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PREVALENCE AND CLINICAL - GENETIC FEATURES OF CONNECTIVE TISSUE DYSPLASIA IN THE UZBEK POPULATION

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ABSTRACT

Connective tissue dysplasia is an issue that requires the attention of specialists from various disciplines (paediatricians, internists, rheumatologists, cardiologists, traumatologists and orthopaedists, gastroenterologists, neurologists, clinical geneticists, as well as general practitioners). The morphological changes observed in the clinical examination of patients are characterised by a variety of clinical manifestations: from benign subclinical forms to multi-organ and multi-system pathologies with a progressive course, based on defects in collagen structure, which lead to changes in fibrous structures and connective tissue basic substance, causing structural and functional disorders of all organs. The genetic aspect of the disease is of particular relevance, in order to determine the burden of this pathology, especially in the Uzbek population. It should be noted that such a problem in the Uzbek population has been insufficiently studied, and the available data are fragmented and irrelevant.

INTRODUCTION

Connective tissue dysplasia is a hereditary connective tissue disorder of a multifactorial nature, united into different syndromes and phenotypes by the commonality of external and visceral features. According to recent research, CTD is not a syndrome or disease, but a pathological condition caused by a genetic disorder of connective tissue formation during the embryonic or postnatal periods. In total, it significantly reduces patients' quality of life and affects the course of other pathologies, resulting in an unfavourable prognosis for patients.

Mutations in genes responsible for the synthesis and formation of the spatial structure of collagen and the formation of intercellular matrix components, or enzyme genes that take part in processes of fibroblogenesis, play a leading role in the development of CTD. A large group of CTD genes has now been deciphered. Most of them are mainly monogenes and involve mutations in the genes responsible for the synthesis of extracellular matrix proteins (collagens of various types, fibrillin, tenascin), growth factor receptor genes, particularly fibroblast growth factor (TGF-β) and MMP. Based on known monogenic defects of the extracellular matrix, these pathological changes could be inherited predominantly with autosomal dominant or autosomal recessive types of inheritance.

The purpose of the study was to assess the incidence and clinical and diagnostic aspects of connective tissue dysplasia in the Uzbek population in order to optimise early diagnosis of the disease.

MATERIALS AND METHODS

A total of 221 persons with signs of CTD, including 90 (40.7%) male and 131 (59.3%) female, aged 18 to 44 years, were included in a population-based study. Diagnosis was based on T.I. Kadurina classification. The control for the compared data was 40 relatively healthy subjects (20.1±1.3), who gave informative verbal consent. The group of examination did not include persons with concomitant pathology as the result of careful study of anamnesis and instrumental examination, in particular persons with cardiovascular diseases, rheumatism, chronic liver, kidney, lung pathologies.

Blood was taken from the ulnar vein in the morning on an empty stomach for serum isolation and Mg+2 ions (in mmol/L) were determined on AF-610-A atomic absorption spectrophotometer (LTD, China), nitric oxide concentration (μmol/L) was measured for the main stable metabolites -NO2-and -NO3-. Endothelial and inducible NO synthase (eNOS and iNOS), peroxynitrite (ONO2-), endothelin-1 (ET-1), vascular endothelial growth factor (VEGF) and its receptors - VEGFR-2 were detected using automatic universal reader with AT-858 enzyme immunoassay

analyzer (LTD, China). Standard ELISA solid state ELISA kits from Human (Austria) were used as well as HLA titers, class II typing was conducted using the DNA amplification method and COL1A1 and MMP12 genes were identified by PCR.

The obtained data were statistically processed on Microsoft Windows using Microsoft Excel-2013 and Statistica 8 software packages. The data are presented as M±m. Significance of differences was determined by Student's t-criterion and considered significant at P‹0.05.

RESULTS AND DISCUSSION

All clinical trials were conducted during the period 2019-2022 on the basis of Samarkand City Medical Association, Samarkand State Medical University Multiprofile Hospital and Sharof Rashidov District Medical Association of Jizzakh region. A total of 221 patients were under observation, in particular 90 (40.7%) male and 131 (59.3%) female, aged under 20 years about 6.8%, 19-32 years - 63.8%, 33 and above - 29.4%, respectively.

