„OVIDIUS" UNIVERSITY IN CONSTANTA
MEDICINE FACULTY

DOCTORAL THESIS

MORPHOLOGICAL AND ETIOPATHOGENIC CONSIDERATIONS ON THE PALMAR APONEUROSIS IN DUPUYTREN'S DISEASE

SUMMARY

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Abbreviations used in the text:

HO^-	- hydroxyl radical
H_2O_2 -	- Hydrogen Peroxide
$ONOO^-$	- Peroxynitrite
$^1O_2$ -	- singlet oxygen
ROS	- reactive oxygen species
$ROO^\cdot$	- peroxyl radical
$RO^\cdot$	- alkoxyl radical
$HOO^\cdot$	- hydroperoxyl radical
HOCl	- hypochlorous acid
MPO	- myeloperoxidase
EPO	- eosinophil peroxidase
XDH	- xanthine dehydrogenase
SOD	- superoxide dismutase
GSH-Px	- glutathione peroxidase
GSH	- glutathione
HSPs	- Heat Shock Proteins
TAS	- Total antioxidant status
IFG	- impaired fasting glucose
IGT	- impaired glucose tolerance
ALT (ALAT)	- alanine aminotransferase
TGP	- glutamic pyruvic transaminase
NH2	- amino group
VLDL	- very low density lipoprotein
LDL	- low density lipoprotein
HTA	- Arterial hypertension
ICC	- chronic heart failure
AVC	- stroke
RX	- radiography
AHC	- family history
IGT	- impaired glucose tolerance
IFG	- impaired fasting glucose
IMA	- acute myocardial infarction
$HX\text{-}Fe^{III}$	- methemoglobin
$X\text{-}[Fe^{IV}=O]$	- feril myoglobin
ABTS®	- 2,2'-Azino-di-[3- ethylbenzthiazoline sulfonic]
HE	- hematoxylin-eosin

Key words: palmar aponeurosis, retraction, oxidative stress, total antioxidant status, fibroblasts, flexion, aponevrectomy.

CHAPTER I

INTRODUCTION

The hand is not only a locomotor segment, it is a sense organ, an organ of creation, being in the highest degree, alongside with the brain, the most distinctive human segment.

The knowledge desire has been and continues to be the essential condition of human existence.

The present study is among the modern concerns of medical research on etiopathogenic aspects and physiopathological mechanisms involved in the development of Dupuytren's disease, incompletely elucidated yet.

Palmar aponeurosis has represented a study subject for researchers, anatomists, surgeons, for almost 200 years since Baron Guillaume Dupuytren in 1831 first described the disease that bears his name. Ignorance of Dupuytren's disease is rooted in not knowing etiopathogenic and physiopathological mechanisms acting both systemically and locally by structural changes in the palmar aponeurosis.

The purpose of this paper is to provide an overview on physiopathological mechanisms involving oxidative stress and morphopathological aspects leading to Dupuytren's disease outbreak.

Alongside these aspects we have also highlighted new epidemiological elements related to the incidence of this disease in Constanța County as well as the therapeutical approach of patients constituting the test group.

By this thesis I tried to summarize and update knowledge regarding this disease, which involves chronic pain of anatomical structures at the palmar aponeurosis level involving physiopathological aspects with microcirculatory effects, effects of hypoxia and overproduction of free radicals.

In selecting the cases and in order to facilitate investigation of patients with Dupuytren's disease enrolled in this study and hospitalized in the Plastic Surgery and Reconstructive Microsurgery Clinic within the Constanța Emergency Clinical County Hospital, I had the support of prof. Dr. Bordeianu Ion, head of the clinic, who guided me with great professionalism throughout this study and who I would like to thank this way.

I. GENERAL

CHAPTER II

2.7. Palmar Aponeurosis

Palmar aponeurosis represents a triangular condensation of deep fascia covering the palm and it is distally anchored to the skin.

The apex of the triangle is continued on the palmaris longus tendon, when this one exists; in the absence of palmaris longus situations, it is anchored to flexors retinaculum. From this point, the fibers radially extend to the fingers bases, then projecting to the index, middle finger, ring finger and auricular and by an underrepresented extension to the pollex.

Transverse fibers interconnect the longitudinal beams which continue to the fingers.

The vessels, nerves and long flexor tendons are located deeply to the aponeurosis at the palm level. [1]

2.7.2 Embriology of palmar aponeurosis

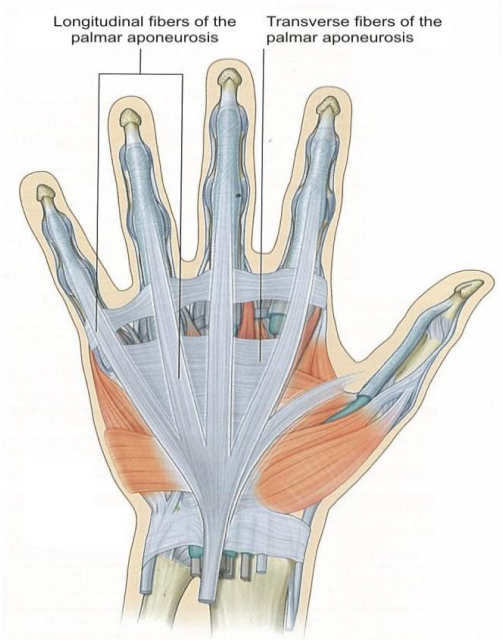

Fig. 11 Palmar aponeurosis (Richard L. Drake, A. Waine Vogl, Adam W. M. Mitchell, 2010)

Palmar aponeurosis represents that permanent structural formation of the hand, that is, ever since the amphibians, the insertion basis of short (intrinsic) muscles of the hand. Note that the palmar aponeurosis is from its beginning aponeurosis, which means it has tendon structure, in direct connection with muscle insertions and must never be confused neither structurally nor functionally with fascia.

