prenatal period, the labour and delivery, the time of onset of the haemangioma and the clinical course prior to the examination, any co-morbidities or symptoms that may be relevant to the planned treatment.

4.2.2. Dermatological status

Location and size were recorded for single haemangiomas, and location, size and number were recorded for multiple haemangiomas. The nature of the lesions and the phase of evolution in relation to their location, depth, shape, consistency and color were taken into account.

4.2.3. Documentary method

- Medical documentation submitted by the patient.

- Photo documentation.

4.2.4. Clinical follow-up

The effectiveness of the treatment, as well as the development of haemangiomas / haemangiomas in patients without treatment, were assessed according to the results reported from clinical observation at the beginning, throughout the course of treatment and at the end of the treatment, or at the end of the follow-up period. Follow-up was at regular intervals during treatment. The clinical follow-up method was applied to 256 children.

4.2.5. Method for analysis and assessment of the impact of the disease on the quality of life - questionnaire 'Haemangioma Family Burden'.

Haemangioma Family Burden (HFB) is a specific dermatological questionnaire developed and validated to assess the impact of childhood haemangiomas on parents, children and family lifestyles (132). 133) It is intended to be filled in by one of the parents and provides information on the overall impact on the quality of life in families with children with haemangiomas.

The questionnaire contains questions that cover six different areas, characterizing the psycho-social functions of everyday life: family life (four questions), relationships and work (three questions), emotions and feelings (three questions), psychology (three questions), treatment (two questions) and general influence (five questions about psychological effect, sexuality, financial influence). The answers to questions 1 to 15 are: definitely yes (3 points), maybe (2 points), definitely no (0 points) and don't know (1 point). The answers to the last five questions, reflecting the influence on the physical and mental state of the parents, are: negative influence (1 point), no (0 points), positive influence (-1 points) and I do not know (0 points).

The global score is from 0 to 50, with a higher score being associated with a greater burden on the daily life of the family,

The method was applied to 186 children.

4.2.6. Method for assessing the severity and efficacy of the treatment

- Scale for assessment of the activity and severity of haemangioma and the efficacy of treatment, developed as part of the study: Haemangioma Activity and Severity Index (HASI). The method was applied to 186 children (Annex *2).*

- Informed consent for initiation of treatment with topical β-blockers. The method was applied to 186 children (Appendix *3).*

4.3. Statistical methods

The collected information was entered and processed with the GraphPad Prism statisticalpackage. Parametric and non-paramethical methods, description of qualitative variables and quantitative variables areused.

A. Descriptive methods and methods of evaluation

The following descriptive statistics methods are used:

1. Variational analysis of quantitative variables – average, median, standard deviation, minimum, maximum.

2. Frequency analysis of quality variables (nominal and rank) that includes absolute frequencies, relative frequencies (in percentage) and their 95% confidence intervals, cumulative relative frequencies (as a percentage)

3. Graphic images: bar charts.

B. Methods of statistical conclusions and verification of hypotheses

In all studies, the formulated zero hypothesis is a lack of influence or connection, while the alternative hypothesis postulates the presence of influence or connection. P<0,0 1 was chosen as a level of significance rejecting the zero hypothesis. The results are described by tables, graphs and numerical dimensions (percentages, coefficients, averages, standard deviation, etc.). The assessment of the statistical reliability in the groups studied shall be carried out by means of the value of 'P' for the chi-squared meaning or the exact Fisher criterion, taking the differences at a level of importance P<0.01 as significant.

1. Non-parametric methods

1.1 *Cross-tabulation analysis* – mutual frequency distribution of two quality variables.

1.2. *Mann-Witney method* – comparing two groups in terms ofthe characteristics of a quantitative variable that does nothavea real distribution.

2. *Student T-test: method* for assessing differences in means between two independent study groups. The test can be applied to samples of different sizes, as long as the variables are normally distributed in each group. The level of significance(P) reached when applying it suggests the probability of an error in accepting the hypothesis of a difference.

B. Other methods

1. Calculation of chance ratios and 95% confidence intervals, such as estimates of relative risks.

4.4. Thrapeutic methods

- Therapy with topical timolol maleate (preparation registered for ocular use) ,in the form of drops with a concentration of 0.5% or a gel-forming solution with a concentration of 0.5% or 0.1%. The parents are instructed to apply the drops on the haemangioma and spread them with a finger, but not to use a cotton swab.

The method is administered to 186 children with haemangiomas.

- Therapy with topical timolol maleate 1% cream, a formulation created specifically for the treatment of infantile haemangiomas. The preparation is applied in a thin layer, two to four times a day, with an initial gradual increase in the number of applications. The method is applied in 8 children.

4.5. Ethics

The implementation of the studies is in accordance with national and international requirements for conducting clinical studies, including the preservation of the anonymity of the participants and the non-disclosure of their personal information. The preparation of the protocols of the studies is in accordance with the principles laid down in the Helsinki Declaration, the requirements for good clinical and laboratory practice, as well as in accordance with the legislation in force in the Republic of Bulgaria.

1. RESULTS OF OUR STUDIES

5.1. Epidemiological study for evaluation of prenatal and postnatal factors associated with an increased risk of developing infantile haemangiomas in the Bulgarian population.

The aim of this epidemiological study was to investigate the etiological factors, prenatal and perinatal, associated with the development of infantile haemangiomas in the Republic of Bulgaria in 256 children with haemangiomas and a control group of 256 children without haemangiomas.

Aims of the study:

1. To investigate epidemiological factors that may lead to the development of infantile haemangiomas, including:

- *Prenatal factors:* method of reproduction (assisted reproduction or natural conception) intake of drugs during pregnancy, episodes of hypoxia during pregnancy, placental position, placental abnormalities, interventions during pregnancy, chronic diseases of the mother, age of mother and father, smoking and others.
- *Perinatal factors*: hypoxia at birth, method of delivery, medication intake during childbirth, need for interventions during delivery, body weight and gestational age at birth.

2. To assess the frequency of predisposing etiological factors in infants and children with haemangiomas compared to a population group without haemangiomas by gender and age.

3. To compare the data for the Bulgarian population about the frequency of established etiological factors with literature data about frequency worldwide and to establish the specificities for the Bulgarian population

Data is collected through a targeted questionnaire formulated for the primary registration of children with haemangiomas, reviewed at the Clinic of Dermatology and Venereology of University Hospital " Alexandrovska"EAD, Clinic Euroderma and other dermatological clinics in the Republic of Bulgaria (*Addendum*. *1).* The questionnaire is filled in by one of the parents and includes the child's name, date of birth, history of the pregnancy,

childbirth and perinatal period and the development of the haemangioma, as well as prior treatment and investigations.

Participation in the study was offered to parents of 298 children with infantile haemangioma. 256 children were enrolled in the study after prior informed consent from the parents. The collected information is consolidated in an Excel Table with encoding of participants for anonymization for statistical analysis.

The control group consists of children without haemangiomas, reviewed for a different skin condition. After prior informed consent, parents fill out a specially formulated questionnaire containing the same questions about the course of pregnancy and childbirth as the questionnaire for children with haemangiomas. The form is anonymous and does not contain personal information about the child and/or parents as it was created for the purposes of the study and not for clinical registration and follow-up for the primary skin condition.

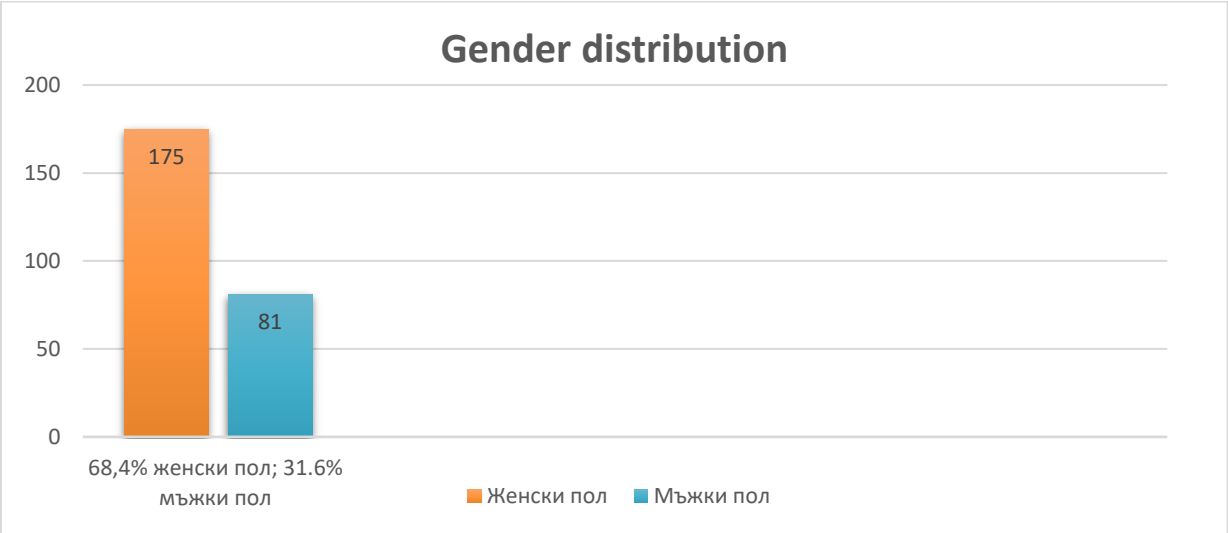

Figure 6. Distribution of patients by gender

Of the 256 children with haemangiomas, 81 are male (31,6%) and 175 are female (68,4%) (P<0,001, z-test) (*Fyghurrah 6*). Контролната група е набрана в съответствие със същото полово разпределение. Всички деца са на възраст между 2 и 18 месеца.

Comparative analysis of prenatal factors associated with the development of infantile haemangiomas

Twenty-one children (8,2%) of the study group were conceived by the in-vitro method (including intracytoplasmic sperm injection/ICSI), compared to 14 (5,5%) from the control group (P=0.22). Multiple pregnancies (including three-embryonic) were reported for 29 (11,3%) haemangiomas and 19 (7,4%) haemangiomas (P=0.13). Problems during pregnancy, including eclampsia and pre-eclapsia, premature contractions, bleeding*, placenta previa* and hospital admission, were recorded for 90 (35,2%) children with haemangiomas and for 16 (6.2%) children without haemangiomas (P-0.001). Placenta abnormalities and *placenta previa* were recorded in 21 of the problematic pregnancies (out of a total of 90) (P-0.001). Prenatal procedures (amniocenesis and chorion biopsy) were reported for 9 (3,5%) children with haemangiomas and 4 (1,56%) without haemangiomas (P=0.16). The intake of tocolytics (magnesium and combined preparations), regardless of trimester and duration, is comparable between the two groups (mothers of 142 children with haemangiomas and 153 children without haemangiomas) (P=0.33). Previous thyroid disease with treatment during pregnancy was noted for 15 mothers of children with haemangiomas and 30 mothers of children without haemangiomas (P=0.02). Smoking during pregnancy was reported in 31 (12,1%) mothers of children with haemangiomas and in 18 (7%) mothers of children without haemangiomas (P=0.05) (Table*2, Figure 7).*

Prenatal factor	Children with haemangiomas (N=256)	Control Group (N=256)	P value (z-test)
Multiple pregnancies	29 (11.3%)	19 (7.4%)	0,13
Pregnancy problems	90 (35.2%)	16 (6.2%)	**<0,001**
Placental anomalies	21 (8.2%)	4 (1.6%)	**<0,001**
Amniocentesis and chorion biopsy	9 (3.5%)	4 (1.56%)	0,16
Assisted reproduction	21 (8.2%)	14 (5.5%)	0,22

Tocolytics during pregnancy	142 (55.5%)	153 (59.8%)	0,33
Smoking	31 (12.1%)	18 (7%)	0,05
Previous thyroid disease	15 (5,9%)	30 (11,7%)	0,02

Table 2. Prenatal risk factors for the development of infantile haemangiomas

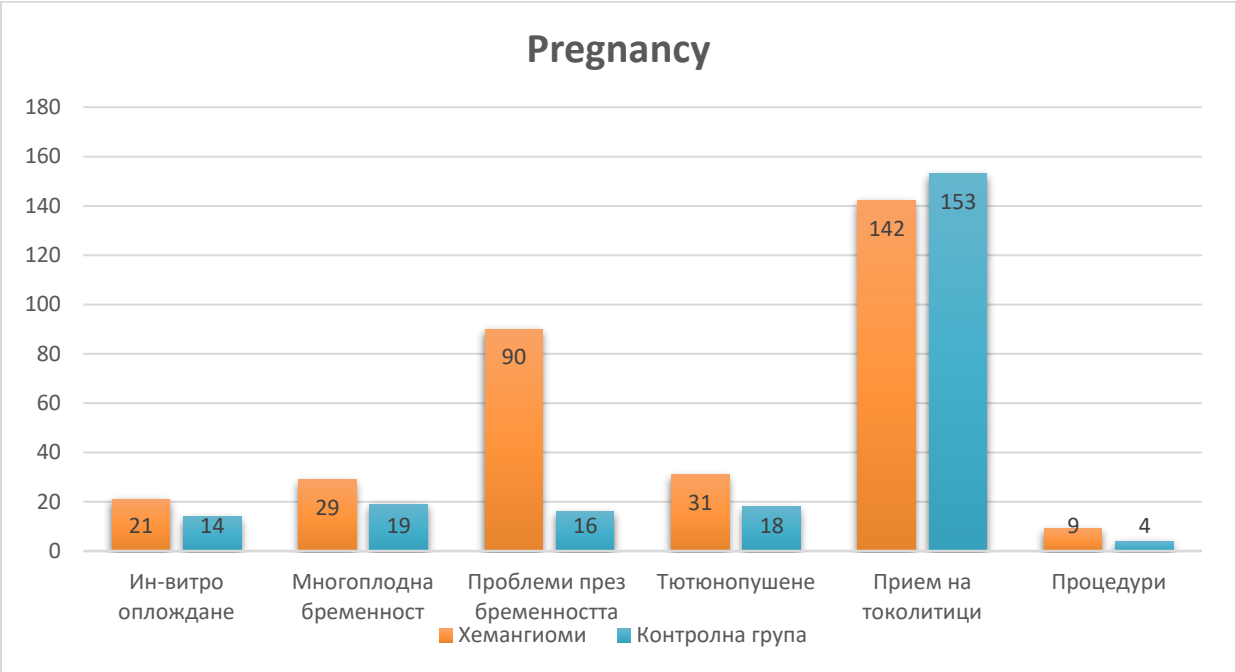

Figure 7. Prenatal risk factors for the development of infantile haemangiomas with distribution in the study and control group

Comparative analysis of perinatal factors associated with the development of infantile haemangiomas *(Figure 8).*

The number of children with haemangiomas born with C-section was 145 (56.6%), compared to 150 (58.6%) for the control group of children without haemangiomas. Nine children with hemagiomas were born with umbilical cord, wrapped around the neck and secondary hypoxia.

Ananalysis of body weight at birth showed a significant difference between the study group of children with haemangiomas and the control group. In the haemangiomas group

there are 67 children with low body weight (under 2499 g) /first and second degree of prematurity according to who criteria (1100 – 2490 g), compared to 21 in the control group (1600 – 2480 g) (*Table 3*).

The children in the haemangioma group were born between 28 and 42 weeks gestation, and the children in the control group were born between 32 and 42 weeks gestation. Before 36 weeks gestation were born 43 (8%) children with haemangiomas and 9 (3.5%) without haemangiomas (P-0.001, z-test) and before 38 weeks gestation were 151 (59%) children with haemangiomas and 49 (19,1%) children without haemangiomas (P–0.001, z-test) (Table *4).*

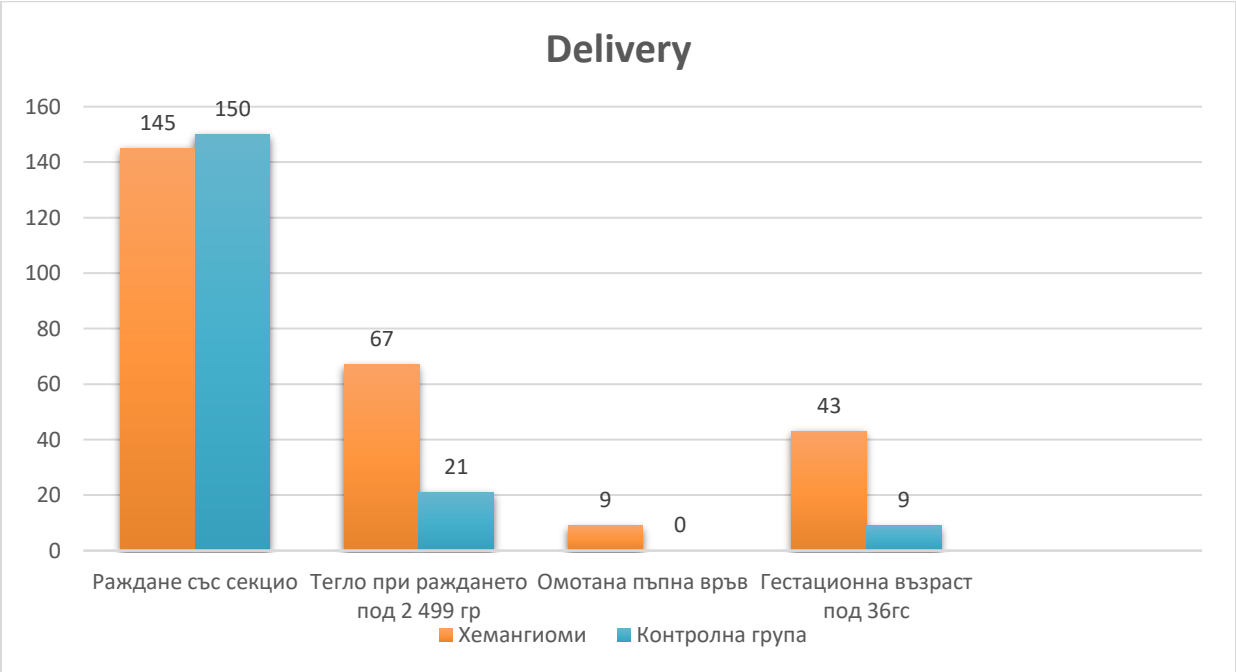

Figure 8. Perinatal risk factors for the development of infantile haemangiomas with distribution in the study and control group