In Samarkand and Jizzakh regions we surveyed 2355 local residents, among whom 221 patients with CTD signs were identified. An analysis of the incidence of CTD in patients in Samarkand and Jizzakh showed no significant difference, with an average incidence of about 9% in both regions, particularly 9.9% in Samarkand and 8.8% in Jizzakh, respectively (Figure 1).

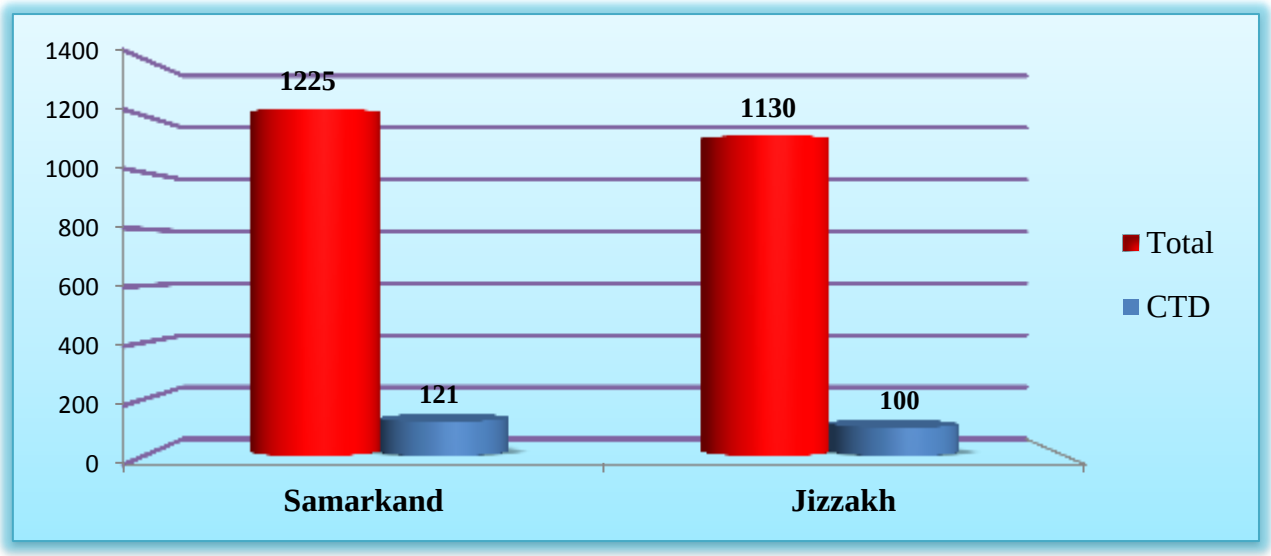

Figure 1. Incidence of CTD in the mentioned regions.

Patients were divided into 3 groups according to the severity of the disease:

Group 1 - mild course 96 patients (up to 3 signs of CTD)

Group 2 - moderate course 90 patients (up to 4-5 signs of CTD)

Group 3 - ыevere course 35 patients (more than 6 signs of CTD).

The distribution of patients according to age and sex is shown in Table 1.

Table 1. Distribution of CTD patients by age and sex

Age, years	**Women**		**Men**		**χ2**	**P**
	abs.	%	abs.	%		
Up to 18	9	4,1	6	2,7	0,48	>0,05
19-32	82	37,1	58	26,2	0,04	>0,05
Over 33	40	18,1	26	11,8	0,11	>0,05
Total	131	59,3	90	40,7	2,30	>0,05

The study showed that the gender distribution of male and female patients was approximately the same, with only a slight predominance of women. We also found that the disease was more prevalent in the 19-32 age group (63.8%).