The palmar aponeurosis is no longer just a special individualized formation, but it represents the fibrous skeleton of the hand, being the one forming the fibrous channels of palm

pulleys, fingers and lumbricals channels, as it also provides the thenar and hypothenar fiber unit. By its dynamic significance, the palmar aponeurosis is considered as a functional structure, which exceeds by far, in many authors' conception, the term usually restricted to anatomical element. This notion of integrated structure is very important because many hand surgeons, younger and some of them even older, consider that there is a separate aponeurosis from the fiber pulleys of the palm.

Palmar aponeurosis consists of transversal fibers and longitudinal fibers resulting the fibers material for building palmar septa and dermis anchoring especially of palmar creases.

CHAPTER III

ETIOPATHOGENIC ASPECTC IN DUPUYTREN'S DISEASE

3.1 Definition. Preliminary notions

Dupuytren's disease is clinically characterized by the retraction (contraction) in permanent and progressive flexion of one or more fingers due to degenerative connective phenomena prevalent at the palmar aponeurosis level and starts with the appearance of nodules and palmar longitudinal clamps. [18]

In order to understand the lesions it is necessary to recall some elements of anatomy, in particular the normal anatomy of a palmar aponeurosis.

It is known that many fibers of the palmar aponeurosis attach themselves to the deep layer of the skin, thus the retraction of the aponeurosis is accompanied by the retraction of the skin ("pits").

Longitudinal fibers of the palmar aponeurosis surround the dorsal area of the basic and medium phalanges, thus explaining the retraction with flexion of the medium phalanges on the proximals and of the latter on the metacarpals.

The circular fibers of the palmar aponeurosis cover the vessels and nerves of the hand thus explaining the retraction of the vessels that penetrate the aponeurosis and resulting in trophic disorders of the subcutaneous cellular tissue and of the teguments.

Palmar aponeurosis relationship with the skin has an anatomical and clinical significance in Dupuytren's disease: palmar skin is thicker because epidermal layers have a greater thickness, the germinative layer is more deeply folded and the blanket formed by fibrous tissue is more pronounced. The palmar creases are lines that connect the skin to the aponeurosis, mostly on the flexion area of the joints and they have a functional significance linked to the main motions. [19]

Palmar aponeurosis is divided by some authors into two components: *superficial palmar aponeurosis* and *deep palmar aponeurosis* (fibrous formations subjacent with pulleys, lumbricals channel and "phalangeal fiber comb").

This passion for classifications has sown a lot of confusion within the surgical population of the medical staff, who consider palmar aponeurosis as a separate structure, attached to palmaris longus and independent from fibrous structures specific to the hand. [7]

3.2 Etiological aspects

Dupuytren's contracture is predominant in white persons, Caucasians and is more common in northern peoples. In 60% of cases, patients' age ranges between the limits of 40 and 60 years. The youngest case met was of 11 years. [7]

The disease occurs in both hands in 40% of cases and the dominant hand does not seem more frequently affected. It may occur in all fingers, but most often the ring and auricular finger but the pollex is not excluded from the statistics. [20]

The authors assert that the disease is found equally in intellectual and manual professions and that the changes affect all the elderly and Dupuytren's disease occurs at third age. It has been found that there is no relation between the type of profession, namely normal request and the disease incidence.

3.3 Pathogenic mechanisms involved in the development of Dupuytren's disease

The hereditary transmission of Dupuytren's disease is an important link in the etiopathogenesis of the disease, leaving open the philosophical and scientific concept which includes the variety of ideas and theories to synthesize them into a unitary conception explaining the pathogenesis and pathology of the disease.

Several etiopathogenetic theories have been issued, but none of them has been completely accepted; there is, nonetheless, an agreement concerning the existence of some predisposing factors related to the disease development.

A number of patients with this disease had a positive family history (***genetic theory***), that means they had ancestors on the male line (80-90% of cases). This explains the delayed appearance of the disease among women. In this category of patients the nodules and joint deformities occur early, about 20-30 years, and they can also show plantar and penile fibrosis.

Genetic predisposition associated with other exogenous factors such as: trauma, chronic intoxications, infections, metabolic disorders, can cause an imbalance in the connective tissue through its disintegration in favor of cell proliferation, resulting in scar tissue.

Repeated microtrauma are considered essential contributing factors. The studies found that the disease is triggered by changes in blood flow and / or innervation, being considered to be a decrease in blood flow at the hand level resulting in the appearance of subcutaneous nodule.

3.5 Oxidative stress involved in Dupuytren disease

Dupuytren's disease is a family fibro proliferative disorder with late onset affecting the hands. It is extremely common in people of northern Europe. Genetic studies have not yet identified the genes involved in disease formation. Oxidative stress and production of free radicals may be important factors in the pathogenesis of Dupuytren's disease. Mitochondrial genes are also included in the control of apoptosis. The affected tissue contains a large number of MYO-fibroblasts, which disappear via apoptosis during normal wound healing. A large number of mitochondria have been observed in fibroblasts derived from the affected tissue. In the light of this evidence, the mitochondrial genome represents a potential location of the genes susceptible for this disorder with late onset. [38]

3.5.1 Preliminary notions on oxidative stress

The notion of "oxidative stress" in the biological systems dates from the early period of research in the activated oxygen domain, initially the studies being focused on oxygen and X radiation toxicity. [38] A large part of the literature on oxidative stress was revised in 1979 by Chance in an article referring to hydroperoxide metabolism in mammalians' organs [39]

The concept of **"oxidative stress"** was first developed by Helmut Sies with synonymous terms of "***oxidant stress***", " ***pro-oxidant stress*** " or "***reductive stress***" based on observations resulting from laboratory experiments conducted at the molecular level [40-42]

3.5.2 Free radicals

Free radicals occur as intermediate metabolic products, in normal life conditions, in all aerobic organisms and they do not induce pathological changes except in the situations where they cannot be controlled by specific endogenous antioxidants.