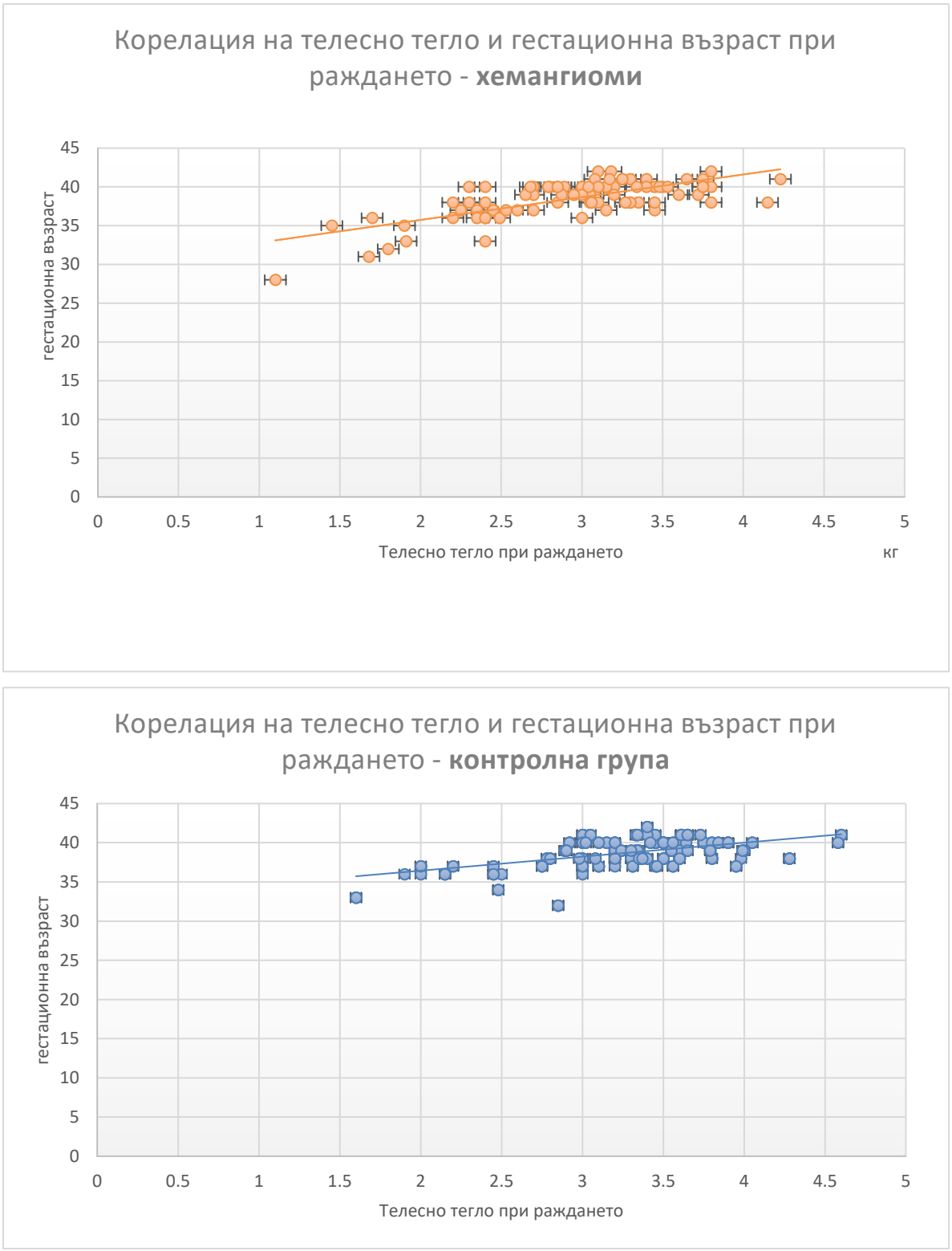

Figure 9. Regression analysis of the relationship between body weight and gestational age at birth

Regression analysis shows a marked correlation between body weight and age at birth, which makes it very difficult to separate the two factors as independent *(Figure 9).*

Body weight at birth			
	Haemangiomas	Control group	**p-value (z-test)**
Over 3500 g	111	109	0,86
3000–3499g	33	99	**<0,001**
2500-2999g	45	27	0,02
Under 2499g	67 (26,2%)	21 (8,2%)	**<0,001**

Table 3. Distribution of children in the study and control group for body weight at birth

Gestational age at birth			
	Haemangiomas	Control group	**p-value (z-test)**
40-38	117	207	**<0,001**
37.6-36	96	40	**<0,001**
35.6-34	28	3	**<0,001**
Before 34 weeks gestation	15	6	0,04
Before 37 (premature-temporary birth according to WHO criteria)	52	19	**<0,001**

Table 4. Distribution of children in the study and control group for gestational age at birth.

5.2. Development of a scale for assessing the activity and severity of haemangiomas, suitable for initial evaluation and follow-up of treatment

The aim of this study was to develop and validate a scale for the assessment of infantile haemangiomas.

The development of an instrument for assessment of infantile haemangiomas is a challenge due to their heterogeneous morphology, clinical evolution and response to treatment. Повечето автори използват субективни визуални аналогови скали (visual analogue scale -VAS) или измерват промените на повърхността, обема или плътността на хемангиома S. R. Janmohamed et al. provided an easy scoring instrument, the Haemangioma Activity Score (HAS), for assessment of the proliferative activity and treatment outcome in infantile haemangiomas. HAS includes characteristics such as lesion depth, change in colour and size of the ulceration. Other assessment instruments were introduced by members of the Haemangioma Study Group, namely the Haemangioma Severity Scale [HSS]) and the Haemangioma Dynamic Complication Scale [HDCS].

The available assessment scales, however, do not include all the objective characteristics of infantile haemangiomas, which can compromise both adequate assessment of the condition- and the assessment of the effect of the treatment. Therefore, we have developed and validated a new index for assessing the activity and severity of infantile haemangiomas - Haemangioma Activity and Severity Index (HASI).

Aims

- To determine the parameters that characterize infantile haemangiomas by severity.
- To determine parameters that can characterise the activity of haemangioma. The parameters should reflect the effectiveness of treatment.
- To validate the assessment scale by determining validity, reliability and clinical applicability.
- To analyse the results and to identify a cut off result where systemic treatment is recommended.

Methods and results

HASI was developed by dermatologists actively involved in the monitoring and treatment of children with vascular tumors and malformations. The index includes two parts: one for activity: HASI (activity) and one for severity: HASI (severity). The activity scale includes all the physical characteristics of haemangiomas, which evolve spontaneously or

change over the course of treatment. This allows accurate assessment of the regression process and registration of minimal changes with the necessary precision. The severity scale includes the characteristics that help with the assessment of the risk for complications and the need for a systematic approach (Table 1). To facilitate dermatologists with limited experience in the application of the index or limited experience with vascular lesions in children, HASI has an additional table for interpretation of the colour and extent of regression (*Figure 10*).

The separation of activity and severity in different scales is necessary, since these parameters do not overlap. Lesions in the active phase may be mild or life-threatening in severity. Similarly, lesions that disrupt function and have severe complications may be in the process of involution. The separation of the two parameters is the basis for a more accurate assessment of the need to follow up and determine the most adequate treatment.

Validation

The validation procedure for each new index for assessing the medical condition is well established and includes an assessment of validity and reliability (138) (139). Validation is extremely important to show whether the index is suitable for use in clinical practice and whether the results are reproducible by different researchers and for different patients.

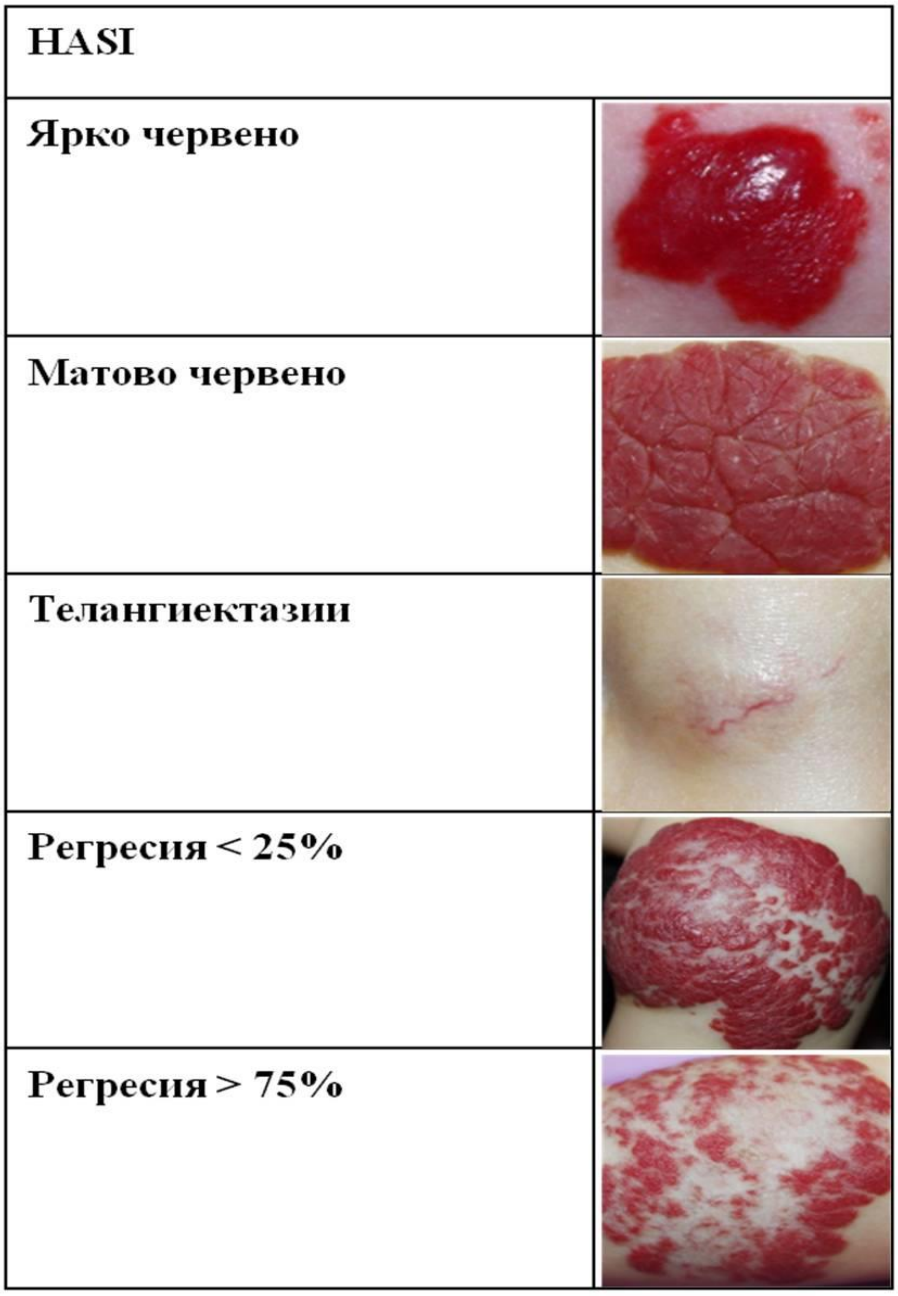

Figure 10. Interpretation of the colour and extent of regression.

Validity

The validity of HASI is confirmed in a series of expert, multicenter consultations with dermatologists and pediatricians. On the basis of expert recommendations, some improvements have been made to the originally proposed index. Susceptibility to change is determined by monitoring patients over time and evaluating at regular intervals. Clinically relevant minimal changes are recorded as a change in HASI.

Reliability

Fifty-nine patients with superficial or mixed haemangiomas (40 superficial and 19 mixed) in the proliferative phase were enrolled in the HASI validation pilot study. Patients were followed for 6 months with an assessment of the condition of the lesions at the initial visit and then every month by two dermatologists independantly.

Intra-rater reliability (reliability for each appraiser individually). An assessment was made on each visit and three days later. The difference between the first and second assessments was negligible with an average of 0.3 to 0.4 for each evaluator.

Inter-rater reliability (reliability for individual evaluators). The correlation between the different evaluators was high. The intraclass correlation coefficient (ICC) was 0.82 (95% confidence interval (CI) = 0.75-0.88) for the activity index and 0.94 (95% CI = 0.91-0.96) for the severity index.

Clinical applicability

The average time to complete HASI is 2.5 minutes after thorough history and physical examination. HASI (activity) does not include ultrasound examination and volume calculation, making it quick and easy to apply in busy clinical practice. In case of suspected systemic involvement, HASI (severity) shall be completed after further consultation with the relevant specialist and imaging studies (ultrasound, traffic police, MRI, etc.).

HAEMANGIOMA ACTIVITY AND SEVERITY INDEX (HASI)

Patient name:..... Date of birth:.....

Date						
ACTIVITY						
Colour	Bright red **(8)**					
	Bright red+matt red **(7)**					
	Matt red **(6)**					
	Single telangiectasias +/- hyperpigmentation **(1)** ***do not score regression**					
	Skin colour **(0)** ***do not score regression**					
Grey-blue areas of regression	1-25% **(-1)**					
	26-50% **(-2)**					
	51-75% **(-3)**					
	≥ 76% **(-4)**					
Flattening	No **(1) **score at initial assessment**					
	Yes **(0)**					
Consistency	Soft **(0)**					
	Tense **(1)**					
Ulceration size	≤ 10 mm^2 **(0.5)**					
	11-30 mm^2 **(1)**					
	≥ 31 mm^2 **(1.5)**					
Ulceration depth	Superficial **(1)**					
	Deep **(2)**					

Deep component	Soft **(1)**					
	Tense **(2)**					
Deep component reduction of volume	< 50% **(2) **score at initial assessment**					
	> 50% **(1)**					
	100% **(0)**					
***TOTAL SCORE ACTIVITY* (0-17.5)**						
SEVERITY						
Number	1 **(0)**					
	2-4 **(1)**					
	≥ 5 **(2)**					
Size	≤ 20 mm^2 **(0)**					
	≥ 21 mm^2 **(1)**					
Type (morphologic subtype)	Localized **(0)**					
	Segmental **(2)**					
Type (depth of involvement)	Superficial **(0)**					
	Mixed/Deep **(1)**					
Localization	Skin **(0)**					
	Mucosa, lips, folds **(1)**					
Involvement of other organs	None **(0)**					
	One **(1)**					
	More than one **(2)**					
Functional impairment (vision, breathing, feeding	No **(0)**					
	Yes **(4)**					
***TOTAL SCORE SEVERITY* (0-13)**						

Severity score	
0	Systemic treatment is not required, monitor as uncomplicated
1-3	Systemic treatment is not required, but active monitoring is advised
≥ 4	Systemic treatment is required

*The initiation of systemic treatment in these cases should be at the discretion of the attending physician; +The presence of a functional disorder alone is a sufficient criterion for initiating systemic treatment.

Table 5. Interpretation of severity results as a guide to the therapeutic approach

5.3. Prospective study of the therapeutic efficacy and safety profile of a new drug for topical treatment of infantile haemangiomas – topically administered timolol maleate.

The aim of this study was to establish the efficacy and the safety profile of timolol maleate - a β-blocker for topical use.

Aims:

- To study the therapeutic effect of topical timolol maleate for the treatment of superficial and mixed haemangiomas in the proliferative phase and in early inolution.

- To optimise the schedule of administration of timolol maleate in terms of adequate dosing and duration of treatment.

- To establish the localized and systemic side effects of topical administered timolol maleate and its safety for use.

- To identify indications for treatment with topical timolol maleate and its place in the scheme of treatment of infantile haemangiomas.

The main criterion for inclusion in the study is the presence of superficial or mixed haemangioma in an active phase, without indications for systemic treatment or after previous systemic treatment. The study excluded haemangiomas in late spontaneous involution and haemangiomas with indications for active systemic treatment (with functional disorders,

deformities and life-threatening complications, Table 1). Three haemangiomas indicated for systemic treatment by clinical evaluation and by HASI, were included due to parents' refusal to initiate systemic therapy.

The study included 186 children with a total of 208 haemangioma after prior informed consent from parents. Of these, 134 (72%) were girls and 52 (28%) were boys aged 2 to 16 months. Of the 208 haemangioma, 151 (72.6%) were on the body and limbs, and 57 (27.4%) were located in the area of the head and neck (mainly affecting the face). By clinical characteristics, 181 (87%) haemangiomas were superficial and 27 (13%) were mixed with a superficial and deep component. One in four (70.2%) haemangiomas were in the proliferative phase, 48 (23.1%) were in the early phase of involution. Fourteen haemangiomas were in the resolution phase after systemic treatment with propranolol (6.7%).

A form as described in section 5.1 has been completed for each patient.

In the studied group, 172 children had no prior treatment and 14 children had prior treatment with propranolol. In the group of children with prior treatment, timolol maleate was administered as a continuation of systemic treatment for additional treatment of residual surface component and to prevent a rebound after discontinuation of systemic treatment.

The distribution of patients is presented in *Table 6. Epidemiological characteristics of the study group.*

The control group included 22 children, 16 girls and 6 boys for whom parents refused topical or systemic treatment. Children in the control group were at similar age as the study group and with infantile haemangiomas in the proliferative phase. A control group corresponding to the number of the study group was not compiled as part of the study due to parents' expressed interest in starting treatment. Children in the control group were followed for 6 months with initial consultation and photo documentation with HASI (activity and severity), and follow ups on weeks 12 and 24 with HASI (activity).

Study participants were followed up every 4 weeks for a period of 6 months (a total of 7 reviews, of which one primary and 6 secondary). During the review to initiate the study, the parents filled in:

1. Registration form for patients with infantile haemangiomas;

2. HASI (activity and severity).

HASI (haemangioma activity) was completed in each subsequent follow up.

Epidemiological characteristics	***Number (percentage) included in the study***	***Number (percentage) of patients who completed the study***
Gender		
Girls (female)	134 (72%)	127 (73%)
Boys (male)	52 (28%)	47 (27%)
Prior treatment with propranolol		
No	172 (93%)	164 (89.4%)
Yes	14 (7%)	10 (10.6%)
Location of haemangioma		
Chapter/Gate	57 (27.4%)	51 (27%)
Body/limbs	151 (72.6%)	139 (73%)
Type on haemangioma		
Superficial	181 (87%)	169 (89%)
Mixed	27 (13%)	21 (11%)
Deep	0 (0%)	0 (0%)
Development phase		
Proliferation phase	146 (70.2%)	135 (71%)
Early spontaneous involution	48 (23.1%)	41 (21.6%)

Involution after propranolol	14 (6.7%)	14 (7.4%)

Table 6. Epidemiological characteristics of the study group

A detailed family and personal history of respiratory and cardiovascular diseases was taken before initiating treatment. With the consent of the parents, clinical pictures of the lesions were taken before starting treatment and in each subsequent follow up examination to assess the efficacy of the treatment. At the beginning of the study the protocol included heart rate and blood pressure measurements and auscultation to rule out a heart defect (in 56 children), but the protocol was changed due to a build-up of literature data and a change in international recommendations for initiation of β-blocker treatment in children with infantile haemangiomas. In accordance with the new recommendations, physical studies of the cardiovascular system were excluded from the protocol in children with no history of cardiovascular defect or normal body weight at birth. Abnormalities were not detected in any of the children who underwent physical examination.