Weight and height parameters and BMI differed in the studied groups of patients, as their weight, height depended on the age of the patients. Anthropometric and phenotypic characteristics of the studied patients showed an average chest circumference of 85.46±7.25 cm, epigastric angle (in degrees) of 86.39±8.54, foot length as a function of height of 0.149±0.08, foot arch height of 7.65±1.15 cm, respectively.

We were interested in examining the frequency of external and internal phenes in the patients of the compared groups. The incidence of musculoskeletal phenes is presented in Table 2.

Table 2. Incidence of musculoskeletal phenes

Phenes		1st group, n=96	2nd group, n=90	3rd group, n=35
Scoliosis (grades I and II)		39,6%	32,2%	34,3%
Kyphoscoliosis (grades I and II)		1,04%	11,11%	31,4%
Hyperlordosis		0%	8,89%	14,3%
Hypermobility of the joints	I	57,3%	48,9%	51,4%
	II	15,6%	22,2%	20%
	III	14,6%	21,1%	17,2%
	IV	3,13%	7,8%	11,4%
Flat feet	∑ of groups	17,65%	22,2%	22,9%
	Transverse	6,25%	6,6%	8,6%
	Longitudinal	11,4%,	15,6%	14,3%
Chest deformities		28,13%	31,1%	48,6%

One of the main manifestations of CTD are spinal deformities. Analysis of the incidence of bone and skeletal phenes in comparison groups revealed the formation of scoliosis of 1st and 2nd degree in Group 1 and 2 in 39.6% and 32.2%, kyphoscoliosis of 1st and 2nd degree in 1.04% and 11.11%, and hyperlordosis in 0% and 8.89% of patients, respectively. Patients of group 3 had scoliosis of 1st and 2nd degree in 34.29% cases, kyphoscoliosis of 1st and 2nd degree - in 31.42%, and hyperlordosis - in 14.3%. As can be seen from the above data, spinal deformities in the form of scoliosis of grade 1 and 2 were more frequent in group 1, in contrast complex deformities were detected in group 3, indicating a severe course of the disease.

Joint changes in people with CTD are manifested by flat feet and hypermobility.

Hypermobility of varying severity was detected in all examined patients of the second and third groups, and only 1/10 of patients in the first group did not have this pathology. Thus, if hypermobility of 1st, 2nd, 3rd and 4th degree joints was detected in 57.3%, 15.6%, 14.6% and 3.13% of the patients in Group 1, in Group 2 patients - 48.9%, 22.2%, 21.1% and 7.8%, and in Group 3 patients - 51.4%, 20%, 17.4% and 11.4% of the examined patients. It is remarkable that all three groups demonstrated a similar decreasing trend in the proportion of patients according to the severity of this phen. Also noteworthy is the relatively narrow distribution of the number of patients with the second and third degrees of hypermobility, which may require revision of the criteria for differentiating the stages of hypermobility.

According to the data, the incidence of flat feet in groups 1, 2 and 3 was 17.1%, 22.2%, 22.9%, respectively. At the same time transverse flatfoot was detected in 6.25%, 6.6% and 8.6% of patients, longitudinal flatfoot in 11.4%, 15.6% and 14.3% of the examined persons. The differences between the groups were statistically insignificant. Analysis of the incidence of flat feet showed no significant difference in the comparison groups.

An analysis of the frequency of chest deformities in Group 1 patients showed their presence in 28.13% of cases. In group 2, 31.1% of patients had this group of pathological changes, while in group 3, 48.6% of patients suffered from chest deformities, which was statistically significantly higher than in group 1 ($P<0.05$). Assessment of the thorax showed that keel-shaped (pectus carinatum) and funnel-shaped (pectus excavatum) forms of the thorax deformity were more common among the examined patients, with no significant difference in the groups.

Based on the above data, it can be assumed that patients with CTD have different clinical variants of musculoskeletal lesions, which in turn complicate the diagnosis and choice of treatment tactics for general practitioners and therapists.