Free radicals are nothing but a manifestation of oxygen molecule duality, which on one hand is essential for maintaining cellular energetic metabolism and maintenance of tissue respiration process, but also, in certain circumstances, it may lose an unpaired electron and the form of this intermediate chemical species - free radicals [50]

The main sources of reactive oxygen species:

- Mitochondrial electron transport;
- NADPH oxidase;
- Cyclooxygenase and lipoxygenase;
- Nitric oxide synthase;
- Myeloperoxidase;
- Xanthine oxidase;
- Catecholamine autoxidation.

II. PERSONAL PART

CHAPTER V

MATERIAL AND METHOD

5.1 Selection of study groups

Determinations which are the subject of this paper were performed on a group test consisting of 35 patients consecutively enrolled between November 2009 - May 2012, aged 50 and 79 years, hospitalized in the of Plastic Surgery and Reconstructive Microsurgery Clinic within the Constanta Emergency Clinical County Hospital with the diagnosis of Dupuytren's disease and a control group consisting of 18 people apparently clinically healthy.

All patients (those that were part of the test group as well as the persons who volunteered, in order to complete control group) were informed about their enrollment within the study and gave written consent by signing the consent form wittingly expressed (Annex 1 Informed consent).

The diagnosis was based mainly on clinical examination of the hands highlighting palmar aponeurosis retraction and determining evolutionary stage.

5.1.1 Structure of test group

5.1.1.1 Distribution of patients according to gender and age group

The test group consists of 31 males (89%) and 4 females (11%).

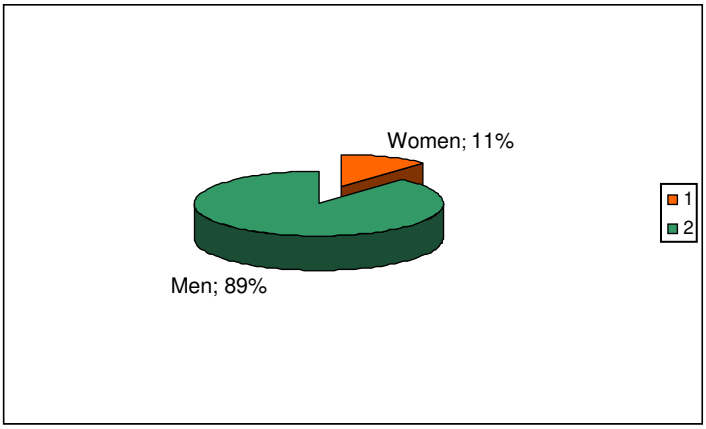

Fig. 21 Structure of test group according to gender

With regard to the distribution of test group patients by age, we considered age ranges 50-59, 60-69,70-79 and it was noted the following distribution:

Table 1 Test group structure on age groups

TEST GROUP	50-59 YEARS	60-69 YEARS	70-79 YEARS
MALE PATIENTS	13	16	2
FEMALE PATIENTS	0	4	0
TOTAL PATIENTS	13	20	2

From the chart and the table presented it results that the peak incidence - at least for patients addressing the physician - is within the age decades 50-59, 60-69 years and it should be pointed out the fact that although Dupuytren's disease can begin at younger ages, we have included in the study group only people who are more than 50 years old.

5.1.1.2 Distribution of patients according to life environment, profession and associated pathology

With regard to the life environment of group test patients, there are 29 persons from urban area and 6 persons from rural area.

With regard to Dupuytren's disease associated pathology in group test patients we distinguish: 27 persons with chronic alcoholism and chronic toxic hepatopathy (ethyl), 24 people diagnosed with chronic pancreatitis, 15 persons with essential hypertension and 20 persons with dyslipidemia.

Table 4 Distribution of group test patients according to the evolutionary stage

	Stage 2	Stage 3	Stage 3-4	Stage 4
TEST GROUP	0	15	3	17

5.1.2. Criteria for inclusion in the study group

They were not restrictive, the only condition being for patients to comply with:

- preliminary medical conditions regarding cooperation during the study (pre, intra and postoperative);
- fall within the age groups set for the study;
- to sign the informed consent (sometimes it is also signed by next of kin, especially in patients over 70 years).

5.1.3. Criteria for exclusion from the study

Criteria for patients exclusion from the study were:

- patients aged over 80 years;
- patients with diabetes mellitus ;
- intercurrent inflammatory diseases, recent surgical interventions or trauma (under three months), the existence of a neoplasm, as conditions that can change acute phase reactants and oxidant / antioxidant balance;
- oral anticoagulant therapy in the last four weeks since anticoagulant therapy may interfere with the quantitative determination of antioxidant status;

- vitamin therapy in the last three months;
- uncooperative patients suspected after preliminary discussions;
- patients who did not agree to participate in this study.

Determinations of Total Antioxidant Status (TAS) were performed on the test group at the hospitalization (preoperative) of study group patients with Dupuytren's disease in Plastic Surgery and Reconstructive Microsurgery Department within the Constanța Emergency Clinical County Hospital, in order to perform surgical treatment. Biochemical determinations of glucose, lipid profile and transaminases were performed within the first 24 hours of hospitalization, ambulatory determined before hospitalization for treatment, including conducting specialized tests (cardiology, nutrition diseases, neurology, pneumology) required by the preanesthetic examination.

Therefore it was possible to capture physiopathological changes characterizing this pathology.

5.1.4. Control group structre

In order to establish a reference system as valid as possible in interpreting results obtained by investigating the test group, the measurement of analyzed parameters (biochemistry, oxidative stress) were also carried out on a control group consisting of 18 clinically healthy patients (9 women, 9 men), aged 40-55 years, who voluntarily participated in this study.