The study protocol included treatment with timolol maleate gel 0.1% five times a day or timolol maleate drops 0.5% twice a day. Parents were instructed to apply one drop of the preparation per square centimeter or up to two drops per application and gently rub it with a finger if necessary, but not with a cotton swab so as not to absorb part of the drug. Possible localized (edema, erythema, irritant and eczematous reaction at the site of application) and systemic (hypoglycemia, decrease in heart rate and blood pressure, respiratory spasm) side effects were discussed with parents with instructions about appropriate monitoring during treatment.

Results

Efficacy

Treatment results from 6 consecutive examinations at 4-week intervals were recorded for 174 children with 190 haemangiomas. Twelve children with a total of 18 haemangioma did not complete the study and there was no data for these children about the activity and severity of haemangiomas at the end of the 6-month study period.

An assessment scale developed and validated by us has been used to assess the activity and severity of haemangiomas: HASI (as described in *Chapter 5.2*).

A significant reduction in HASI was recorded at each 4-week review in the study group with active treatment with timolol maleate. From an average HASI score of 13.43111 prior to initiation of treatment, at the end of the 6-month study period, HASI showed a decrease of an average of 10.653332 to an average of 2.777778 (a decrease of 79.3%). For the control group, with a similar initial HASI, the reduction was to an average of 7.818182 for 24 weeks of follow-up (decrease by 41.3%). The results showed that the most significant response of the haemangioma was noted in the first 4 weeks of treatment, with some parents reporting an improvement within a few days of treatment, with 3.39778 points (25.3%) from 13.43111 to 10.03333, which corresponds to reports in the literature. (*Table 7, Figures 11-13*).

In the group of children with haemangiomas, after a 6-month treatment/follow-up period, 48 (25.3%) haemangiomas had complete involution, compared to 2 (9%) in the control group.

Representative cases for the haemangioma group are illustrated in *Figures 14-22.* Representative cases for the control group are illustrated in Figures *23 to 25.*

Poor therapeutic response in haemangiomas without prior treatment was observed in 4 children (4 haemangioma), of which two haemangiomas were localized on the lips, affecting the mucous membranes or the transition zone. This type of haemangiomas are indicated for systemic treatment due to the risk of anatomical deformation, depth of haemangioma in soft tissues and potential for increased absorption of the topical treatment when applied to mucous membranes.

	Timolol treatment group		**Control group**		
	HASI **Mean (median)**	**Reduction in HASI from initiation of treatment**	**HASI** **Mean (median)**	**Reduction in HASI from the start of follow-up**	**P value** ***(unpaired Student t-test)***

Primary review	13,43111 (14) *SD=2.3*		13,31818 (13,75) *SD=2*		
Week 4	10,03333 (10)	3,39778			
Week 8	8,955556 (9)	4,475554			
Week 12	7,666667 (8) *SD=2.7*	5,764443	11,09091 (12) *SD=2.9*	2,22727	P<0,0001 (CI 4.6-2.18) (standard error=0.613
Week 16	6,311111 (7)	7,119999			
Week 20	4,511111 (4.5)	8,919999			
Week 24	2,777778 (2) *SD=2.3*	10,653332	7,818182 (8) *SD=3.2*	5,499998	P<0,0001 (CI 6:17-4) (standard error=0.542

Table 7*- Assessment of clinical activity of the haemangioma at initiation of treatment and during monitoring of the timolol maleate treatment group and the control group. *(SD : standard deviation; CI: confidentiality interval.)

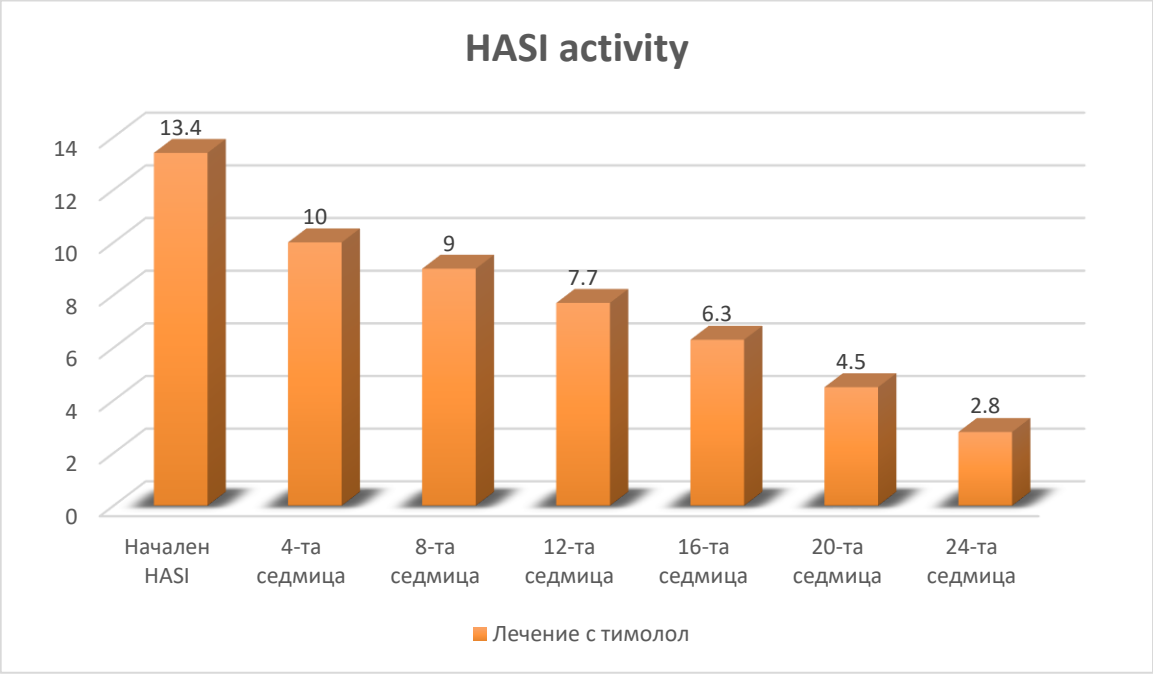

Figure 11. HASI activity score for the timolol maleate treatment group

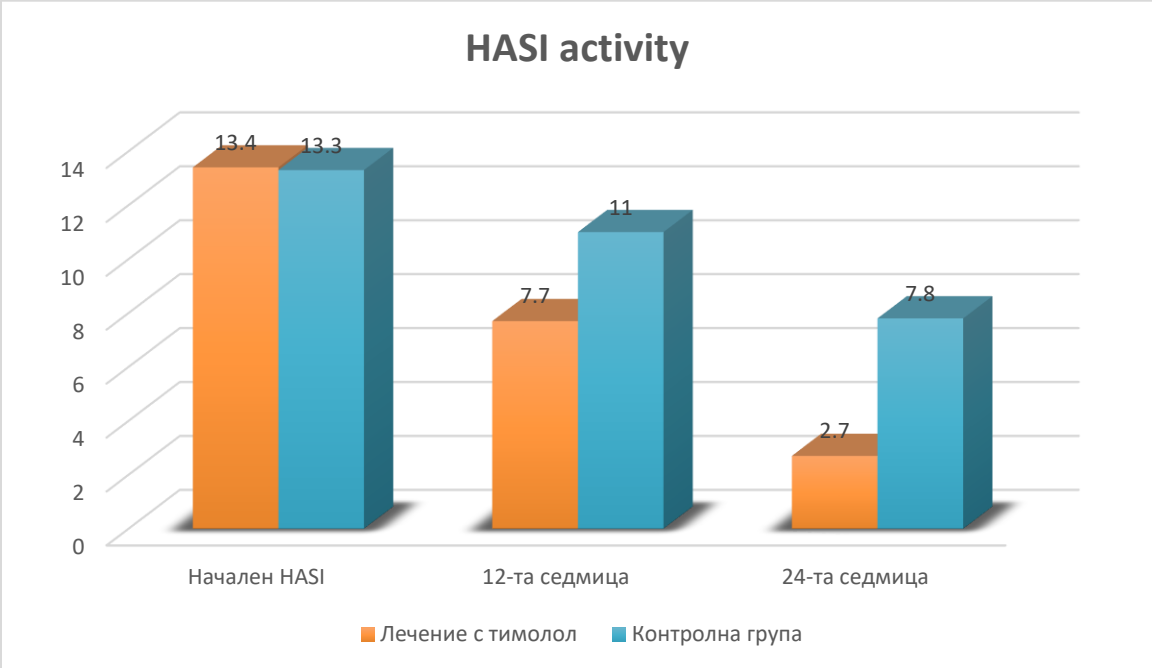

Figure 12. HASI activity score for the timolol maleate treatment group and the control group at initiation of treatment and at 12th and 24th weeks

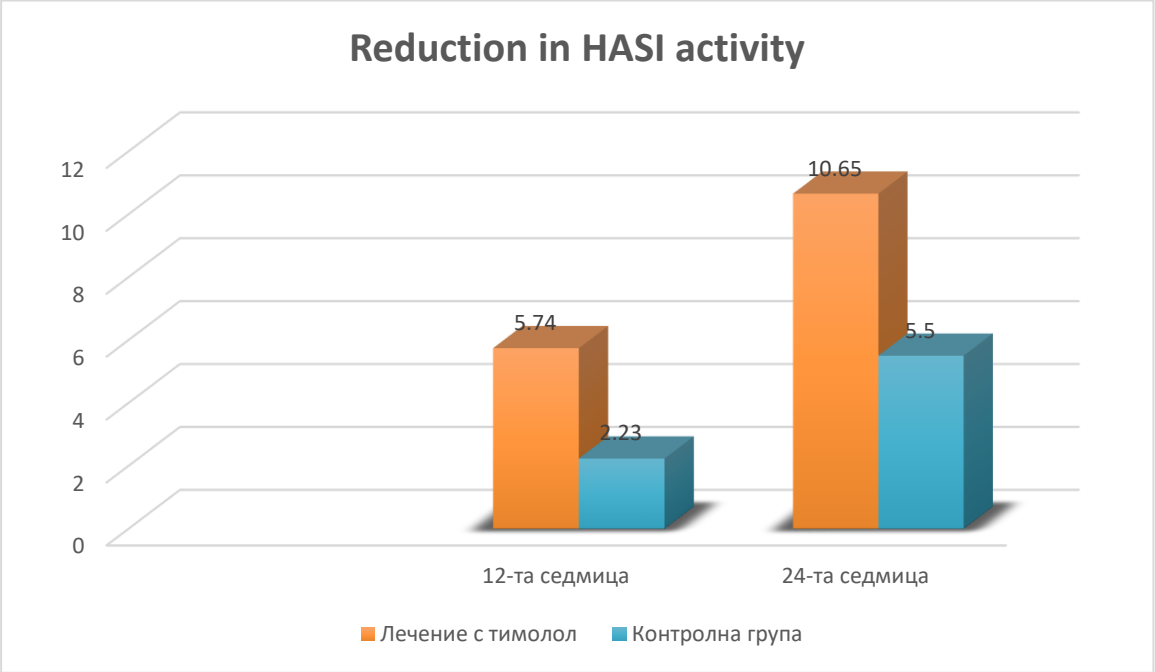

Figure 13. Decrease in HASI activity compared to the initial assessment for the timolol maleate treatment group and for the control group at Week 12 and Week 24

Fading color and switching from bright red to matte red color and changing consistency from tense to soft are the initial changes that are observed as a result of treatment with topical timolol maleate. The color change is gradual with the development of regression zones until complete resolution of lesions with or without residual changes. Change in consistency was seen in all patients after 4 weeks of treatment.

Treatment with timolol maleate showed better efficacy in plaque-like lesions lesions as compared to nodular lesions, as well as in lesions in the proliferation phase compared to lesions in the involution phase

No significant decrease in HASI was recorded in children on previous treatment with systemic propranolol, which can be explained by the stage of involution. However, in most children the lesions are almost completely gone after a 6-month period of subsequent topical treatment. In addition, none of the 10 children who completed the study had a rebound phenomenon of haemangiomas after stopping system propranolol and switching to topical therapy.

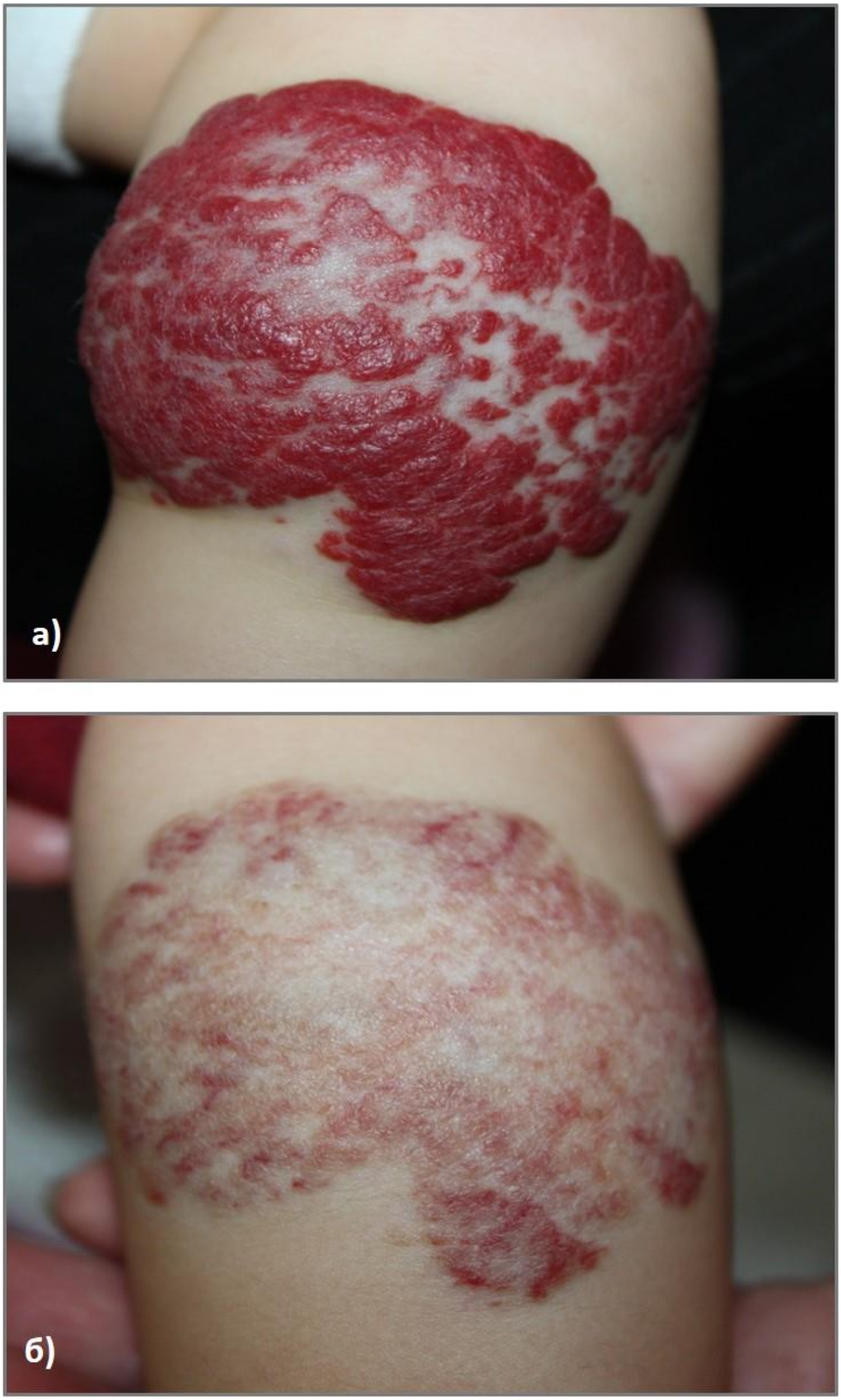

Figure 14. Mixed haemangioma with significant improvement: a) prior to initiation of treatment and b) after 6 months of treatment

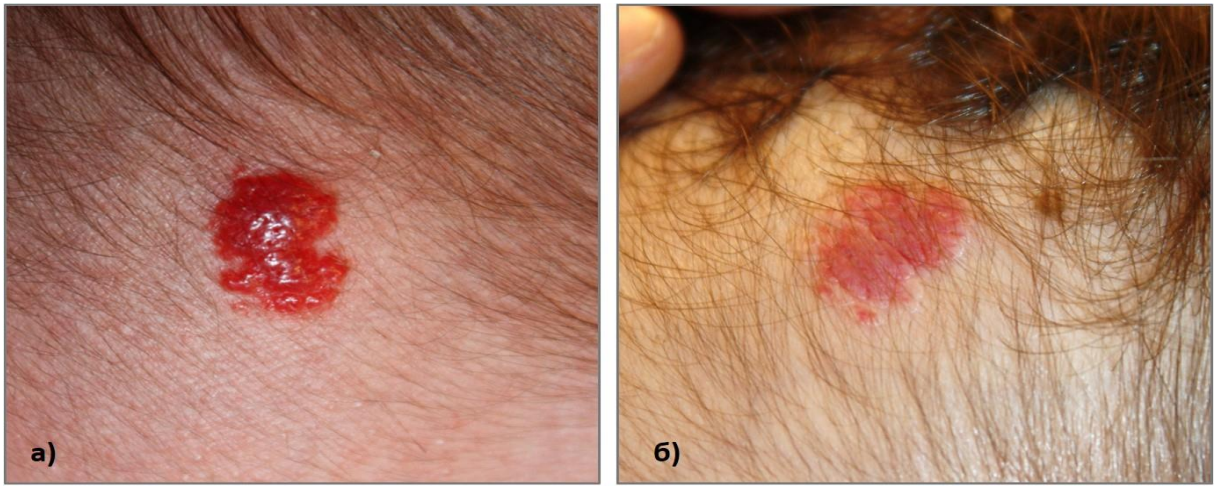

Figure 15. Superficial haemangioma in the proliferation phase (a) prior to initiation and (b) after 12 weeks of treatment (complete clearance after 24 weeks of treatment)