Analysis of the occurrence of internal phenes in patients with CTD showed that ocular manifestations of the disease were characterised by the development of

various degrees of myopia, which occurred in almost 1/3 of patients in all compared groups. However, the degree of myopia clearly coincided with the severity of the disease. Thus, the first degree of myopia was detected in 23 (23.96%) group 1 patients and the second degree in 3 (3.1%) patients. Astigmatism, anisometropia and retinal degeneration were not detected in the patients of this group. Grade 1 myopia was found in 14.5% of group 2 patients ($P<0.01$), grade 2 - in 15.6% ($P<0.01$). However, in Group 3, 1st degree of myopia were observed in significantly smaller number of patients than in the previous two groups (2.8%) while 2nd grade of this pathology - in 20%. Patients in this group were characterised by more severe and persistent pathological eye changes in combination with moderate to severe myopia - astigmatism (31.4%) and retinal degenerative changes (28.6%). Ocular manifestations in the form of myopia of various degrees and astigmatism tended to develop in group 2 patients, while more profound disturbances were common in group 3 patients.

The frequent occurrence of internal phenotypes in our study subjects, depending on the severity of the disease, was also confirmed by the presence of concomitant diseases or comorbidities. Thus, chronic bronchitis was detected in 5.2%, 6.7% and 11.4% of patients in groups 1, 2 and 3, pyelonephritis - in 6.25%, 7.8% and 14.3%, biliary dyskinesia - in 18.75%, 22.2% and 31.4%, nephroptosis of both kidneys of 1st and 2nd degree - in 6.3%, 12.2% and 17.2%, vegetative vascular dystonia - in 49%, 62.2% and 74.3%, respectively.

Of particular note are gastroduodenal pathology (5%), liver pathology (1.8%), lumbar spinal osteochondrosis (4.1%), anaemia (9.5%), osteoarthritis (6.8%) and others (n=221).

The predominance of internal phenes in group 3 patients was confirmed by the statistically significant occurrence of comorbidities in group 3 patients compared to group 1-2.

Thus, analyzing the clinical manifestations of CTD, we can assume that the external phenes of CTD were characterized by small anomalies, skeletal, skin and joint forms, whereas the internal phenes were represented by visual disturbances, abnormalities of the cardiovascular and pulmonary systems, abdominal and kidney organs, and especially the autonomic nervous system.

One of the main causes of deepening pathological changes in connective tissue dysplasia is disturbances in its morphological structure represented by the extracellular matrix, collagen and elastin. Leading world scientists have recently emphasized the relevance of studying the regulation of extracellular matrix fibrillar proteins, i.e. magnesium ions. Mg^{+2} deficiency contributes to the disturbance of joints, bones, cardiovascular system and heart valve apparatus, increases the frequency of myxomatous degeneration of prolapsing mitral valve leaflets and

heart rhythm disturbances (16). In this regard, we also investigated the serum magnesium, oxyproline, levels in patients with CTD. The studies carried out on this subject revealed a tendency for a decrease in blood magnesium levels, we detected a significant decrease in magnesium content in patients with comorbidities, in particular in the combination of CTD. In this group of patients its level in blood serum decreased by 1.2 times (P<0.001) relative to values of practically healthy persons (Table 3).

Table 3. Serum Mg+2 ion, glucosaminoglycan, hyaluronidase levels and oxyproline excretion in CTD patients, M±m

Indicators	Control, n=20	1st group, n=96	2nd group, n=90	3rd group, n=35
Mg^{+2}, mol/l	0,912±0,022	0,902±0,022	0,759±0,038*^	0,623±0,038*^
Glucosaminoglycan, µmol/l	4,861±0,098	5,079±0,040	5,323±0,095*^	5,452±0,066*^
Hyaluronidase, µmol/l	203,50±2,04	211,00±7,60*	222,30±5,89*^	231,41±6,21*^
Total oxyproline, µmol/l	21,79±0,55	25,03±0,66*	27,09±0,42*^	29,02±0,52*^

* - p<0,05 compared to the control group,
^- p<0,05 compared to the patient group