5.1.5. Collection of biological material for the study

All determinations were carried out on blood collected by venipuncture within the first 24 hours of admission to the Plastic Surgery and Reconstructive Microsurgery Department of Constanta Emergency Clinical County Hospital.

Total antioxidant status was determined from blood samples collected in tubes with heparin. To determine the lipid profile (total cholesterol, HDL-cholesterol, triglycerides) we used blood collected on EDTA-K3 (2 ml), which was centrifuged at 3000 rot / min, using the resulting plasma. The other biochemical determinations were carried out in serum.

5.2. Determination methods

5.2.1 Epidemiological studies. Descriptive surveys

Epidemiological studies can be classified into observational and experimental.

Observational approaches study how events occur in a natural way; the investigator measures, but he does not interfere. This category includes the so called descriptive and

analytical studies. Descriptive studies are limited to the description of disease occurrence in human populations, and this is often the first step of epidemiological investigation. Analytical studies go deeper by analyzing the relationship between health and other variables. Except for the simplest descriptive studies, the vast majority of epidemiological studies are analytical studies. [3]

5.2.2. Biochemical and oxidative stress markers determinations

5.2.2.2. Serum determination of the Total Antioxidant Status (TAS- Total Antioxidant Status)

Measurement of total serum antioxidant compounds is based on their property to inhibit specific enzymatic oxidation reaction. ABTS® (2,2 '-Azino-di-[3-ethylbenzthiazoline sulphonate]) is incubated with a peroxidase (metmioglobin) and H2O2, being produced the $ABTS^{®+}$ radical cation. It has a teal color, relatively stable, which is measured at 600 nm. The antioxidants present in the analyzed sample induce suppression of this color production, to a degree proportional to their concentration level.

Normal values - are considered to be normal values, values between 1.30 - 1.77 mmol/l plasma; it is recommended that each laboratory should establish its reference values that reflect age, sex, diet and geographical area of the population.

5.2.3. Morpho biometric determinations

5.2.3.1. Technique used to perform cyto histological examination and microscopic analysis

Morpho-functional cells examination is done on microscopic preparations. A microscopic preparation is done according to the purpose of analysis, namely: they may be fresh or temporary (extemporaneous) preparations and permanent or fixed preparations.

SURGICAL TREATMENT

EXCISION OF PALMAR APONEUROSIS

Dissection is proximally led, to the top of the palmar aponeurosis triangle where the long palmar muscle tendon is inserted. Medial and lateral palmar septa are carefully cut off, releasing the palmar aponeurosis edges. At its apex it is transversely sectioned.

Aponeurosis cut edges are caught with a clamp and pulled distally. Sectioning is continued carefully, aponeurosis can be split into small longitudinal strips, carefully dissected from the deep structures.

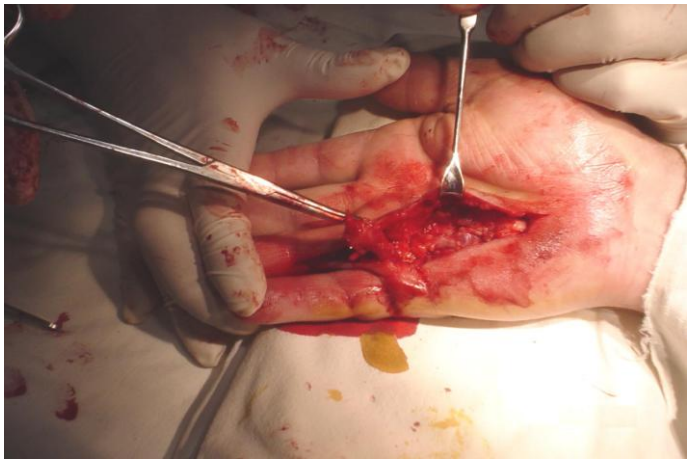

Fig.75 Subtotal anponeurotomy, after removing the hemostatic strap (image from the casuistry of Plastic Surgery and Reconstructive Microsurgery Department, Constanța Emergency Clinical County Hospital)

In the distal part dissection is driven until the superficial transverse ligament of the metacarpus. [19]

Hypertrophied digital expansions of the aponeurosis are carefully excised not to damage the vessels and nerves, which can have different routes. Having established the route of the nerves and vessels, fibrosis mass is excised and extracted. The process is repeated for each finger. The excision of these digital hypertrophy is facilitated by practicing "Z" incisions favorably exposing the region.

Throughout these interventions the skin will be carefully handled as its viability may be compromised. [20]

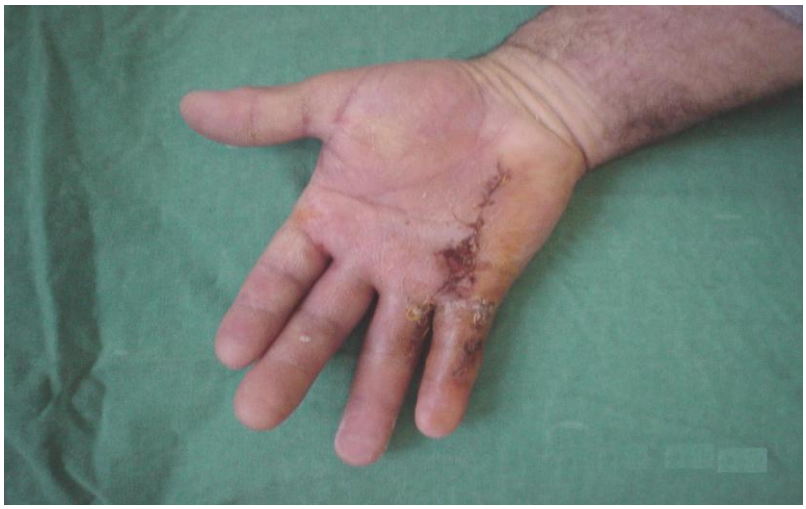

Fig.76 Postoperative appearance – (image from the casuistry of Plastic Surgery and Reconstructive Microsurgery Department, Constanța Emergency Clinical County Hospital)

5.4 Data statistical processing

Data obtained from each determination have been entered in tables being processed and statistically analyzed, and then graphically represented.