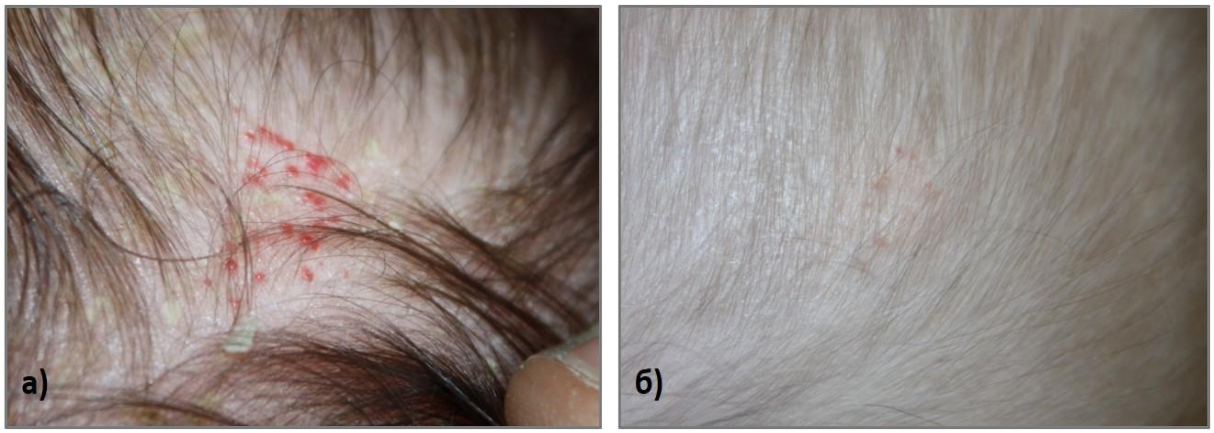

Figure 16. Complete resolution of haemangioma in early stage of development in a boy at 11 weeks: a) before treatment and (b) after 8 weeks of treatment

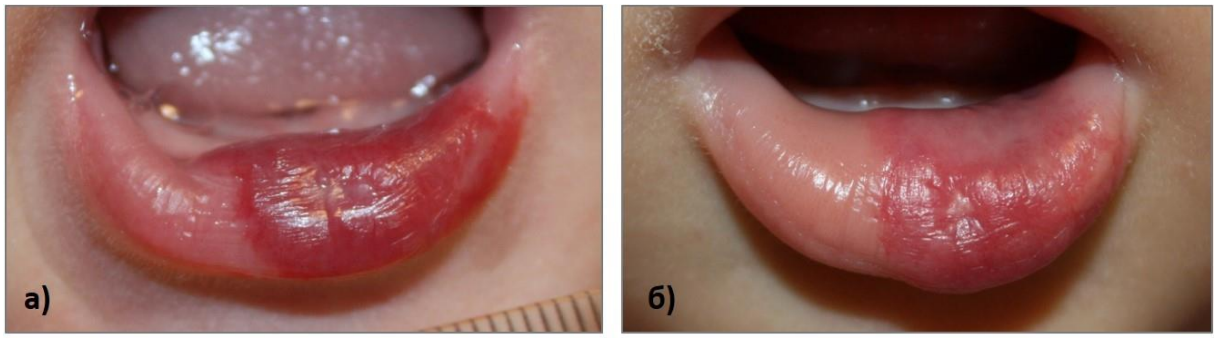

Figure 17. Mixed haemangioma with topical treatment due to parental refusal of systemic treatment: a) before starting treatment and b) after 6 months of treatment

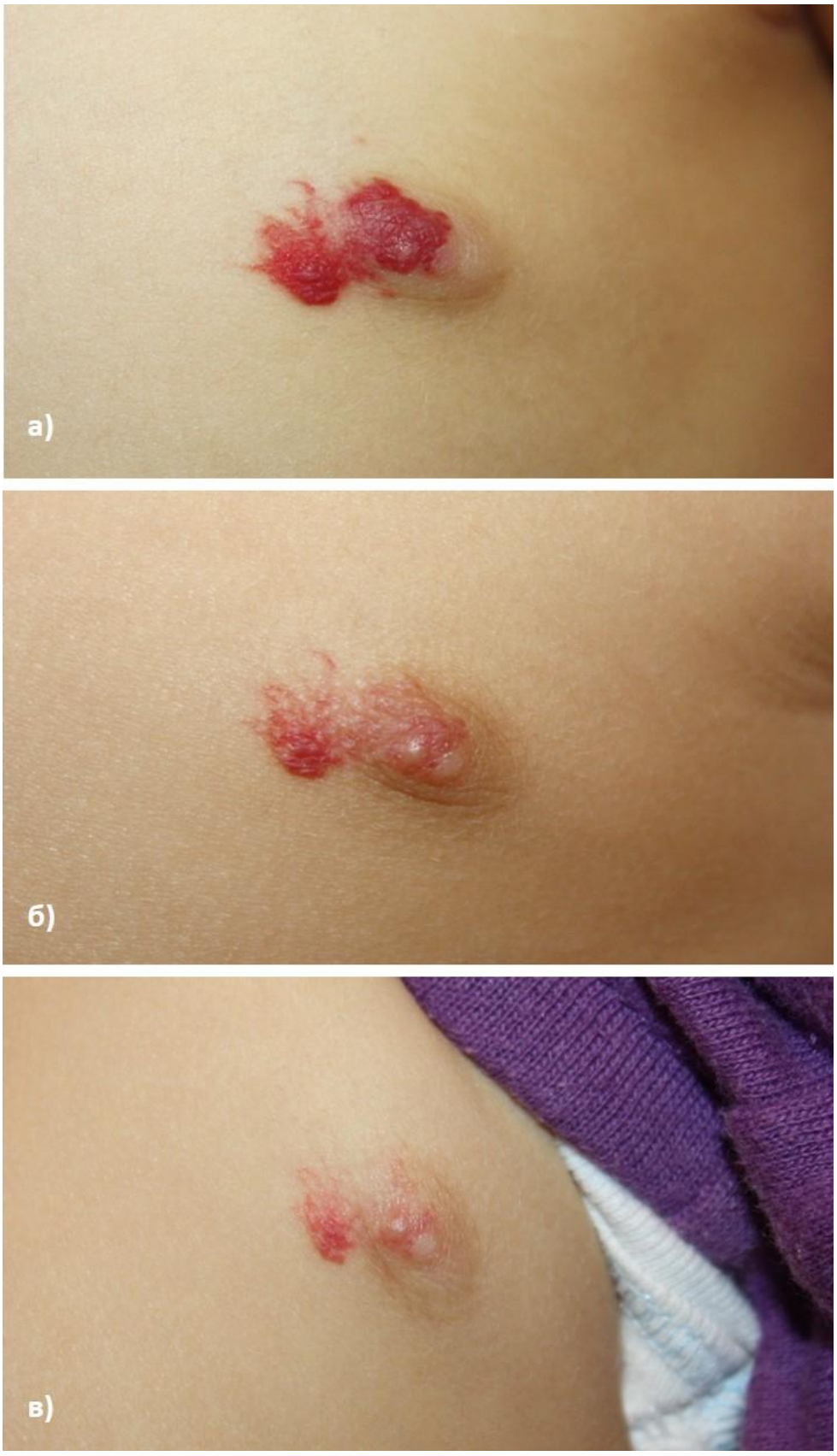

Figure 18. Girl aged 5 months: a) at the start of treatment; (b) after 3 months of treatment; (c) after 6 months of treatment

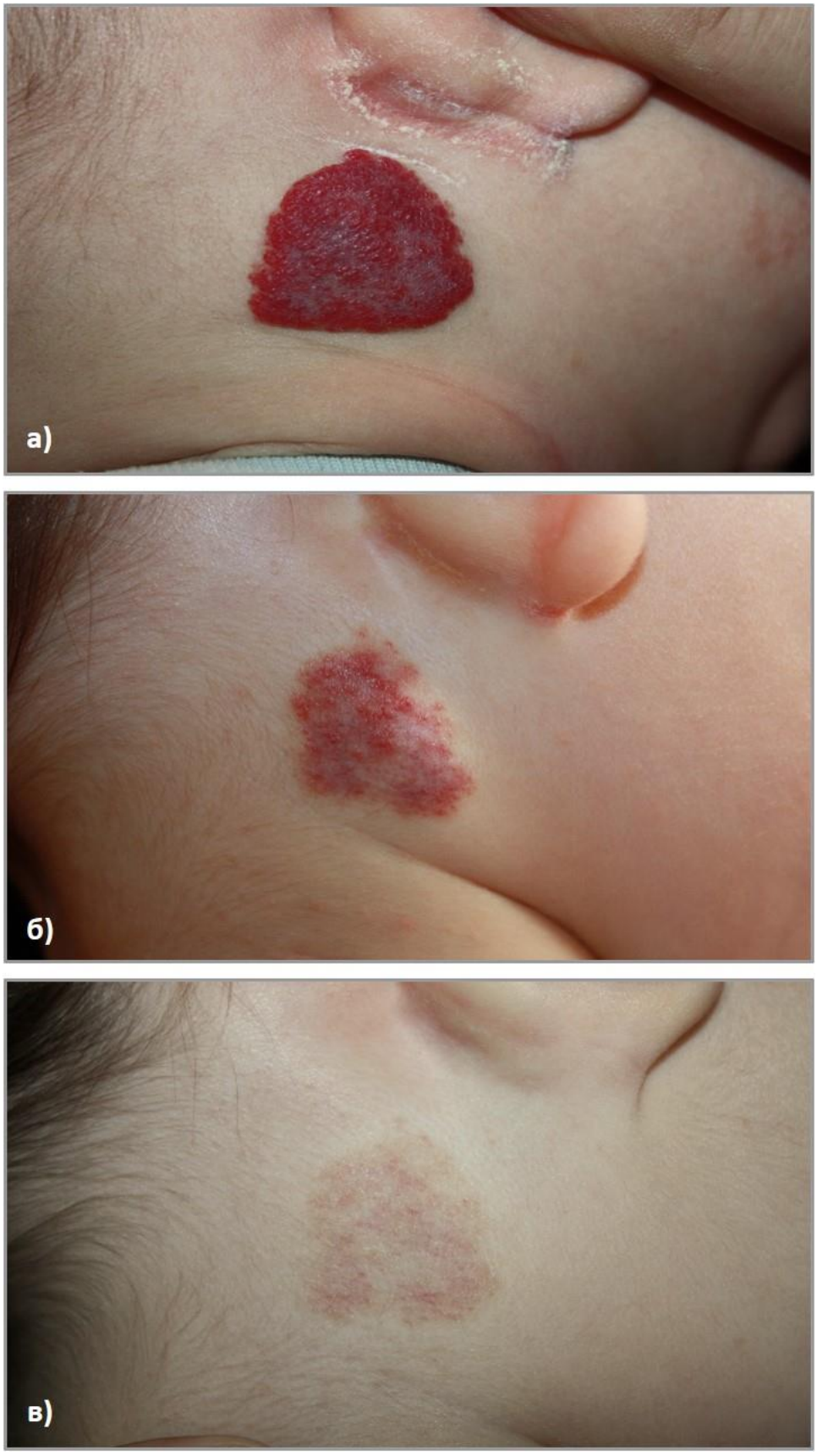

Figure 19. Boy aged 5 months: a) at the start of treatment; (b) after 3 months of treatment; (c) after 6 months of treatment

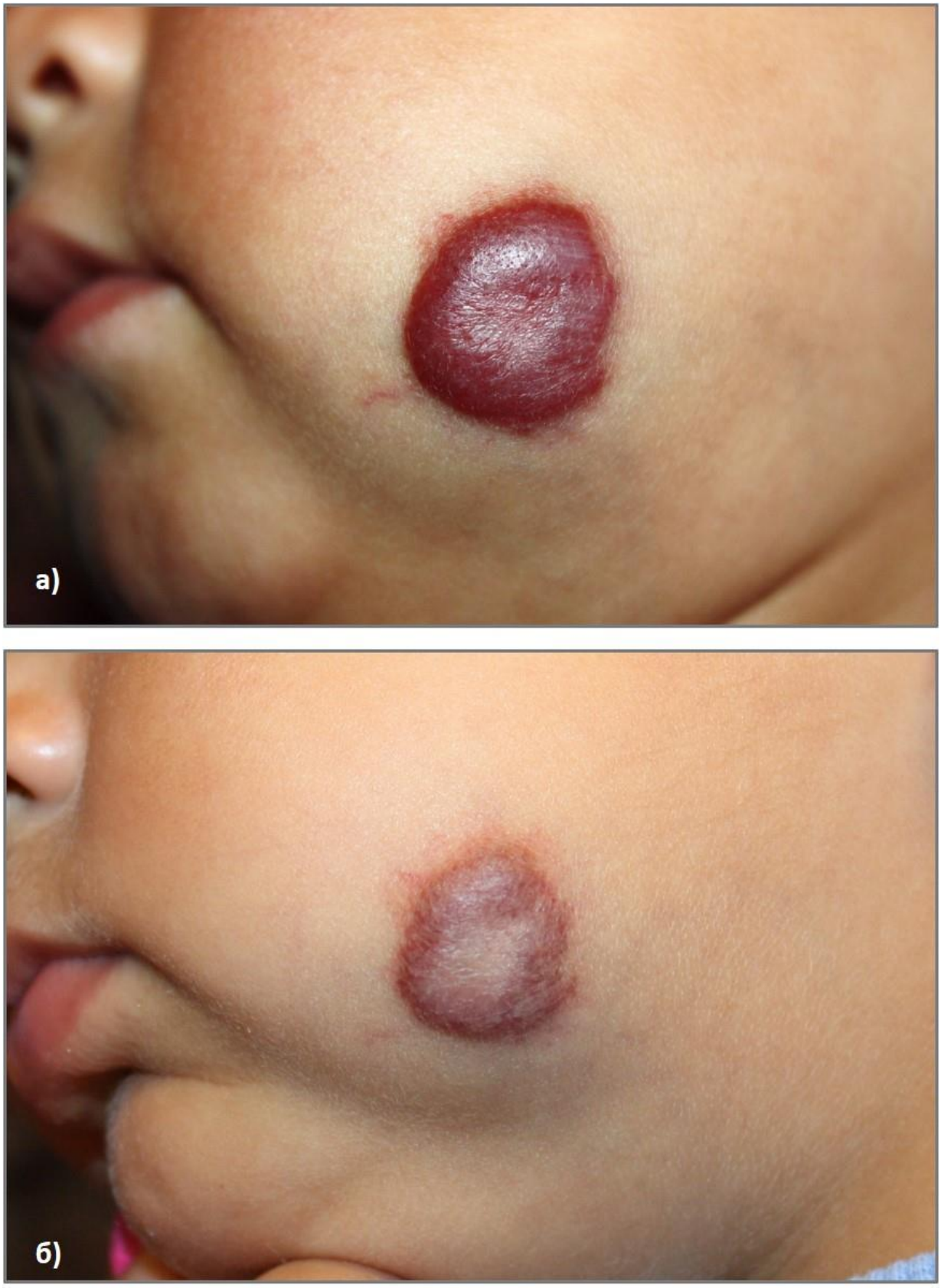

Figure 20. Boy aged 4 months (a) at the start of treatment; (b) after 6 months of treatment

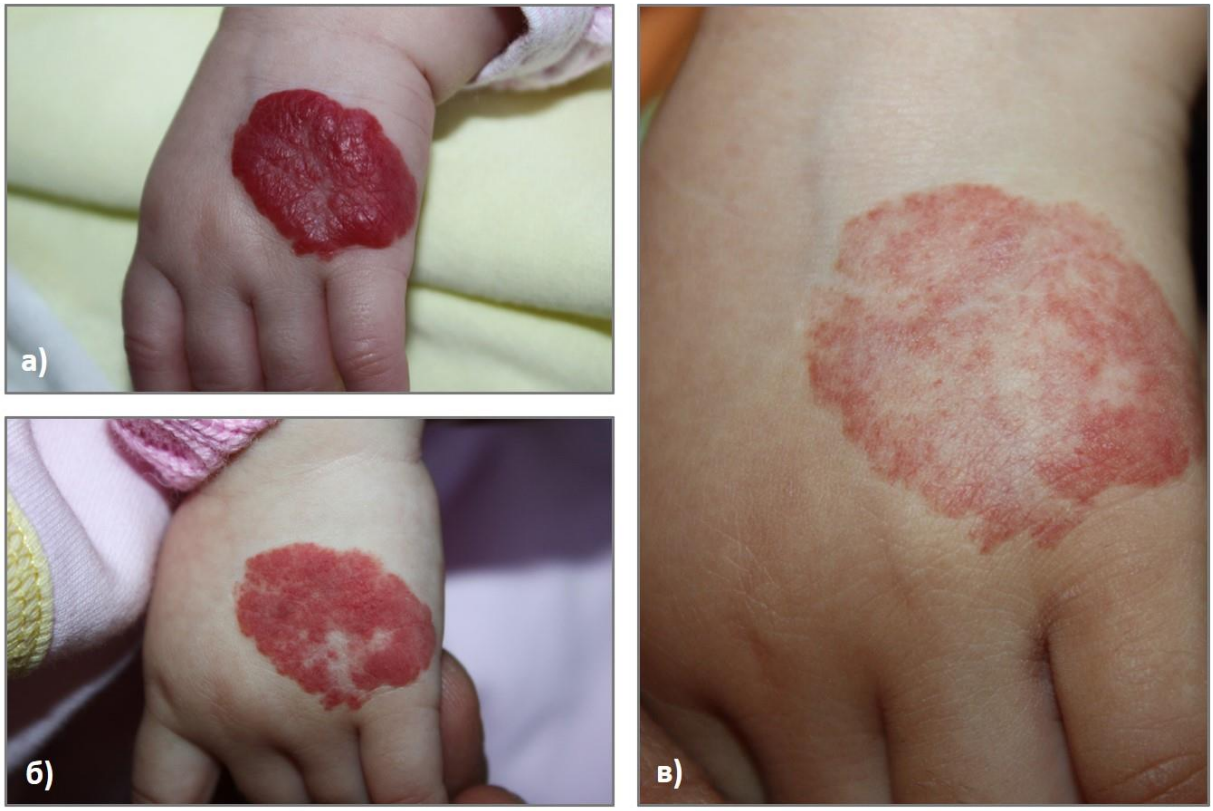

Figure 21. Mixed haemangioma in the proliferation phase: a) before initiation of treatment with propranolol; (b) after 24 weeks of treatment with propranolol and prior to initiation of timolol treatment; (c) after 24 weeks of timolol treatment following systemic treatment