According to the data of Table 3, low level of magnesium leads to pathology of endothelium, disorders of volumetric organization of collagen and elastin, which cause abnormal formation of extracellular matrix components. Thus, it is possible to suppose that low level of magnesium in group 3 patients is one of the triggering mechanisms of collagen formation, if one takes into account that low level of magnesium leads to endothelium damage, disorders of elastin and collagen organization responsible for the formation of extracellular matrix components, as well as enzymes participating in the process of fibrillogenesis. Low level of magnesium in patients with CTD could be one of the definite factors of the pathology clinical progression. It is also worth mentioning the increased activity of proteolytic enzymes on the background of decreased Mg+2 ions in the blood, especially the excretion of total oxyproline significantly increased in subjects with CTD by 14.9% in group 1, by 24.3% in group 2 and by 33.2% in group 3 compared with the values in virtually healthy subjects.

One of the difficult issues of therapy is the assessment of the severity of CTD. Many authors attribute this to the variety of clinical manifestations of CTD, due to the involvement of various organs and systems, especially the cardiovascular system, in the pathological process. A number of authors point to the primary role of endothelial dysfunction in the progression of CTD. The authors believe that this is due to an imbalance in the polymorphism of extracellular matrix protein genes. There are also opinions that endothelial dysfunction is caused by impaired local production of nitric oxide by endotheliocytes as well as intensification of oxidative stress (3,8). A characteristic feature of endothelial dysfunction is impaired microcirculation, hypoxia, reperfusion, and the consequent activation of vasculogenesis, angiogenesis and vasoactive substance formation. Vascular endothelial growth factors play an important role in this process (Vascular endothelial growth factor, VEGF) (15,16). A decrease of VEGF in cells leads to an activation of endothelial apoptosis. This process in turn causes lumen obstruction and regression of vascular growth. In view of the above, we studied the content of pro- and anti-angiogenic factors in the serum of patients with DCF depending on the degree of cardiac valve regurgitation.

Studies carried out in this regard showed an increase in serum VEGF content in patients. Thus, the content of this factor in patients of Group I increased by 1.1 times, in patients of Group II - by 1.27 (P<0.001) times, and in Group III - 1.38 times, indicating the activation of vascularization processes. This is confirmed by the increased concentration of its receptors in the serum of the examined patients. Thus, the content of VEGF-R1 increased in 1.2 (P>0.05), 1.42 (P>0.05) and 1.59 (P<0.01) times, and VEGF-R2 increased in 1.08, 1.18 and 1.24 (P<0.05) times, respectively to 1st, 2nd and 3rd group (Table 4).

Table 4. Concentration of pro- and anti-angiogenic factors in serum of patients with CTD, M±m

№	1st group, n=96	2nd group, n=90	3rd group, n=35	Control, n=20
VEGF, ng/ml	151,92±2,58***	162,11±2,51***	176,13±2,98***^^^	138,58±1,69
VEGF-R1, ng/ml	0,610±0,055	0,700±0,049	0,799±0,038***^^	0,502±0,028
VEGF-R2, ng/ml	4,20±0,12	4,55±0,21	4,80±0,34*	3,879±0,265

Note: * - differences relative to the control group are significant (* - P<0,05, *** - P<0,001); ^ - differences relative to the group of Grade I patients are significant (^^ - P<0,01, ^^^ - P<0,001)

However, we identified some distinctive features of changes in these receptors: changes in VEGF-R1 levels were more pronounced. This is probably due to the specificity of the effect of these receptors on growth factors. For example, VEGF-R1 mainly binds to both VEGF and placental growth factor PIGF, while VEGF-R2 binds only to VEGF. Whereas VEGF-R1, after binding to VEGF, inhibits angiogenesis, VEGF-R2 accelerates this process and the inducer of this process is HIV1 Tat.