For each quantifiable parameter **specific statistical indicators** were mathematically determined.

The main indicators characterizing a series of data are either central tendency indicators or indicators characterizing data dispersion around a mean value. A data series consists of a set of values that we note: $x_1, x_2, \dots, x_n$.

Student t test was determined using *EXCEL* tool. In order to obtain the final ***p*** probability we introduced the values of the two series on a spreadsheet. The "T" TEST function was introduced into a cell specifying in order:

- areas containing data of the two series of values;
- value: 1 or 2 – to indicate whether the test is one-tailed or two-tailed;
- in case the test is two-tailed, then in the case of hypothesis H_0 rejection it is considered that there are differences between the averages of the two characteristics without specifying which of the two averages is higher;
- test type: 1, 2 or 3:

1 - whether data sets are dependent;

2 - whether data sets are dependent and it is supposed that the populations have the same dispersion;

3 - whether data sets are independent and it is supposed that the populations have the different dispersions. [6]

Graphical representation of obtained data

The Bland-Altman plot, or difference plot, is a graphical method to compare two measurements techniques. In this graphical method the differences (or alternatively the ratios) between the two techniques are plotted against the averages of the two techniques. Alternatively the differences can be plotted against one of the two methods, if this method is a reference or "gold standard" method.

CHAPTER IV
RESULTS

Dupuytren's disease is a retraction of the palmar aponeurosis and its digital expansions of still unknown etiology. It affects mainly males, especially after 40 years. The evolution, unpredictable, outbursts and it results in a lack of joints expansion of the fingers, the flexion remaining possible.

Nonspecific local manifestations: palmar teguments induration, persistent pruritus sensation, followed by the appearance of a tumor that develops with the formation of a clamp and irreducible retraction of the fingers in flexion represent the onset elements of the disease.[26]

Dupuytren's disease diagnosis, both the positive and the differential one, is a clinical diagnosis, usually simple, based on fundamental characteristics of the disease - the presence of nodules and clamps. The clinical course is characterized by cyclicity, with outbursts of activity, interrupted by periods of stagnation, varying in duration.

The patient has to be monitored for at least six months from the diagnosis date and best time for surgery is chosen during the stagnation period of the disease. The interval from the onset of the disease to hospitalization varies, being from 3 years to 10 years, most of the patients trying different conservative treatment methods before presenting to service of Plastic Surgery and Reconstructive Microsurgery within Constanta Emergency Clinical County Hospital.

6.1. Epidemiological tests results

6.1.1. Epidemiological aspects in Dupuytren's disease. Retrospective and prospective studies

To know the different aspects of the Dupuytren's disease in Constanța County, we performed a retrospective study on domestic casuistry surgically treated in the specialized section within Constanta Emergency Clinical County Hospital during the period January 2006 - October 2009 as well as a prospective study during the period November 2009 - May 2012, which revealed a number of 124 cases.

Surgical intervention is not necessary for establishing the diagnosis, which is reserved only for those patients to whom the retraction in flexion produces functional deficiency, depriving them from daily activities.

We have followed from a theoretical point of view the etiopathogenic conditions of hospitalized cases, hereditary transmission of the disease and from a practical point of view the surgical cure of the disease as well as the recovery of the functionality of the affected hand.

The anamnesis has provided data on the onset time of the lesions, the evolution in time, degree of kinship between several sick people of the same community, pathogenic connections with patients' profession or other associated conditions.

RETROSPECTIVE STATISTICAL STUDY (*January 2006 - October 2009)*

For this study we analyzed a group of 89 patients diagnosed with Dupuytren's disease in the Plastic Surgery and Reconstructive Microsurgery Department of the Constanța Emergency Clinical County Hospital.

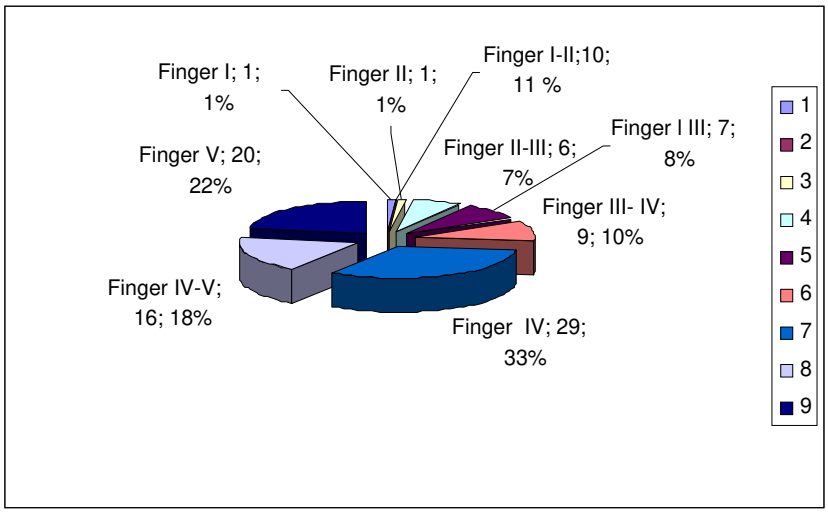

Fig. 38 Patients distribution according to initially affected finger

Table 10 Patients distribution according to associated pathology

Associated conditions	2006	2007	2008	2009
Ethylism (chronis toxic hepatitis)	***14***	***10***	***6***	***4***
Chronic pancreatitis		***10***	***3***	***1***
Diabetes mellitus	***1***			***1***
Obesity		***1***		
Epilepsy			***1***	
HTA	***3***	***1***	***2***	
ICC		***4***		