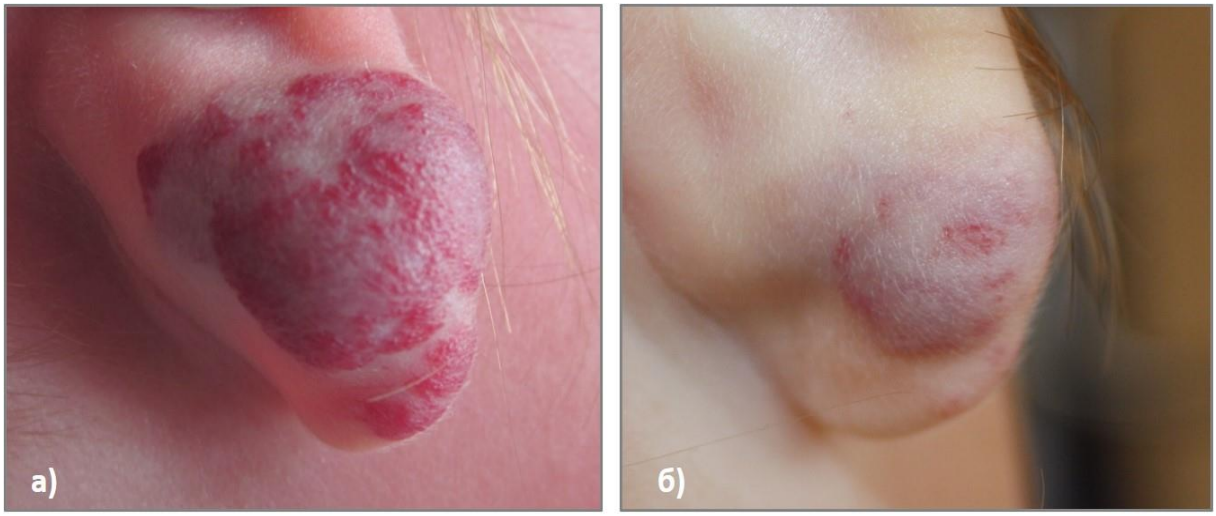

Figure 22. Haemangioma in the involution phase: (a) prior to initiation of treatment and (b) after 6 months of treatment

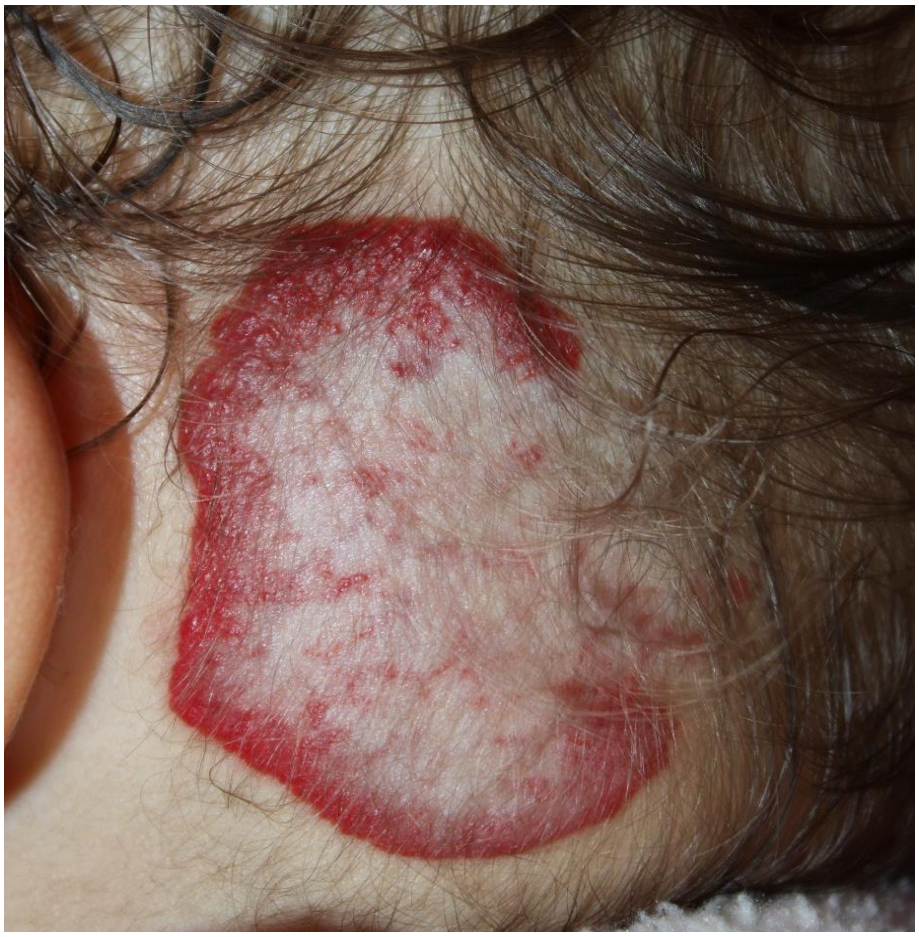

Figure 23. Haemangioma of the control group left without treatment (child 8 months old)

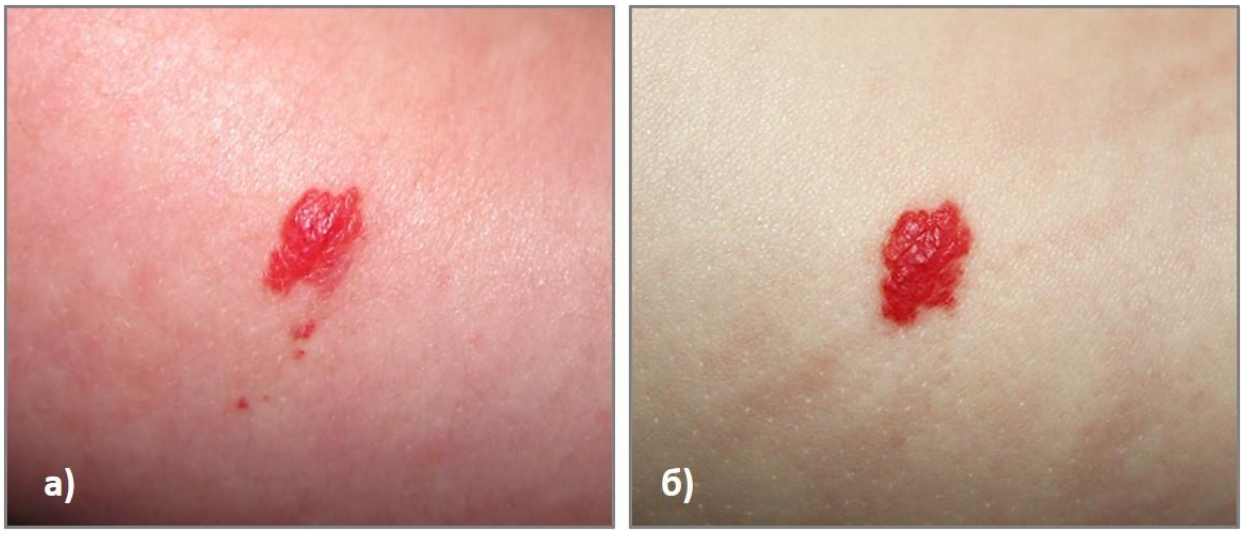

Figure 24. Control group. Arm haemangioma in a child aged 2 months: (a) at the beginning of follow-up and (b) 8 months of age/6 months of follow-up

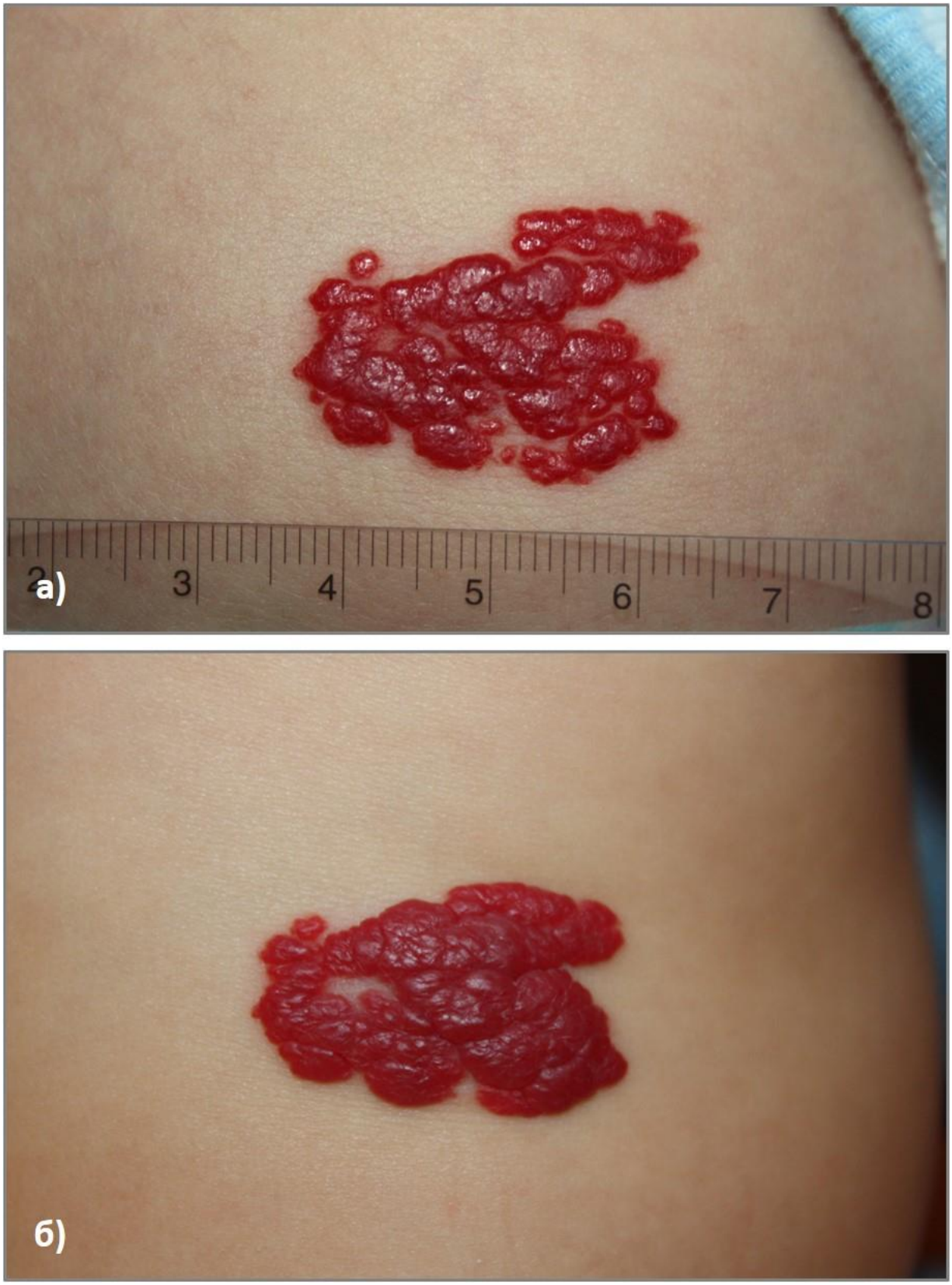

Figure 25. Control group. Child aged two months: (a) at the beginning of follow-up: (b) at 5 months (3 months of follow-up)

Safety

Side effects were not recorded in any of the subjects, including patients with multiple or large haemangiomas who received a higher amount of the medication.

5.4. Study on the efficacy and safety of timolol maleate 1% emulsion/gel

Целта на това пилотно отворено, несравнително, нерандомизирано проучване е да се установи ефективността и профила на безопасност на тимолол малеат под формата на емулсия/гел с концентрация 1%, създаден за епикутанно приложение. Препаратът е разработен в R&D лабораторията на фирма „Биотрейд България“ ООД, като емулгелът е избран като носител поради добрата пенетрация. The purpose of this pilot open, non-comparative, non-randomized study was to establish the efficacy and safety profile of timolol maleate in the form of an emulsion/gel with a concentration of 1%. The preparation was developed in the R&D laboratory of Biotrade Bulgaria Ltd. and the emulgel was selected as a carrier due to the enhanced penetration.

Aims:

- To study the therapeutic effect of topically administered timolol maleate 1% emulgel for treatment of superficial and mixed haemangiomas in the proliferative stage and in early involution.

- To establish the localized and systemic side effects of topically administered timolol maleate 1% emulgel and its safety.

The main criterion for inclusion in the study was the presence of superficial or mixed haemangioma in an active phase, without indications for systemic treatment. From the study were excluded haemangiomas in the late stages of resolution and haemangiomas with indications for active systemic treatment (associated with functional disorders, risk of disfigurement and life-threatening complications, *Table 1*).

The study included 8 children with a total of 8 superficial or mixed haemangiomas after prior informed consent from parents. Of these, 6(72%) were girls and 2 (28%) were boys aged 2 to 10 months.

Parents were applying the medication following a gradual dose increase regimen. Timolol was applied on the haemangioma in a thin layer once a day during the first week, twice a day during the second week and from the third week and until the end of treatment – three times a day.

Similar to the study with timolol maleate drops (*section 5.4*), a detailed family and personal history of respiratory and cardiovascular diseases was obtained before starting therapy. With parental consent, clinical pictures of the lesions were taken before starting treatment and at each subsequent consultation to assess the efficacy of treatment. No heart rate or blood pressure was measured prior to the start of therapy

Possible localized (edema, erythema, irritant and eczematous reaction at the site of application) and systemic (hypoglycemia, decrease in heart rate and blood pressure, respiratory spasm) side effects were discussed with parents with instructions about appropriate monitoring during treatment.

There was a significant improvement in haemangiomas over a period of 5-8 months of treatment, and in some patients already after 4 weeks of treatment, a significant response to haemangioma was observed (Figures*26-29*).

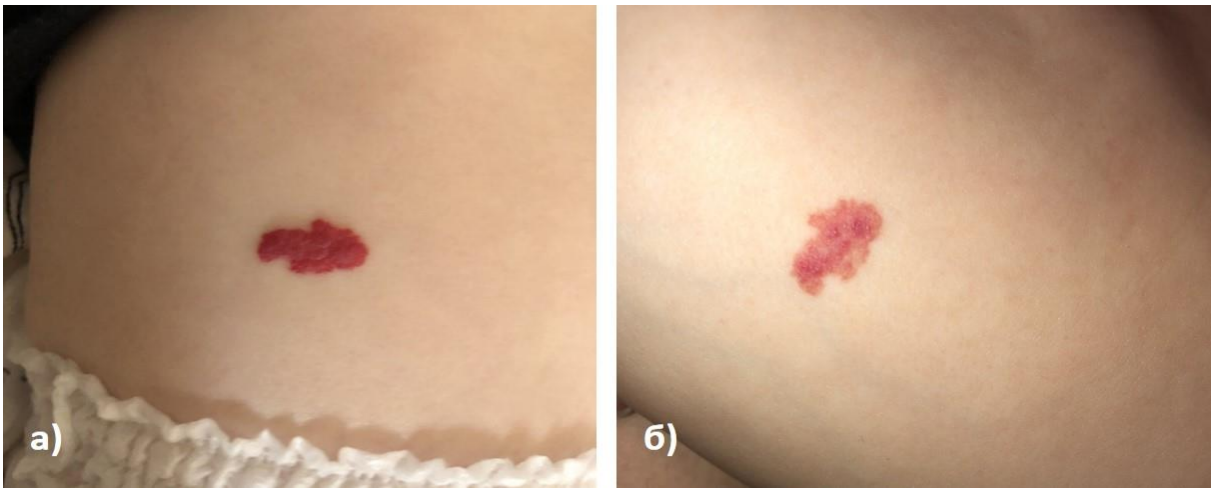

Figure 26. Child at age 4 months; (a) prior to initiation of treatment; (b) after 4 weeks of treatment.

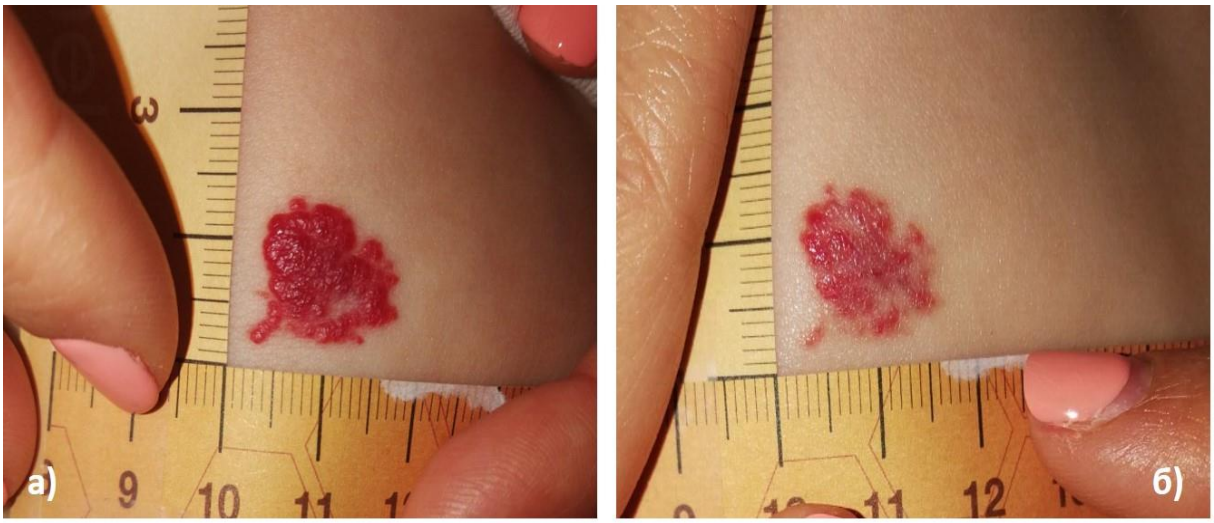

Figure 27. Child aged 2 months; (a) prior to initiation of treatment;(b) after 4 weeks of treatment. The haemangioma disappeared completely after 8 months of treatment.

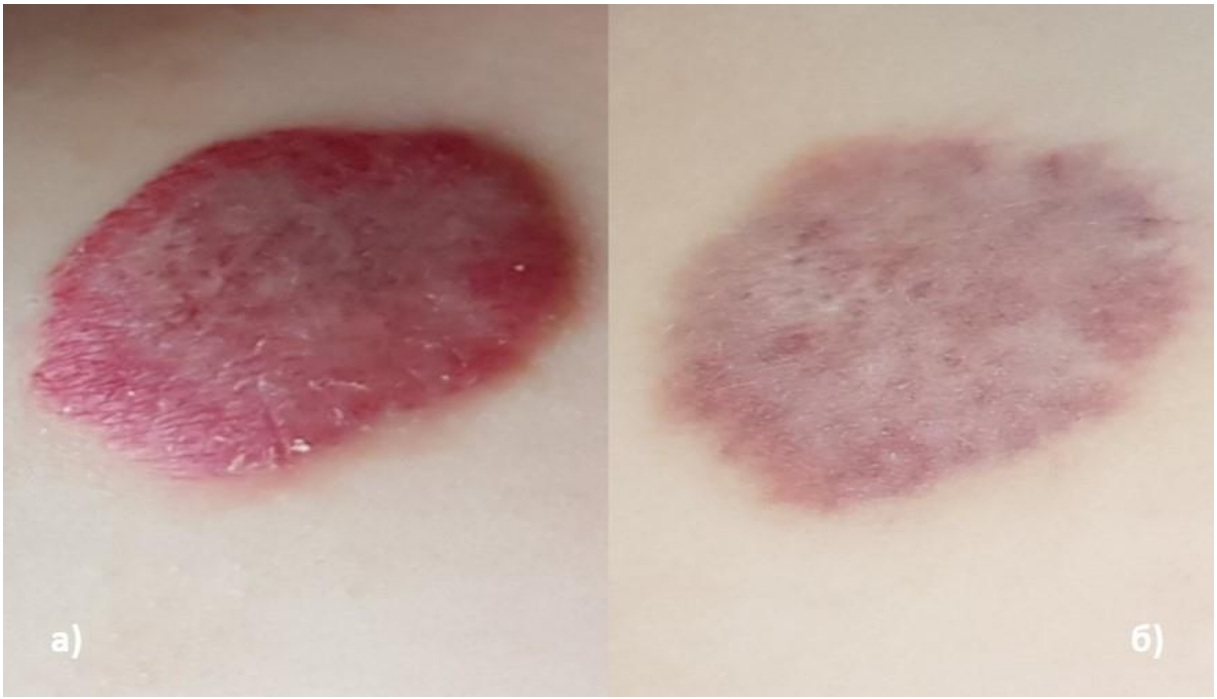

Figure 28. Child aged 8 months:(a) before treatment; (b)after 5 months of treatment

Despite the higher concentration and better penetration as compared to timolol maleate 0.5% drops, no systemic side effects were observed during treatment.