One of the conditions for angiogenesis is an increase of endothelial permeability. The vascular endothelium provides barrier, secretory, haemostatic and vasotonic functions. It plays an important role in inflammatory reactions and vascular wall remodeling. The increase in its permeability is mainly attributed to the effect of nitric oxide, which is synthesized by endothelium under the action of specific NO synthases: eNOS and iNOS. NO synthesized by endothelial cells and released into the bloodstream acts as a vasodilator regulating blood rheological properties and arterial pressure. In view of the above, we investigated some parameters of NO system in patients with MVP (Mitral valve prolapse) with different degrees of regurgitation. The findings showed an increase of stable nitric oxide metabolites by 1.12 ($P>0.05$), 1.34 ($P<0.001$) and 1.41 times in Group 1, Group 2 and Group 3, respectively. The detected changes are probably a compensatory response of the organism of patients to the presence of certain chronic myocardial ischemia in this group of patients. This coincides with an increase in serum VEGF levels in patients, leading to some myocardial vascularization.

Thus, patients could have endothelial dysfunction due to an imbalance in the NO system. The imbalance in the NO system is due to overexpression of iNOS and accumulation of ONO2-, inhibition of eNOS activity.

An individual's genetic predisposition to and resistance to collagen formation disorders depends on the diversity of the major histocompatibility complex (HLA) genes and the polymorphism of the Col1A_1 and MMP12 genes. The Col1A_1 gene codes for the α1-chain of collagen type I, which is responsible for strengthening and maintaining many body tissues including cartilage, bone, tendons, skin and sclera. Type I collagen is the most abundant form of collagen in the human body. MMP12 is the gene that codes for the protein MMP12 (Macrophage Metal Elastase - in the human genome, the gene is located on the short arm of chromosome 11). MMP12 substrate proteins include elastin (connective tissue protein) as well as a range of intercellular matrix proteins, including type IV collagen of the major histocompatibility complex (HLA). In our study of the occurrence of Col1A_1 and MMP12 gene polymorphisms in individuals from the Uzbek population, the CC allele of the Col1A_1 gene and the AA allele of the MMP12 gene were identified in the control group (figure 2).

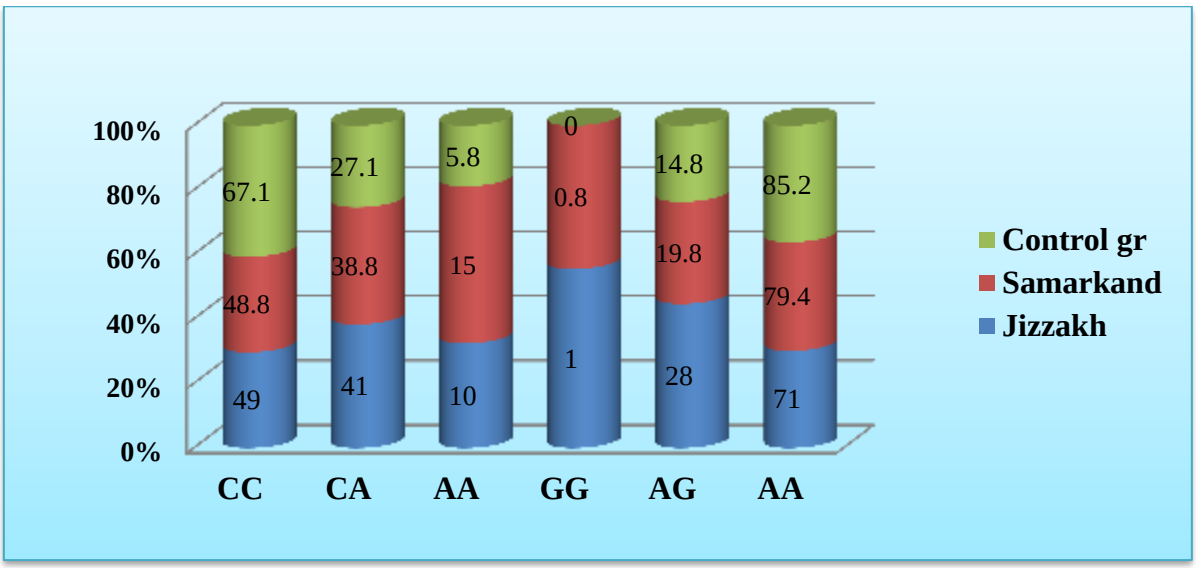

Figure. 2. The incidence of Col1A_1 and MMP12 polymorphisms in the Uzbek population among studied groups

The present study showed that the Col1A_1 genotype in CTD patients is represented predominantly by the A allele (84.16%), equally as a homo- and heterozygous phenotype. The AA genotype of the Col1A_1 gene was associated with an expected risk (ER) of severe CTD (ER - 8.3, $p<0.001$) (Table 2).