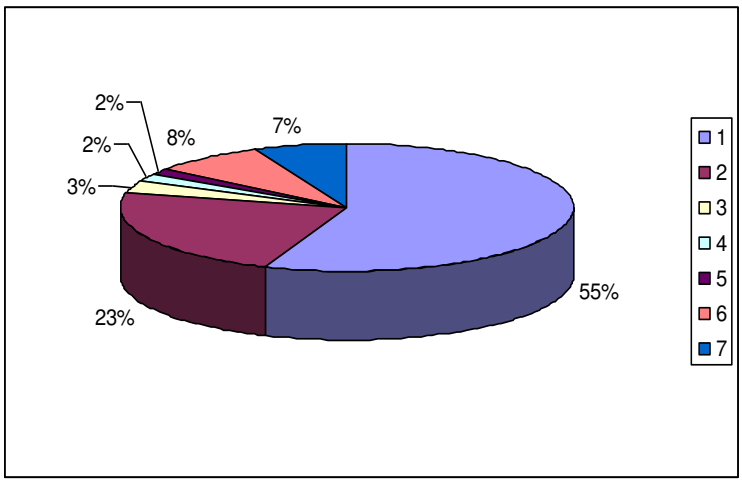

Fig.39 Patients distribution according to associated pathology

The table and figure above show that etylism is the main favorable factor, accounting for 55% (34 patients) of all cases, followed by chronic pancreatitis 23% (14 patients), diabetes mellitus, 3% (2 patients), obesity 2% (1 patient), epilepsy 2% (1 patient), HTA 8% (5 patients), ICC 7% (4 patients).

h) The role of family factor:

With regard to family medical history of Dupuytren's disease in patients involved in our study, we found that out of 89 patients, 40% (36 patients) had genetic implications.

6.2 Biochemical determinations results

The emphasis in this study focused primarily on oxidative component with excessive production of reactive oxygen species (ROS). Because direct highlighting of ROS requires the use of difficult to reach techniques, we indirectly performed their study by measuring the activity of antiradical protective systems namely the total antioxidant status (TAS).

Data obtained at the hospitalization of patients diagnosed with Dupuytren disease, after statistical processing, are presented below in tables and graphs, the results being subsequently analyzed comparatively with the values obtained in the control group as well as the data from the literature until present time.

6.2.2 Study of hepatic cytolysis indicators

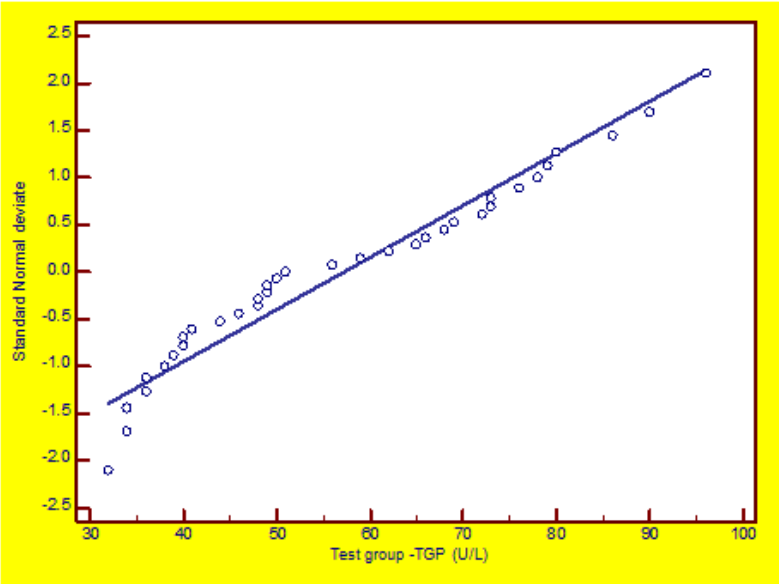

Fig. 56 Distribution of TGP serum values in the test group according to the method of analysis Plot of normal distribution of analyzed values

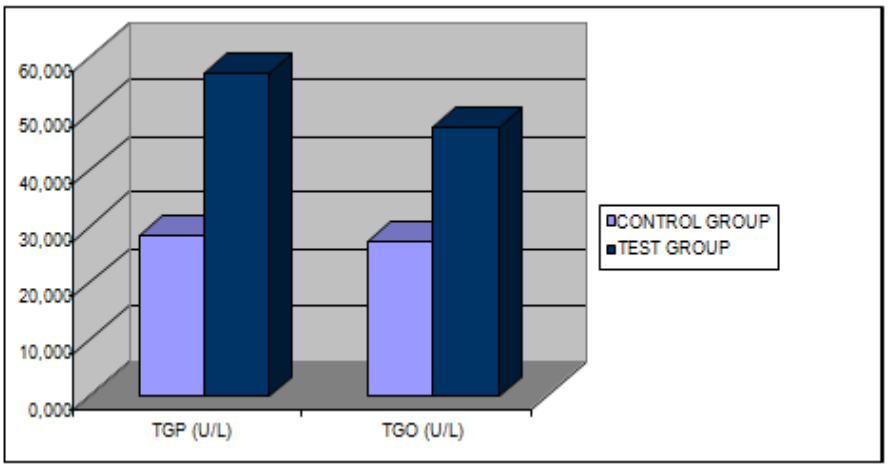

Fig. 57 Comparative graphical representation of the average values of TGP and TGO to the test group and control group

It is revealed a statistically significant increase in transaminases (TGO and TGP) in test group patients as the important sign of hepatic cytolysis present mainly in patients with chronic toxic hepatopathy (ethyl) suggesting chronic hepatic pain.

As a method to compare data obtained from blood measurements TGO on the 2 groups (graphically revealed above by *Box-and-Whisker* diagram) it is observed a significant increase of these values to the test group (patients suffering from toxic hepatopathy - ethyl and Dupuytren's disease) compared with control group.