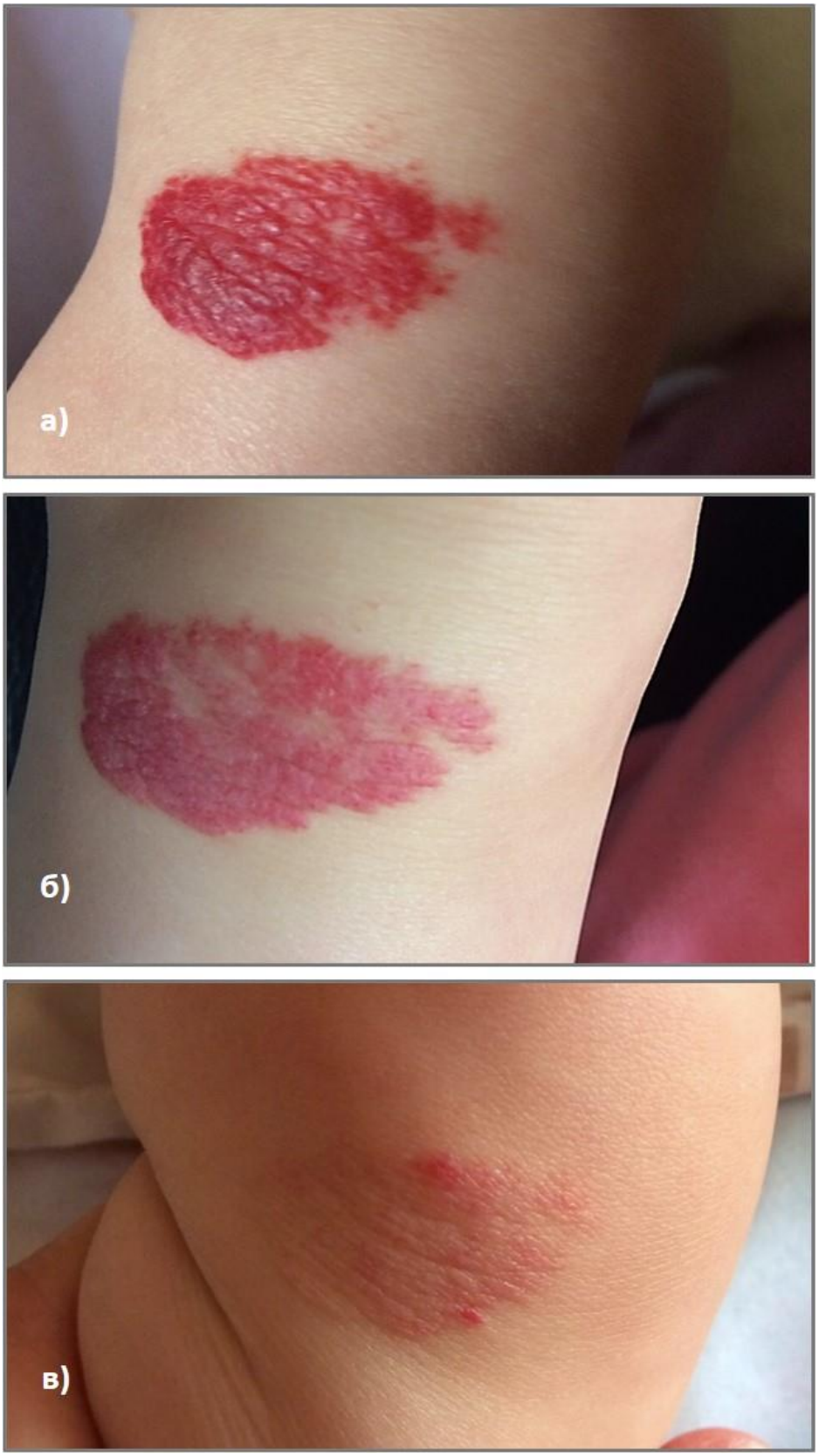

Figure 29. Child aged 3 months: a) before starting treatment; b) after 3 months of treatment; (b) after 6 months of treatment

5.5. Study to assess parents' perception of the psycho-social impact of infantile haemangiomas on the quality of life of the family

The purpose of this study was to determine the impact of infantile haemangiomas on the parents, on the child and on the lifestyle of the family for the Bulgarian population and to compare it with the available data from the world literature.

Tasks:

- To determine the burden of infantile haemangiomas on the psycho-social functions of the daily lives of patients and their parents by filling out a questionnaire "Haemangioma Family Burden";

- To Identify specific groups where the burden is greater and there may be a need to consult a clinical psychologist;

- To compare the data for the Bulgarian population with the data from the world literature;

- To compare the impact of infantile haemangiomas on the family compared to the impact of atopic dermatitis as a disease with a well-studied psycho-social negative impact. For the study, a questionnaire was used, which was pre-validated and applied to assess the impact in children with haemangiomas, in various European countries and the United States of America: Haemangioma Family Burden (HFB). The questionnaire was translated into Bulgarian and described in Chapter 4. Methods (*Table 8*).

Haemagioma Family Burden Questionnaire

1. Our child's haemangioma makes us think about future plans	☐ *Definitely YES*	☐ May be	☐ *Definitely NOT*	☐ I don't know
2. Our child's haemangioma complicates family life	☐ *Definitely YES*	☐ May be	☐ *Definitely NOT*	☐

				I don't know
3. Our child's haemangioma causes serious tension in the relationship between me and my partner	☐ *Definitely YES*	☐ May be	☐ *Definitely NOT*	☐ I don't know
4. My child's haemangioma turned my life around	☐ *Definitely YES*	☐ May be	☐ *Definitely NOT*	☐ I don't know
5. Sometimes we spend less time with our other children because of the child's haemangioma	☐ *Definitely YES*	☐ May be	☐ *Definitely NOT*	☐ I don't know
6. My child's haemangioma had an impact on my career	☐ *Definitely YES*	☐ May be	☐ *Definitely NOT*	☐ I don't know
7. I had to stop working because of my child's haemangioma.	☐ *Definitely YES*	☐ May be	☐ *Definitely NOT*	☐ I don't know
8. My child needs a lot of attention and love because of the haemangioma	☐ *Definitely YES*	☐ May be	☐ *Definitely NOT*	☐ I don't know
9. Because of haemangioma, my child is more vulnerable than other children	☐ *Definitely YES*	☐ May be	☐ *Definitely NOT*	☐ I don't know

10. My child needs more attention because of the haemangioma	☐ *Definitely YES*	☐ May be	☐ *Definitely NOT*	☐ I don't know
11. I'm more protective with my child because of the haemangioma	☐ *Definitely YES*	☐ May be	☐ *Definitely NOT*	☐ I don't know
12. People's reactions to our child's haemangioma depress me	☐ *Definitely YES*	☐ May be	☐ *Definitely NOT*	☐ I don't know
13. I feel guilty about our child's haemangioma	☐ *Definitely YES*	☐ May be	☐ *Definitely NOT*	☐ I don't know
14. I often feel frustrated and helpless after seeing a doctor about our child's haemangioma	☐ *Definitely YES*	☐ May be	☐ *Definitely NOT*	☐ I don't know
15. I have come to terms with our child's haemangioma	☐ *Definitely YES*	☐ May be	☐ *Definitely NOT*	☐ I don't know
In your opinion, did your child's haemangioma have any effect on your				
16. The desire to have another child	☐ *Negative impact*	☐ not	☐ *Positive influence*	☐ I don't know
17. Your sex life or sexuality	☐ *Negative impact*	☐ not	☐ *Positive influence*	☐ I don't know

18. Your financial capabilities and budget	☐ *Negative impact*	☐ not	☐ *Positive influence*	☐ I don't know
19. Your mood	☐ *Negative impact*	☐ not	☐ *Positive influence*	☐ I don't know
20. Your optimism	☐ *Negative impact*	☐ not	☐ *Positive influence*	☐ I don't know

Table 8. Impact of the haemangioma on the quality of life of the family

A total of 48 families of children with infantile haemangiomas were included in the study. Of the 48 children, 19 had haemangiomas on the head and neck and 29 had haemangiomas of the body and limbs; 14 had large haemangiomas (defined as larger than 5 cm), and 34 had small haemangiomas, with 4 of the large haemangiomas on the head and neck area. Of the 48 children, 31 (65%) were girls and 17 (35%) were boys. Parents fill out the questionnaire anonymously, noting the localization and size of the haemangioma. The questionnaire refers to the active phase of development of haemangioma and the early phase of resolution (age of children from 0 to 12 months).

The families of 48 children with mild to severe atopic dermatitis have completed a questionnaire with the same content, but referring to atopic dermatitis, not haemangiomas. Children with atopic dermatitis were selected for a control group due to the known negative impact of this condition on the psycho-social parameters of daily quality of life.

The overall score for families with children with haemangiomas ranged from 0 to 35, an average score of 7.52 (SD±7.04). There was a statistically significant difference between families with children with haemangiomas on the head and neck area (visible to others), in which the total mean score was 11.63 (SD±9.02), and families whose children had haemangiomas on the extremities and body, for whom the total mean score was 4.83

(SD±3.45) ($p<0.001$). A statistically significant difference was also observed for variations in the size of the haemangioma, with the mean score for large haemangiomas being 14.21 (SD±8.14) and for small haemangiomas - 4.76 (SD±4.02) ($p<0.001$).

In the group of families with children with atopic dermatitis, the total score ranged from 0 to 35, with an average score of 19.8 (SD±11.07) ($p<0.001$).

5.6. Rare clinical cases

5.6.1. Development of dermatitis/eczematous reaction in the area of the haemangioma/predilection of atopic dermatitis for the area of haemangioma

In the study group of children with haemangiomas, 4 cases of development of dermatitis reactions in the haemangioma area were observed. The group included three girls and one boy aged 8 to 14 months. Two of the children were on topical therapy with timolol maleate and two had no topical treatment. Significant improvement was achieved with short-term treatment with potent topical corticosteroids (*Figures 25 and 26*).

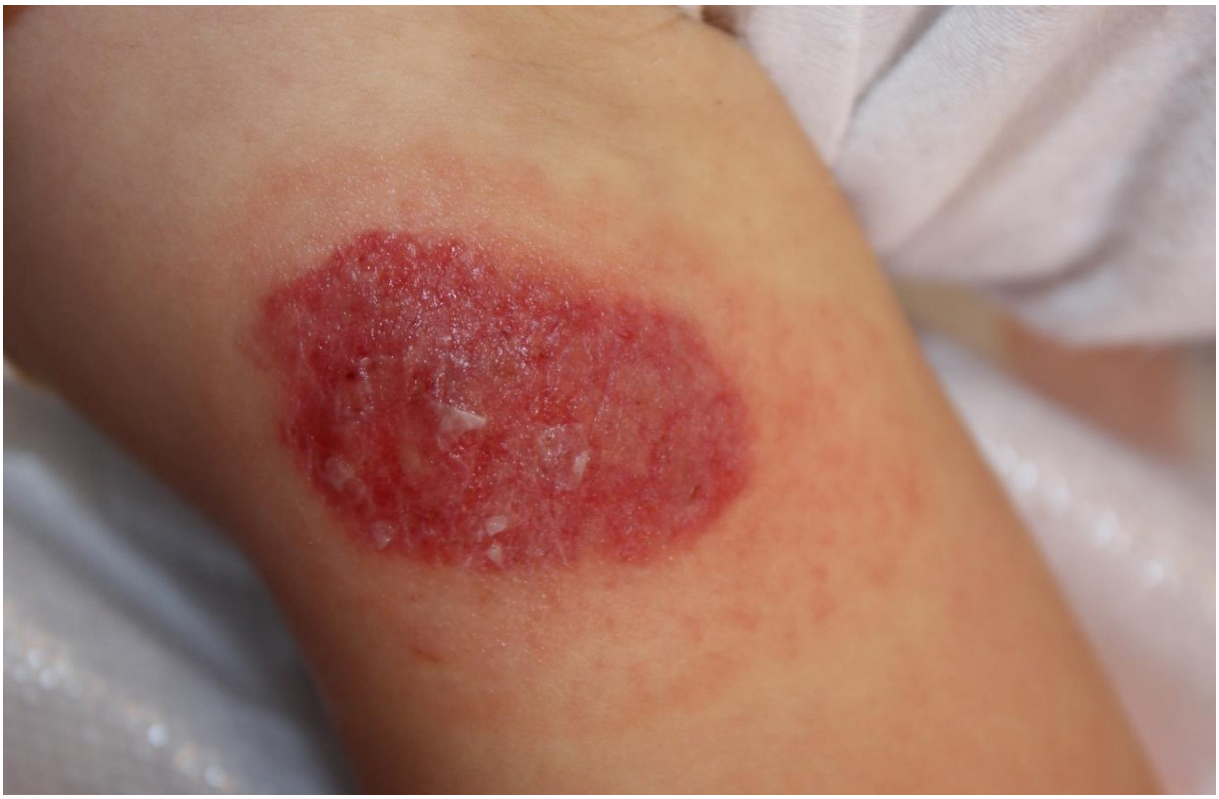

Figure 25. Acute dermatitis/eczematous reaction in the area of an infantile haemangioma

One of the children had prior propranolol therapy and residual changes after discontinuation of systemic therapy for which topical treatment with timolol was initiated (*Figure 26*). Spontaneous resolution was not observed while the eczematous changes were persistent, regardless of the administration of timolol. After complete clearance of the dermatitis/eczema with topical momethasone furoate, treatment with timolol gives a good result with the complete disappearance of the resistant vascular component

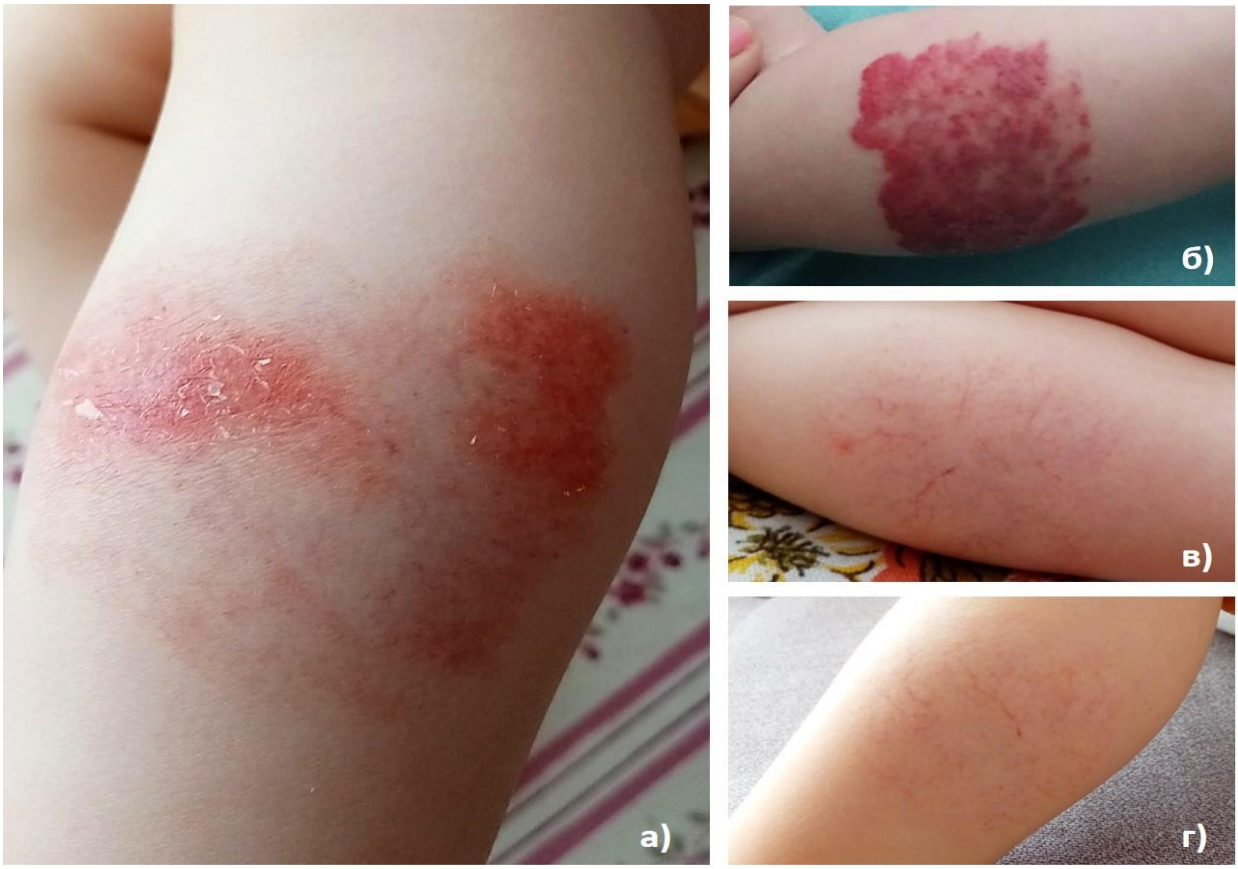

Figure 26. Dermatitis/Eczematous reaction in a child with haemangioma after treatment with propranolol: a) dermatitis/eczema plaque with erythema and desquamation in the area of residual haemangioma;(b) initial phase of haemangioma at the age of 6 weeks; c) after treatment of dermatitis/eczematous reaction with mometasone furoate; d)after

treatment with timolol maleate for 4 weeks and with complete disappearance of the vascular changes after 8 weeks of treatment

In the literature, there are limited reports of development of dermatitis reactions on vascular malformations or haemangiomas. It has been suggested that eczematous changes develop as the increased number of vessels leads to increased blood flow with an influx of cells of inflammation and a cytokine response. This hypothesis may explain the predilection of dermatitis/eczema in children who are predisposed to atopic deramitis, both in the form of a personal family history and a family predisposition. The children in our study group had a family history of atopy.