The MMP12 genotype is represented predominantly by the A allele, more in the form of a homozygous genotype. No differences were observed in the distribution of patients according to the severity of CTD among those with different MMR12 genotypes.

The distribution of patients according to Col1A_1 genotype (AA - 43.44%; AC - 40.72%, CC - 15.84%) revealed significant differences between the groups in the frequency of various degrees of CTD ($x2=70.20$, $p<0.001$, Table 2). Comparison of patients with homozygous and heterozygous genotypes demonstrated differences between AA and AC genotypes (×2 2×3=36.56, $p<0.001$) and no differences between CC and AC genotypes (×2 2×3=4.02).

It has been discovered that there is a statistically significant increase in the frequency of HLA class II genes, in particular in the first and second lines of consanguinity, among patients with CTD. The frequency of class II HLA phenotypes in a sample of patients with CTD and a control group was analyzed. It was found that there was a statistically significant increase in the frequency of class II HLA genes in patients with CTD. Allele 0501 of DQA1 gene was mostly observed in patients with signs of CTD. Interpretation of DQB1 HLA class II gene results in patients with CTD showed that allele 0201 was common. Consequently, it can be hypothesised that all early diagnostic methods in patients who present with signs of CTD should be performed to further prevent possible.

Thus, a positive association with higher RR values of the DQA1, DQB1 and DRB1 genes was observed in CTD. Studies have demonstrated that associations of these genes were detected more frequently in patients with spinal deformities, myopia, flat feet, MVP and myxomatous MV degeneration. This, in turn, suggests that early diagnosis and prevention of the manifestation of possible complications can be achieved by performing these tests.

We analysed the heritable manifestation of patients with CTD depending on the severity of the disease, where we studied the genealogical tree of patients according to the incidence of the signs of the disease. The study of probands revealed the dependence of genetic factors in the formation of CTD. Thus, if in the 1st group of patients the frequency of incidence of CTD signs in the 1st, 2nd and 3rd lineage was revealed in 11 (11.45%), 9 (9.4%) and 8 (8.3%) patients out of 96 patients, then in Group 2 they were detected in 13 (14.4%, $P<0.01$), 14 (15.6%, $P<0.05$) and 11 (12.2%, $P<0.01$) of 90 patients, while in Group 3 - in 12 (34.3%, $P<0.01$), 7 (20%, $P<0.05$) and 5 (14.3%, $P<0.01$) of 35 subjects.

CONCLUSION

The study of magnesium concentration revealed a tendency for its decrease in patients with CTD in comparison groups, as well as increased proteolytic enzymes activity and excretion of total oxyproline, which might indicate a high level of CT structural degeneration. Endothelial dysfunction and stimulation of angiogenesis are the morphological substrate of connective tissue disorganization progression in patients. The degree of severity of clinical symptoms of CTD was related to the frequency and number of combinations of external phenes: joint hypermobility (96%), changes in the spine (51.6%), thorax (32.6%), flat feet (20.4%) and internal phenoms: myopia (28%), heart anomalies, ECG and EchoCG rhythm disturbances in severe forms were more frequent in group 3 patients, which was associated with connective tissue weakness. The present study demonstrated that the Col1A_1 genotype in DST patients is represented predominantly by the A allele (84.16%), equally as a homo- and heterozygous phenotype. The AA genotype of the Col1A_1 gene was associated with severe DST OR (OR - 8.3, $p<0.001$). The MMP12 genotype is represented predominantly by the A allele, more in the form of a homozygous genotype. No differences were observed in the distribution of patients according to the severity of CTD among those with different MMR12 genotypes. And also positive associations with higher RR values of DQA1, DQB1 and DRB1 genes were observed in patients with CTD. The relationship between HLA class II genes (allelic variants of the DRB1 gene *14 and/or *15, 13/14) and clinical manifestations of CTD in the form of external and internal (musculoskeletal and SSS) phenotype changes was found.