6.2.3 Results interpretation of oxidative stress markers (total antioxidant status)

Evidence suggests that reactive oxygen species (ROS, reactive oxygen species) may play an important role in the pathogenesis of Dupuytren's disease. [38] ROS are able to react with unsaturated lipids and initiate self-perpetuating chain of peroxidation reactions of membrane lipids. [39]

Free radicals can cause oxidation of protein sulfhydryl groups and also it is possible the splitting of nucleic acids components. [40]

Local antioxidants inhibit or delay oxidative deterioration of carbohydrates subcellular proteins, lipids and DNA. There is evidence that antioxidants can achieve protection against free radicals, which are responsible for damage caused by reperfusion (post local persistent vasoconstriction with hypoxia) and lipid peroxidation and therefore they can inhibit damage to palmar aponeurosis. [41]

Antioxidant status represents a critical tool to evaluate redox status. Antioxidant status or related antioxidants may play an important role in protecting the body against free radicals mediated damage and can minimize the damage caused by ROS during reperfusion (after hypoxia). [42] Free radicals are highly reactive molecules generated by biochemical redox reactions that take place as part of normal cells metabolism. The human body has multilevel synergistic defense mechanisms including two major classes of protection against cellular ROS. [43] Scavenger enzymes, namely superoxide - dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) represent the enzymatic component and the non-enzymatic component includes a large number of natural antioxidants and synthetic compounds (glutathione system and vitamins), which have increased capacity to neutralize free radicals. [38]

Antioxidant protective systems of the body act in certain stages of formation of free radicals and are metabolically interconnected to secure regeneration and antioxidant capacity at a high level and in all cells of the body:[44,45]

- primary antioxidants (superoxide dismutase, catalase, glutathione peroxidase) prevent the formation of new free radicals;
- secondary antioxidants (such as vitamin E, C, beta - carotene, uric acid, bilirubin, albumin) elimate new free radicals, which can initiate chain reactions;
- Tertiary antioxidants (such as DNA repartition enzymes, methionine sulfoxide reductase) repairing cellular structures altered by free radicals attack. [46]

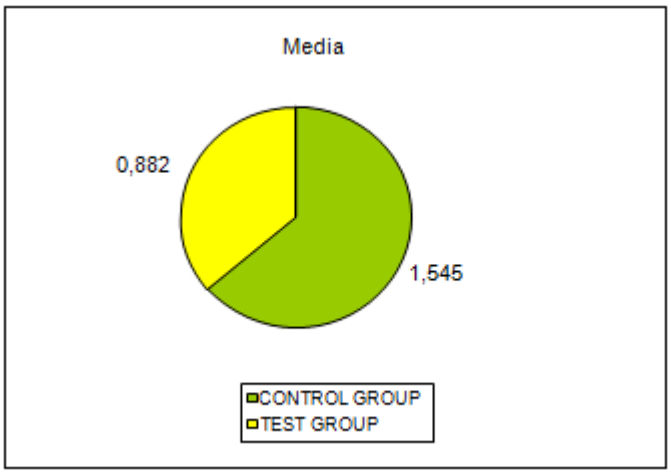

Fig. 61 Graphical distribution of the average values of TAS in the test group and control group

Table 22 Comparative analysis of the average values of total antioxidant status in both groups

TAS (mmol/l)	CONTROL GROUP	TEST GROUP
Average	1,545	0,882
Standard deviation	0.186	0.246
pT		<0.0001

In my study, I evaluated the degree of involvement of oxidative stress in patients with Dupuytren's disease and chronic toxic hepatopathy-ethyl reflected by modifying the activity of antioxidant systems as well as the estimation of these values comparatively with values obtained in a control group.

The overview on antioxidant defense system variations in patients with these chronic diseases was estimated by total antioxidant status (TAS.

The total antioxidant status represents the assessing method of global antioxidant protective mechanisms from blood serum in response to the production of reactive oxygen species within a systemic and local chronic inflammatory process which can favor in the case of these patients the onset and the development Dupuytren's disease.

The results are presented below in tables and graphs and statistically processed, being considered significantly statistic the changes for which we obtain a pT <0.05 (two-tail).

Table 23 Comparative analysis of average serum values of transaminases and total antioxidant status in the 2 groups

AVERAGE VALUES			
GROUPS	TAS (mmol/l)	TGP (U/L)	TGO (U/L)
CONTROL GROUP	1,545	28,278	27,222
TEST GROUP	0,882	57,229	47,400

There is a statistically significant decrease in TAS in test group patients compared with controls group.

This indicates that antiradical protection mechanisms are more slowly stimulated in patients with chronic hepatic compared with control group.

In addition, our study demonstrates there are no changes in the plasma level of total antioxidant status according to gender.

We can assert therefore that free radicals and reactive oxygen species are involved in the pathology of Dupuytren disease.

TAS values in patients with Dupuytren are very low comparatively with those of control group, the upper limit level being below the median for the control group, which shows a pronounced impairment of antioxidant mechanisms in these patients

6.3 Results and discussion on cellular microscopic morphometry studies in patients with Dupuytren's disease

Anatomopathologically, a part or the entire palmar aponeurosis undergoes continuous thickening and contracting, pulling the metacarpophalangeal joint and proximal interphalangeal joint in progressive and fixed flexion. Superjacent skin creases forming deep depressions. They have an important role to locate the node that always lies proximal to depression.

The most powerful superficial proliferation is located in the distal palmar crease. Short fibers that attach palmar fascia to the tegument aggressively proliferate, leading to progressive loss of fat and sweat glands, complying with tendons and vasculonervous packages so that the two structures, palmar aponeurosis and tegument, initially separated, become a plate where the epidermis is placed directly on the modified palmar fascia.