Дерматитни изменения могат да се наблюдават и в резултат на контактна алергия към приложеното лечение. L. Sacchelli и съавт. описват случай на контактен дерматит към тимолол малеат очни капки, като контактната алергия е доказана с алергологично тестуване. При четирите описани деца, лечението с тимолол малеат е продължено след успешно лечение на дерматитната реакция с локални кортикостероиди. Не са наблюдавани последващи реакции, което изключва контактна алергия.Dermatitis changes can also be observed as a result of contact allergy to the applied treatment. L. Sacchelli and co-attach describe a case of contact dermatitis to timolol maleate eye drops, with contact allergy proven with allergy testing. In the four children described, treatment with timolol maleate was continued after successful treatment of dermatitis reaction with local corticosteroids. No follow-up reactions were observed, which excluded contact allergy

5.6.2. Treatment of infantile haemangiomas with brimonidine

The patient is a 5-day-old girl born naturally at term from an uneventful pregnancy. At birth, the parents noticed a red spot on the left cheek, which grew for a few days. After diagnosing segmental maxillary haemangioma, we started treatment with a combined preparation containing timolol maleate 0.5% and brimonidine 0.2%, administered twice a day. Brimonidine is an α-antagonist which could be used as an alternative to treatment with a particularly good effect in ulcerative haemangiomas. M.B. Chu et al. described three cases of successful treatment of haemangiomas with the combined preparation. Only one child had episodes of hypothermia initially developping after completion of treatment.

The toxicity of brimonidine in children is well described. M. L. Becker et al. studied 176 children aged 0 to 5 years with recorded brimonidine poisoning. The latter was predominantly associated with accidental ingestion of the drug, but also with ocular use. The most commonly observed symptoms were drowsiness (40,9%), ataxia (4,5%), irritability (4,0%), bradycardia (4,0%), hypotension (4,0%), myosis (3,4%) In 11 children, naltrexone was administered as a brimonidine antagonist.

In our patient, there was significant improvement from treatment with almost complete clearance of the haemangioma at the age of 11 months (*Figure 27*). No side effects were recorded. Brimonidine in combination with timolol is an alternative for the treatment of infantile haemangiomas with a significantly favorable safety profile.

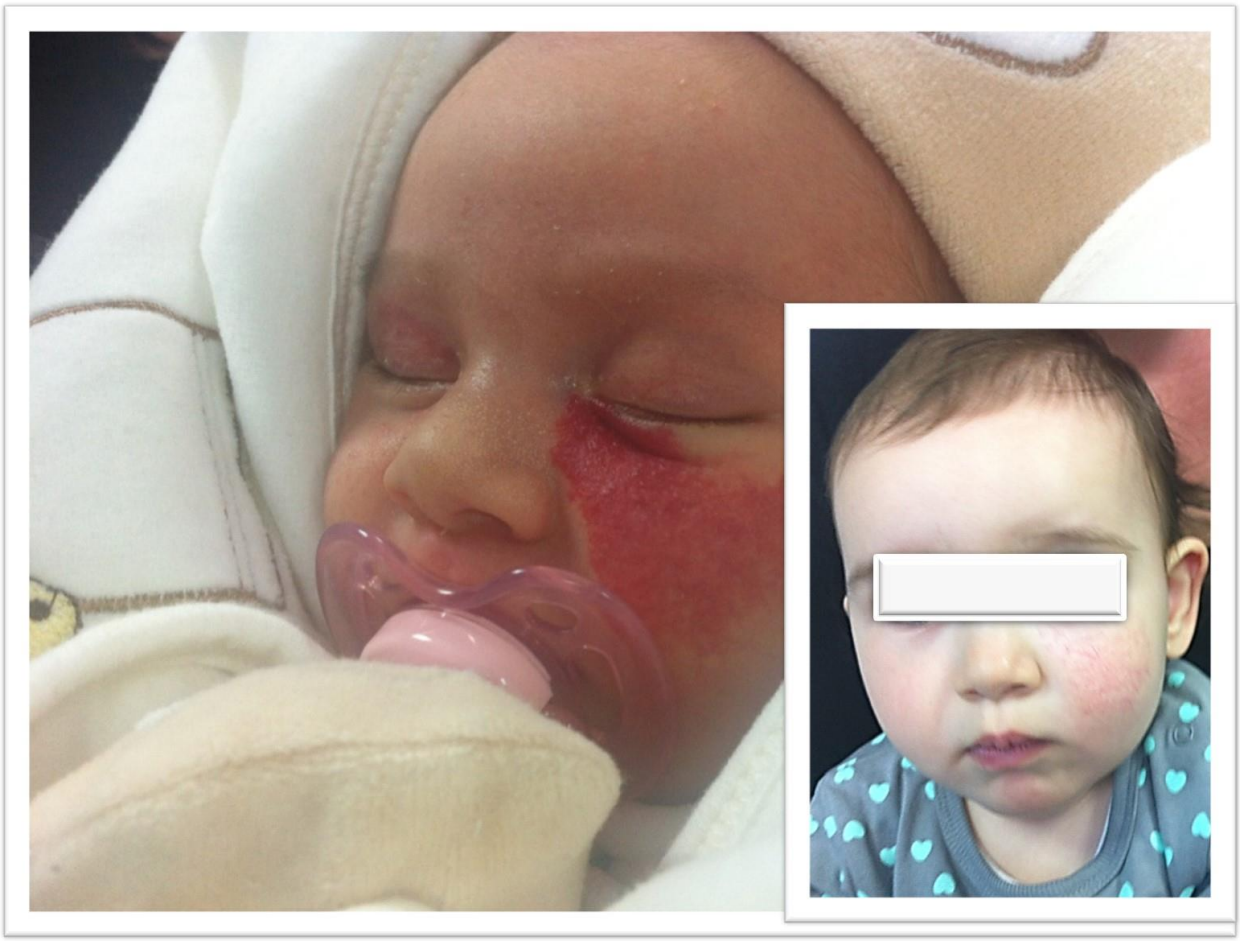

Figure 27. Child with maxillary segmental haemangioma at 5 days and 11 months after treatment with brimonidine

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IV. DISCUSSION

6.1. Epidemiological study for evaluation of prenatal and postnatal factors associated with an increased risk of developing infantile haemangiomas in the Bulgarian population

The results of our study in 256 children with haemangiomas and 256 children without haemangiomas show significant similarities for the Bulgarian population with literature data regarding the established prenatal and perinatal risk factors associated with development of infantile haemangiomas. There are also some specific features for our population.

The increased incidence of haemangiomas in girls, which represent 68.4% of the study group, is confirmed. Although female predominance is well known and described in the literature, there is no clear explanation for this phenomenon.

Comparative analysis of prenatal factors associated with the development of infantile haemangiomas

Assisted reproduction by the IVF method (including intracytoplasmic sperm injection/ICSI), and intrauterine insemination are not associated with an increased incidence of haemangiomas in the Bulgarian population, with only twenty-one children (8.2%) with haemangiomas being conceived by the IVF (or ICSI) method, compared to 14 (5.5%) children in the control group (P=0.22). Globally, there are significant differences in the statistical significance of this association, with some studies showing a link not established in other studies. Systematic meta-analysis of epidemiological studies of risk factors associated with the development of haemangiomas confirms the heterogeneity of the study results.

The mechanism of assisted reproduction does not imply hypoxia, or subsequent differences in fetal development relative to natural conception. The likelihood of multiple pregnancies in assisted reproduction procedures is higher, which also increases the risk of giving birth with lower body weight and childbirth before 40 weeks gestation. The higher incidence of these independent risk factors most likely increases the number of children with haemangiomas after IVF or similar procedures without the assisted reproductive methodology itself being an independent risk factor. In addition, it could be assumed that for parents requiring assisted reproduction, there might be an increased incidence of other

underlying problems, including vascular, which might lead to secondary hypoxia of the fetus and the development of haemangioma.

Multiple pregnancies (including three-embryonal) are not associated with an increased risk in our population, as a factor independent of body weight. This differs from data in the literature that show a clear correlation between multiple pregnancies and the frequency of development of haemangiomas. The difference in this trend may be due to the lower number of patients with multiple pregnancies in our study.

Problems during pregnancy, including eclampsia and pre-eclapsia, premature contractions, bleeding, placenta previa and hospital admission for retention show a clear association with the development of haemangiomas, which is explained by the hypoxic stimulus associated with these factor. In our study, placental abnormalities were a statistically significant risk factor for the development of a haemangioma, independent of weight and gestational age at birth. The placenta is a major source of oxygen for the fetus, and abnormalities in its development, including in the placental vascular network, lead to reduced blood supply to the fetus. Globally, pre-eclampsia/eclampsia is not a risk factor.

Prenatal procedures (amniocentesis and chorion biopsy), intake of tocolytics (magnesium and combined preparations), regardless of trimester and duration, as well as previous thyroid disease with treatment during pregnancy and smoking during pregnancy do not correlate with the development of infantile haemangiomas. This is consistent with the trend in the world.

The absence of impact of smoking is an interesting fact, since the effect of smoking on the vessels of the placenta and fetal-maternal circulation is well known. Changes in the capillary volume of the placenta when smoking are due to a decrease in the average capillary diameter, not the total length, which reduces placental blood flow, reduces the exchange surface for gases and nutrients and increases the risk of intrauterine reparation. Despite the higher number of mothers reporting smoking during pregnancy in the group of children with haemangiomas (12.1% vs. 7%), the difference between the two groups did not reach statistical significance according to our chosen P criterion (P=0.05).

The lack of an association described in the literature and an association in our study in the Bulgarian population may be the result of the small number of women using cigarettes during pregnancy, as well as the number of cigarettes per day, as it is known that the effect on the placenta is dose dependent. Hypothetically, the risk of smoking for occurrence of of haemangiomas may be lower due to the constant hypoxia and the development of adaptive mechanisms of the fetus from early intrauterine development.

Comparative analysis of perinatal factors associated with the development of infantile haemangiomas

The method of delivery, Cesarean section or spontaneous vaginal delivery without complications, is not registered as a risk factor for the development of haemangiomas, which corresponds to the data from the literature. Our study described nine children with hemagiomas born with umbilical cord wrapped around the neck and secondary hypoxia, with similar complications not described in the group of children without haemangiomas.

As the main risk factors for the development of haemangiomas in the Bulgarian population, our study identified low body weight at birth and degree of prematurity. B. A. Drolet et al. described. 40% increase in the risk for infantile haemangioma for every 500 g of reduced body weight. In our study group, this trend was confirmed, with 43.75% (N=112) of children with haemangiomas having a body weight below 2,999 g, compared with 18.75% (N=48) of the haemangioma-free group. An increased incidence of haemangiomas was observed for each group, and statistically significant result was not achieved only for the group with body weight from 2 999 g to 2 499 g.

In our study, 59% (N=151) of children with haemangiomas were born before 38 weeks gestation, compared to only 19.1% (N=49) of children without haemangiomas.

A regression analysis shows a pronounced correlation between body weight and gestational age at birth, making it difficult to differentiate the two factors as independent. Tissue hypoxia and oxidative stress are well-known pathophysiological reactions in premature birth, developing as a result of oxygen resuscitation, blood transfusions,

phototherapy, nutrition, high metabolic activity, inflammation and infections and immature antioxidant system. Although the etiopathogenesis of infantile haemangiomas is not yet fully understood, one of the hypothesis for their development is the presence of tissue hypoxia which stimulates the secretion of growth factors.

6.2. Development of a scale for assessing the activity and severity of haemangiomas, suitable for initial evaluation and follow-up of treatment

A limited number of evaluation scales have been proposed and introduced into medical practice in order to record clinically significant changes of haemangiomas in nursing and childhood, as opposed to numerous indices for other dermatological diseases such as lupus erythematosus and atopic dermatitis. Currently, there are two evaluation scales in the literature, one for activity and one for severity, none of which is widely introduced in clinical practice. The lack of a uniform and validated standard assessment method is the result of several factors. On the one hand, clinical examination is usually sufficient to monitor infantile haemangiomas, and on the other – the number of clinical trials until 2008 was limited due to spontaneous regression and the limited safety and efficacy of conventional treatments. Since 2008, when S. Laute-Labreze et al. showed efficacy of β blockers for the treatment of infantile haemangiomas, they have become the focus of scientific studies again. The latter requires the development of an evaluation scale that is validated and standardised for use in clinical trials. We have developed and validated HASI after a series of expert consultations to facilitate our research. The scale can be used by dermatologists and pediatricians who treat children with haemangiomas without the need for prior training.

HASI assesses the objective physical status and does not include an assessment of the mental impact on children and parents due to the low objectivity of the psychological criterion. It is desirable to avoid subjective criteria when a precise assessment of the outcome of a treatment is required.

Correct interpretation of the results obtained using an evaluation scale can be achieved only after its widespread use in different patients, in different settings and with different observers. The interpretation of HASI should be separate for both scales - HASI (activity) and HASI (severity). A change in activity over time is accurately reflected, but the

number of patients in our validation study is not enough to clearly classify the improvement according to the change in outcome. However, a preliminary summary analysis of the available data from this and from other studies using the index (unpublished data) shows that a result of 15 or more on the activity scale is an indicator of systemic treatment.

The severity assessment, on the other hand, is based on stable parameters, such as type, number and involvement of other organs, and can be interpreted on the basis of the current knowledge for the evolution of haemangiomas. The result of severity can be successfully used to choose a therapeutic approach (*Table 5*). In the case of signs and symptoms of systemic involvement or where there are suspicions of such because of localisation or morphological characteristics, the severity can only be assessed after an appropriate imaging examination and HASI (severity) should not necessarily be determined at the initial visit. Since additional studies are an indispensable part of the diagnostic process in high-risk cases, they are usually carried out before starting treatment, regardless of whether an evaluation system is used or not.

Compared to other available evaluation scales, HASI is more objective and has a higher reproducibility rate than VAS as it includes well-defined clinical characteristics rather than a comprehensive assessment. Compared to HAS, HASI includes an additional consistency assessment, introduces a different method of color quantification and has a separate severity section. The change in the consistency of the superficial component of a haemangioma from tense to soft is usually the first indication for treatment efficacy. These changes can correlate with changes in colour. Although not the most important sign of therapeutic response, the change in consistency indicates regression and has its place in the assessment criteria. HSS and HDCS are reliable for assessing the severity of infantile haemangiomas, including the severity of complications, but do not include the assessment of activity.

This validation study has several limitations, namely the small number of patients and the participation of a small number of researchers. HASI is better suited for direct evaluation of patients, since photographic images often do not allow measurement of available erosion/ulceration and, when poor quality, an accurate assessment of the consistency and deep component cannot be made. The quantification of regression areas is approximate and

leaves room for subjectivity and slight deviation for different evaluators. However, our study showed no statistically significant differences between individual specialists, which proves that the level of subjectivity is within acceptable limits.

Our HASI validation studies show promising results in terms of clinical benefit and relevance in practice. HASI has also been used in the study of treatment with topical timolol maleate described in section 5.3. The index is easy and fast to apply, gives a clear idea of the activity of haemangioma at a time and does not take a significant time from the clinical consultation. The proposed evaluation scale can be used by dermatologists and pediatricians and introduced as a routine standard method for uniform and objective determination of the activity and severity of infantile haemangiomas.

6.3 Prospective study of the therapeutic effect and safety profile of a new drug for topical treatment of infantile haemangiomas – timolol maleate

Timolol maleate is a non-selective β-blocker. Although the mechanism of action of β-blockers in infantile haemangiomas is not fully understood, it is currently assumed that their effectiveness is due to three complementary pharmacodynamic mechanisms that are activated at different times – vasoconstriction, inhibition of angiogenesis and induction of apoptosis. Clinically, these three mechanisms translate into rapid change of the surface colour of the haemangioma, growth cessation and regression.

The administration of any new treatment must comply with the ethical principles of medicine and an effective balance of efficacy and safety. The purpose of our study was to identify the efficacy of treatment relative to spontaneous involution and the benefits of early treatment, as well as the safety profile. The safety of treatment, namely the type and frequency of adverse reactions, also determine the follow-up pattern. In medical conditions or tumours where spontaneous improvement is expected, it is difficult to assess the efficacy of a treatment modality, since there is no objective method that filters the improvement resulting from spontaneous involution and improvement as a result of the applied treatment. The introduction of a control group reduces the risk of error in such studies as comparative improvement between the two groups is looked at rather than an improvement from baseline.

Timolol maleate is not registered as a preparation for the treatment of haemangiomas, but is used outside the registered indication for the treatment of open-angle glaucoma and secondary glaucoma. The preparations that are applied are solutions and gel-forming solutions in the form of eye drops. Therefore, it is important to explain to parents that the medication is applied onto the skin and how much to apply for treatment.

In patients on treatment with timolol, early change in colour of the surface component is observed usually within the first days/weeks of treatment, which is due to vasoconstriction of the vessels. This was also noted in our study, with most significant decrease in HASI by 25% in the first 4 weeks of treatment. Some parents reported improvement within a few days of treatment, which corresponds to reports in the literature and the expected vasoconstrictive effect.

Vasoconstriction is usually followed by blockage of proangiogenic factors (VEGF, bFGF, MMP-2, and MMP-9), which limits the growth of the haemangioma. The long-term effects of β-blockers are the result of induction of apoptosis in endothelial cells, which is mediated with predilection by β2-receptors. Similar mechanisms are described with spontaneous involution, however, these occur at a later stage - reduction in VEGF and bFGF, both in the haemangioma itself and in the patient's blood. Unlike apoptosis in treatment with β blockers, however, T. Itinteang et al. showed that haemangioma endothelial cells undergo spontaneous differentiation to adopocytes, which correlates with loss of pref-1 expression. This also explains the delayed spontaneous involution, residual fibro-fatty tissue and the worse cosmetic outcomes.

Our study is the first of its kind in the Republic of Bulgaria focusing on the application of topical therapy for infantile haemangiomas and our observations and results confirm published studies from different countries. At the end of the 6-month follow-up period, there was a 79.3% decrease in HASI in the timolol maleate treatment group, compared to 41.3% in the control group, which is explained by the natural spontaneous involution of haemangiomas. In the group of children with haemangiomas, after a 6-month treatment/follow-up period, 48 (25.3%) children with haemangiomas had complete involution, compared to 2 (9%) children in the control group. Similar results were described from a randomised study in 41 children aged 9 weeks, 19 on timolol 0.5% gel and 22 on

placebo (17 at the end of the study) who experienced complete involution observed in 36.8% of children treated with topical timolol and 1 (4.5%) child in the placebo group. Other studies have shown a similar trend for rapid involution, minimal residual cosmetic and functional changes and a reduced risk of ulceration and secondary infection with subsequent scarring.