REFERENCES

1. Abduraxmanov DS, Kurbaniyazov ZB, Davlatov SS, Rakhmanov KE. Criteria for choosing surgical treatment of patients with ventral hernias and obesity. Int J Pharm Res. 2020;12:4057–4066. DOI: 10.31838/ijpr/2021.13.01.598.
2. Bozorov AG, Ikhtiyarova GA, Davlatov SS. Biochemical markers for prediction of premature labor in urogenital infections. Int J Pharm Res. 2020;12:4894–4897. DOI: 10.31838/ijpr/2021.13.01.674.
3. Davlatov SS, Khamdamov BZ, Teshaev SJ. Neuropathic form of diabetic foot syndrome: etiology, pathogenesis, classifications and treatment (literature review). J Nat Remedies. 2021;22(1-2):147–156.
4. Davlatov S, Rakhmanov K, Qurbonov N, Vafayeva I, Abduraxmanov D. Current State of The Problem Treatment of Mirizzi Syndrome (Literature Review). Int J Pharm Res. 2020;12:1931–1939. DOI: 10.31838/ijpr/2020.SP2.340.
5. Davlatov S, Teshayev Sh, Fayziev X, Khamidova N. Inguinal hernia: Modern aspects of etiopathogenesis and treatment. Int J Pharm Res. 2021;13:970–976. DOI: 10.31838/ijpr/2021.13.02.147.
6. Kudratova DS, Ikhtiyarova GA, Davlatov SS. Medical and social problems of the development of congenital malformations during a pandemic. Int J Pharm Res. 2021;13(1):756–760. DOI: 10.31838/ijpr/2021.13.01.130.
7. Ma B, Lawson AB, Liese AD, et al. Dairy, magnesium, and calcium intake in relation to insulin sensitivity: approaches to modeling a dose-dependent association. Am J Epidemiol. 2006;164(5):449–458.
8. Martinez-Selles M, Garcia-Fernandez MA, Larios E, et al. Etiology and short-term prognosis of severe mitral regurgitation. Int J Cardiovasc Imaging. 2009;25(2):121–126.
9. Shodikulova GZ, et al. Clinical and laboratory features of the course of upper gastrointestinal pathology with connective tissue dysplasia. Conference. 2022;6(6):118–119.
10. Shodikulova GZ, et al. The Correlation among Osteoporosis, Calcium-Phosphore Metabolism and Clinical Symptoms of Main Disease in Patients with Rheumatoid Arthritis. Ann Rom Soc Cell Biol. 2021:4185–4190.
11. Shodikulova GZ, Samatov DK. Peculiarities of clinical picture in patients with upper gastrointestinal pathology with connective tissue dysplasia syndrome. Front Med Sci Pharm J. 2022;2(02):43–49.
12. Ueshima K. Magnesium and ischemic heart disease: a review of epidemiological, experimental, and clinical evidences. Magnes Res. 2005;18(4):275–284.

13. Yosefy C, Ben Barak A. Floppy mitral valve/mitral valve prolapse and genetics. J Heart Valve Dis. 2007;16(6):590–595.
14. Zacchigna L, Vecchione C, Notte A, Cordenonsi M, et al. Emilin1 links TGF–beta maturation to blood pressure homeostasis. Cell. 2006;124(5):929–942.
15. Zikrievna SG, Bakhtiyarovna BZ. Occurrence of clinical options of undifferentiated connective tissue dysplasia in Uzbek population. Int J Psychosoc Rehabil. 2020;24(2):1347–1359.