Treatment with timolol in our study group showed higher efficacy in plaque lesions as compared to nodular lesions and during the proliferation stage as compared to the involution stage. In the literature, thinner superficial haemangiomas were reported to respond better to treatment with topical timolol and treatment of such lesions was associated with a lower systemic concentration compared to thicker/nodular haemangiomas at the same administered amount. One hypothesis is that this is the result of diffusion of the β-blocker in the capillaries, with a larger amount of vessels leading to greater systemic absorption with greater local loss of the drug. It can be speculated, however, that the drug does not reach in depth enough to affect the deeper vessels, and this minimizes efficacy.

For all haemangiomas included in the study, we observed an improvement at weeks 12 and 24 from the beginning of treatment. After the initial fading of the color and switching from bright red to matte red color and changing the consistency from tense to soft, the color change becomes more gradual with delineation of zones of regression until complete involution of the lesions with or without residual changes.

Our study also included children with large haemangiomas and haemangiomas of the mucous membranes/transition zones, which warrant systemic treatment. Although longer treatment and stricter monitoring is required due to the risk of absorption of a larger amount of medication in these cases, topical timolol maleate can be applied in this clinical when there are contraindications to systemic treatment or refusal by parents for systemic treatment. With larger haemangiomas, treatment can last 12-18 months.

Timolol maleate has a good safety profile, despite significant systemic absorption, even with the administration of negligible low doses. The expected side effects are mainly the result of β-adrenergic blockade and include bradycardia, hypotension, hypoglycemia, changes in sleep patterns and irritation at the site of administration. B. A. Drolet et al.

showed systemic bioavailability of timolol in 93% of the 76 children studied after administration of one to two drops per day of 0.5% timolol solution. Although in 80% the plasma concentration was >0.2 ng/mL, a concentration at which a systemic effect of β-blockade is expected in adults, no side effects or effects of blocking of β-adrenergic receptors were observed in children, except for one case of asymptomatic bradycardia at a blood concentration of timolol of 0.79 ng/ml.

J. Borok et al. also showed significant systemic absorption of timolol maleate on average 3.45 hours after administration of a dose after two weeks of treatment in 24 children. What is interesting about this study is that there was a statistically significant relationship with the administered dose per kilogram of body weight. Like other studies, the presence of timolol in the blood does not correlate with clinical manifestation of side effects.

Significant bradycardia was recorded in 4 children (out of 22 enrolled in the study) with Holter following administration of timolol. In two of the children, the episodes were short and asymptomatic and not time-related to the topical treatment. Only two of the children had symptomatic episodes of bradycardia and both were born prematurely and weighed less than 2,500 g when initiating treatment at a dose higher than the average for the study group.

No significant side effects and discontinuation of treatment due to the development of adverse reactions have been reported in efficacy studies.

Our study did not include blood sample tracking for bioavailability of timolol. Before prescribing treatment with timolol, detailed information was given to parents about the possible side effects of treatment resulting from systemic absorption and local affect. It was explained to parents how to recognize the signs of bradycardia, hypotension and hypoglycemia. There have been no reports from parents of side effects associated with treatment or of abnormal symptoms and signs in the child or a change in behaviour. Treatment was not discontinued in any study participant due to the development of adverse reactions

In our study group, children on prior treatment with systemic propranolol did not have a significant decrease in HASI, which can be explained by the stage of involution. In most

children, residual lesions are almost completely gone after a 6-month period of topical treatment after systemic treatment with propranolol. In addition, rebound phenomenon of haemangiomas after stopping systemic propranolol and switching to topical therapy was observed in any of the included 14 children. Our observations are consistent with reports in the literature, although the number of studies with a transition from systemic to topical treatment is limited. D.B. Mannschreck et al. analized 30 children on systemic propranolol therapy and subsequent therapy with topoical timolol. For them, treatment with propranolol was initiated at an average age of 3.9 months and lasted for an average of 7.5 months, significantly shorter than the duration required for the propranolol-only treatment group (9.7 months). In these children, topical treatment with timolol maleate was included simultaneously with a decrease in the dose of propranolol, and no relapse of the haemangioma, requiring re-initiation of propranol, was observed.

There is also literature data on timolol maleate used as a continuation of systemic therapy with β-blockers. Z.L. Zhao et al. described 41 children with residual telangiectasia and superficial vessels after treatment with atenolol. Satisfactory regression with topical treatment was observed in 33 children, and the remaining 9 were given laser therapies after unsatisfactory results of one-month topical treatment.

Discontinuing systemic therapy and switching to subsequent topical treatment should be done carefully taking into account the severity of haemangioma, the presence of a deep component and the systemic effects of treatment with propranolol. Timolol maleate does not penetrate deeply enough to affect deep vessels and prevent recurrence of deep haemangiomas, but it can affect superficial vessels. Timolol maleate can also be used as an alternative to propranolol in case of side effects that require discontinuation of the systemic treatment.

The dosing of timolol maleate is not standardized globally. This is due to its relatively recent introduction into practice and the lack of registration of the product with an indication for the treatment of haemangiomas. The frequency of administration and dose vary in different studies and reports from clinical practice. Most often the treatment is administered once or twice a day, 1-2 drops, with or without occlusion. Timolol maleate was considered relatively safe for infants born around term at a dose lower than 0.2 mg/kg/day in one study,

but other authors advised treatment to adhere to two drops daily. However, the amount of timolol may vary with squeezing of the drop container although it is assumed that one drop of 0,5 % solution contains 0,25 mg of timolol. A 2017 study included five volunteers who squeezed one drop of timolol solution from 8 drop containers, repeated three times. A statistically significant difference in the amount of timolol was observed between the individual participants, as well as in the same participant when repeating the experiment three times.

6.4. Study on the efficacy and safety of timolol maleate 1% emulgel

The lack of a preparation containing timolol maleate, specifically developed and approved for skin use, requires the use of preparations for ocular use which are not always an appropriate form for skin administration with optimal absorption and stable excretion of the drug. In this pilot study, a newly formulated drug containing timolol maleate was used at a concentration of 1% with an emulgel base. The study was open, non-comparative and non-randomized to assess the efficacy and safety profile and to determine the parameters for subsequent studies.

Emulgel is a combination of an emulsion (oil/water or water/oil) with a gelling agent. It is thixotropic, easy to apply, spreads easily, has a longer shelf life and is more pleasant to apply for patients. The emulgel has better stability than most other skin formulations (cream, ointment and powder) and provides a gradually controlled release of the drug, which improves exposure, especially in drugs with shorter half-life and a narrow therapeutic window. Irritant and allergic reactions can be observed as side effects of the base.

Our study showed extremely good tolerability, efficacy and safety with administration of timolol maleate 1% emulgel, and the results were comparable to the results when timolol maleate was administered as drops for ocular administration. No side effects were observed in any of the children despite the higher concentration. This may be due to the more gradual release of the active medicinal substance when using the emulgel as a base. However, the study did not include plasma levels or urine levels of timolol maleate. A limitation of the study is the small number of patients who took part, but the preparation shows satisfactory results for planning future studies.

Based on the results of our studies and the clinical experience with patients followed up for treatment of haemangiomas of varying clinical severity and at different stages of development, as well as in accordance with literature data, we formulated criteria for the administration of timolol maleate in haemangiomas in nursing and childhood (*Table 9*).

PLACE OF TIMOLOL IN THE MANAGEMENT OF INFANTILE HAEMANGIOMAS	
METHOD OF ADMINISTRATION	*0.25% gel/drops* - two to four times a day starting once a day and gradually increasing the dose *0.5% drops* – two to three times a day starting once a day and gradually increasing the dose* *1% cream* - two to three times a day starting once a day and gradually increasing the dose
INDICATIONS FOR ADMINISTRATION	- First line of treatment for small haemangiomas in cosmetically sensitive areas and haemangiomas at risk for ulceration, which are not indicated for systemic treatment. - Continuation of treatment after systemic therapy. - Together with systemic propranolol with expected synergistic action and shortening of treatment. - Treatment of large haemangiomas in the reluctance of parents or contraindications to systemic treatment.
Tracking	***In small haemangiomas in the proliferation stage:*** - At 2nd, 4th and 6th weeks to assess the effectiveness and need for systemic treatment - Every 2 months thereafter, to assess the effectiveness of the treatment ***In small haemangiomas in the involution phase:*** - Week 4 for performance assessment

	- Every 2 months thereafter, to assess the effectiveness of the treatment ***In large haemangiomas in the proliferation phase:*** - At 2nd, 4th and 6th weeks to assess the effectiveness and need for systemic treatment - Every 4 weeks thereafter, to assess the effectiveness of the treatment ***In large haemangiomas in the involution phase:*** - At Week 4 to assess the effectiveness and need for systemic treatment - Every 2 months thereafter, to assess the effectiveness of the treatment ***As a continuation of treatment with systemic propranolol:*** - At Week 2, 4 and Week 6 to evaluate effectiveness and probable relapse - Every 2 months thereafter, to assess the effectiveness of the treatment
DURATION OF TREATMENT	6-12 months depending on the response
ADDITIONAL RECOMMENDATIONS	**if necessary, it may last longer, but minimal benefit is expected in the course of the expected spontaneous resolution) ✓ *Discontinuation of treatment during a febrile episode or systemic disease in the active phase* (infectious or inflammatory).

	✓ *In premature children and children weighing less than 2500g at birth, at the start during the course of the treatment, close monitoring of blood pressure and heart rate,body temperature and signs of hypo dlycemia is recommended*. ✓ In the presence of a family history of psoriasis, assess the benefit-risk balance when applying topical timolol.

Table 9. Place of timolol in the management of infantile haemangiomas: indications, follow-up and duration of treatment

*Each drop of timolol 0.5% (0·05 mL) contains 0.25 mg timolol

6.5. Study to assess parents' perception of the psycho-social impact of infantile haemangiomas on the quality of life of the family

The impact of medical conditions and diseases on the quality of life has been the theme of many studies in recent years, as psycho-social effects in patients in every section of medicine are increasingly recognized. For dermatological diseases, the psycho-social burden is higher due to the visibility and the exposure in society. A. Y. Finlay and G. K. Khan established in 1994 a Quality of Life Index in Dermatology (DLQI), which is a quantitative, easily applicable, practical method of accounting for the impact of different dermatoses on the quality of life of patients in the past week. DLQI is convenient for both clinical practice and research but takes into account only the impact of the disease on the patient themself and is not applicable to assess the impact of the disease on other family members. The World Health Organization has been using the burden of disease concept since 1990 to globally collect and evaluate health information.

Infantile haemangiomas have a long-term effect on the life of the family. In the early stages of development, parents have to deal with questions about guilt and worry, whether treatment will be necessary and what will be the residual cosmetic defects when the child grows up. The decision to initiate systemic treatment with potentially serious side effects, the burden of follow-ups and the frequent visits to the doctor, as well as the associated cost often bother parents of children who require treatment. Residual cosmetic defects and

deformities have an adverse psychological impacton both parents and adolescent children. However, data in the literature to assess these effects is limited.

J. L. Tanner et al. conducted interviews with parents of 25 children aged 8 months to 5 years with haemangiomas of the face of more than 1 cm. The interview was organized in four separate directions: emotions and adaptation of parents, experience in social interactions, problems in child-parent interaction and satisfaction/dissatisfaction with treatment. The results showed feelings of denial, fear, stigma and lack of acceptance from other family members.

To better assess the overall impact of haemangiomas on the family, including psycho-social influence on individual family members, two main tools were created and validated: the Haemangioma Family Burden and the Quality-of-Life Instrument for Infantile Haemangiomas.

Haemangioma Family Burden (HFB) is intended to be filled in by one of the parents and presents the overall impact on quality of life in families where there are children with haemangiomas. The questionnaire covers six different domains characterizing psycho-social functions of everyday life: family life, relationships and work, emotions and feelings, psychology, treatment and general response (psychological effect, sexuality, financial influence). An international study in 639 children and their families showed that the degree of impact and burden to the family increased with an increase in the severity of haemangioma ($p < 0.001$) and for lesions in the head and neck area and in visceral haemangiomas.

Our study involved 48 families and the results obtained fully support the concept of a significant impact of haemangiomas on the psycho-social functions of the daily lives of patients and their parents. The overall score is statistically significantly higher in families whose children have haemangiomas in the head and neck area (visible to others) as compared to families whose children have haemangiomas in the area of the limbs and body. Statistically significant difference is also observed depending on the size of the haemangioma. Although the severity is not comparable to the severity of atopic dermatitis, the presence of haemangioma in the visible areas of the body definitely changes the quality of life of the family.

VII. CONCLUSIONS

1. In the Bulgarian population, as risk factors for the development of haemangiomas are identified: female sex, prematurity, low body weight at birth, problems during pregnancy and placental abnormalities.
2. In the Bulgarian population we did not find an association between haemangiomas and the use of methods of assisted reproduction, diagnostic procedures during pregnancy (amniocentesis and chorion biopsy), intake of tocolytics, mother's disease, smoking, multiple pregnancy and method of delivery.
3. Topical treatment of haemangiomas with timolol maleate is effective in reducing the size of the haemangioma and accelerating the involution. With active treatment with timolol, the risk of a pronounced cosmetic defect and residual changes, as well as the risk of ulceration and secondary infection, is reduced.
4. Topical treatment with timolol maleate, following systemic treatment with propranolol is an effective method of lowering the risk of rebound after systemic treatment discontinuation.
5. Timolol maleate has a good safety profile with a minimal risk of side effects, mainly in premature infants and children with low body weight at birth and when initiating treatment.
6. In children with underlying cardiovascular problems and conditions associated with bronchospasm, as well as in premature infants and those with low body weight, timolol maleate should be up-titrated gradually and under close monitoring, due to the likelihood of significant systemic absorption.
7. Haemangiomas in children, and in particular those of large size and located in the head and neck area, have an effect on the psycho-social functions of everyday life in patients and their parents.

VIII. CONTRIBUTIONS OF THE DISSERTATION WORK

1. Original

1.1. For the first time in the Republic of Bulgaria, the frequency of prenatal and perinatal etiological factors for the development of infantile haemangiomas was assessed for the Bulgarian population.

1.2. For the first time in the Republic of Bulgaria was introduced an innovative therapy for topical treatment of infantile haemangiomas with timolol maleate with a study on the efficacy and safety of this treatment.

1.3. For the first time in the Republic of Bulgaria was proposed a regimen for topical treatment of haemangiomas and the place of topical timolol maleate was determined in the haemangioma management ladder.

1.4. For the first time in the Republic of Bulgaria, the burden of infantile haemangiomas on the quality of life of the family and the impact on parents was determined.

1.5. For the first time in the Republic of Bulgaria, a newly formulated preparation for topical treatment of infantile haemangiomas – timolol 1% emulgel, was introduced.

2. Scientific and theoretical

2.1. A scale has been established to assess the severity of the haemangioma, to determine the choice of treatment and to track the efficacy of the chosen method and the need to change treatment.

2.2 For the first time in the Republic of Bulgaria, a form for standardized registration of children with infantile haemangiomas was introduced, which will facilitate the creation of a database for the Republic of Bulgaria.

3. Scientific, practical and confirmatory

3.1. A diagnostic algorithm for behavior in different types of haemangiomas relative to size, number and anatomical localization has been formulated

3.2. On the basis of own studies, an analysis of the factors associated with the development of infantile haemangiomas was carried out and the data was compared with the data from the literature.

3.3. On the basis of own studies, the favorable profile of efficacy and safety of topically administered timolol maleate in children with haemangiomas has been confirmed.

3.4. On the basis of its own studies, it has been confirmed, in line with the global trend, that infantile haemangiomas have a significant impact on the life of the family and on the psycho-social functions of the parents.

IX. SCIENTIFIC PAPERS AND COMMUNICATIONS RELATED TO THE DISSERTATION

I. Papers in international jounals

1. **Semkova K,** Kazandjieva J. Reaching a consensus on scoring instruments for infantile haemangioma: are we there yet? Int J Dermatol. 2016; 55: e417-418.
2. **Semkova K,** Kazandjieva J, Kadurina M, Tsankov N. Haemangioma Activity and Severity Index (HASI), an instrument for evaluating infantile haemangioma: development and preliminary validation. Int J Dermatol. 2015; 54(4): 494-498.
3. Semkova K, Kazandjieva J. Topical timolol maleate for treatment of infantile haemangiomas: preliminary results of a prospective study. Clin Exp Dermatol. 2013; 38: 143-146.

II. Papers in Bulgarian journals

1. Казанджиева Ж, **Семкова К,** Пилософ В, Переновска П, Масларска М, Матеев Г, Цанков Н. Хемангиоми в кърмаческа и ранна детска възраст – диагностика и лечение. (Консенсус на българската експертна група за лечение на хемангиоми). Дерматология и венерология. 2017; 2: 32-46.

III. Presentations

1. **Semkova K,** Gergovska M, Kazandjieva J. Haemangioma Activity and severity index. World Congress of Dermatology, Vancouver, Canada, 2015
2. **Semkova K,** Gergovska M, Kazandjieva J. Topical beta-blockers for large infantile haemangiomas as an alternative to systemic treatment. 10th EADV Spring Symposium, Cracow, 2013
3. **Semkova K,** Kazandjieva J. Development and preliminary validation of an outcome instrument for infantile haemangiomas – the Haemangioma Activity and Severity Index (HASI). 21st EADV Congress, Prague, 2012
4. **Semkova K,** Kazandjieva J. Timolol maleate for infantile haemangiomas: preliminary results of an open label, prospective study. 19th International Workshop on Vascular anomalies, Malmo, Sweden, 2012

5. Kadurina M, **Semkova K,** Shef A, Kazandjieva J. Treatment of uncomplicated infantile haemangiomas - topical beta-blockers, lasers or a wait-and-see approach. What works best? 9th EADV Spring Symposium, Verona, 2012
6. **Semkova K,** Kazandjieva J. Topical treatment of infantile haemangiomas with the beta-blocker timolol maleate. Report of three cases. AAD 70th annual meeting, San Diego, 2012
7. **Semkova K,** Kazandjieva J. Treatment of infantile haemangiomas with topical timolol gel. Junior Member Session, 20th EADV Congress, Lisbon, 2011.
8. **Semkova K,** Kazandjieva J. Topical timolol for treatment of infantile haemangiomas: a prospective study. 26th BSPD Annual Symposium, Nottingham, UK, 2011
9. **Semkova K,** Kazandjieva J, Marina S. Topical treatment of Infantile haemangiomas with timolol maleate. Sofia Dermatological Days, 2011
10. **Semkova K,** Nikolova A, Kazandjieva J. Propranolol for treatment of infantile haemangiomas. 12th National Congress for GPs and pediatricians, 2011.