By optical microscopy studies it was tried to identify cyto-morphological changes induced in the palmar aponeurosis. In the literature there is little information about how fibrosis modifies the fibroblasts structure. [50]

An issue pursued in cyto-histological studies performed in test group patients operated in the Plastic Surgery and Reconstructive Microsurgery Clinic of Constanta Emergency Clinical County Hospital was changing the ratio of the number of fibroblast cells and collagen area.

It was also made an assessment regarding the cells size, aspect which can be related to the operating mode and respectively to the level or type of collagen secretion. [51.52]

Following this assessment were identified several architectural types, depending on the type and abundance of fibroblast cells.

I- the sector of active, proliferating, oval, elongated, with abundant cytoplasm and large nuclei fibroblasts II – the sector with few fibroblasts arranged in islands among the collagen structures

III- the sector with disorganized collagen structures and lacunar areas associated with neovascular elements;

IV- cells with irregular shape and non-uniform nuclei, possibly in apoptotic phases

The images below represent sections through Dupuytren nodules - color hematoxylin - eosin (HE), zoom 400X.

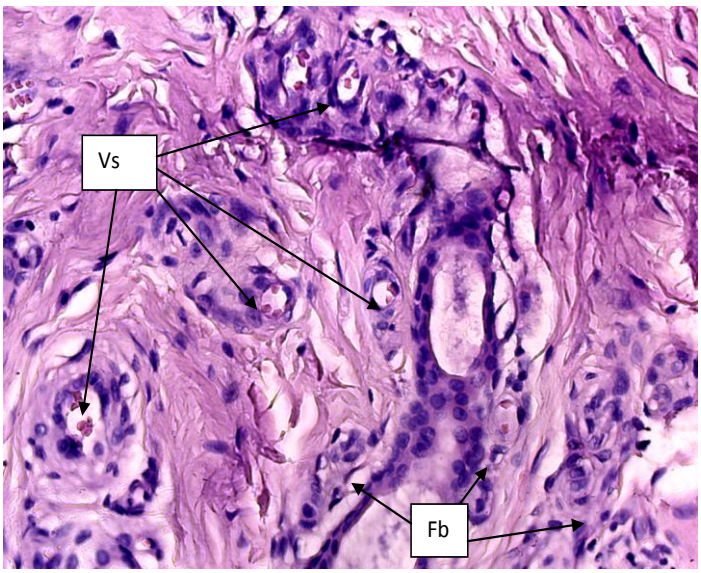

Fig. 65 Neovascular areas located by connective stroma (Fb=fibroblasts, Vs=blood vessels)

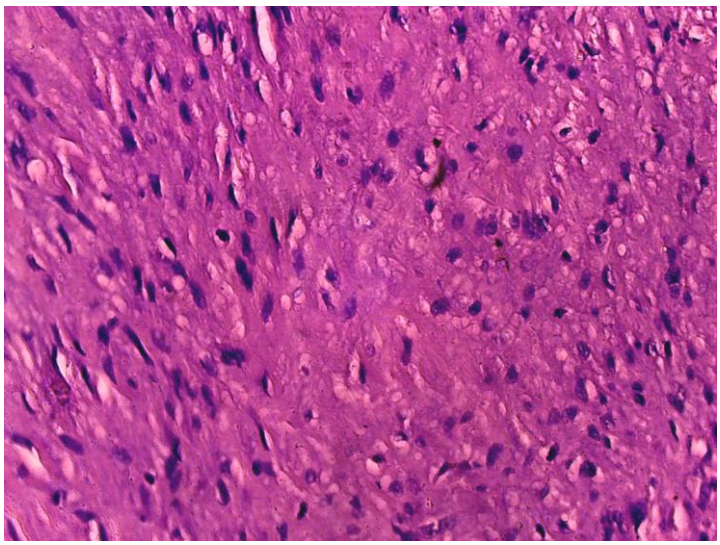

Fig. 66 Area with fibroblasts uniformly located without neovascular structures, elements lacunary in collagen part

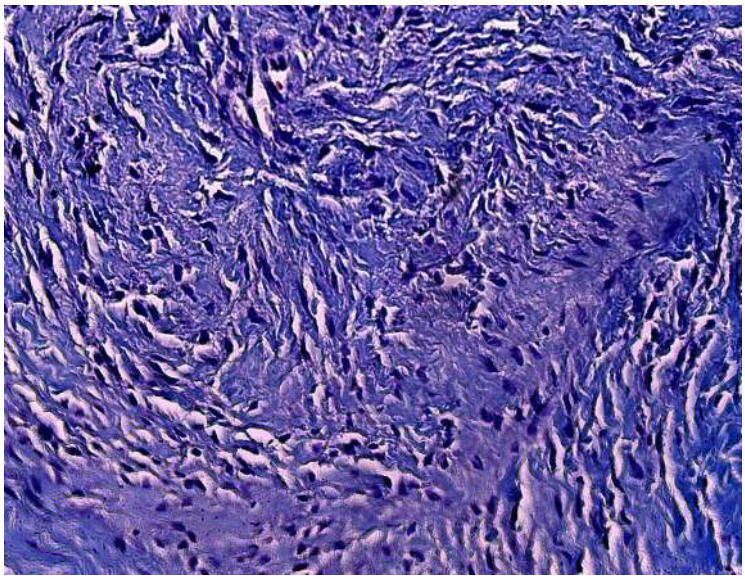

Fig. 68 Non-uniform collagen areas and possibly apoptotic fibrocytes

Observations on cyto-histological evidence allowed as well to perform an assessment regarding the number of cells on the analyzed area of tissue, being quantified for proliferative areas between 7.05 fibroblast cells / μm^2 and 2.01 fibroblast cells / μm^2 (Table No.24.), values explaining the differences in secretory activity.

In areas with abundant collagen and few fibroblasts it is found either a uniform distribution of fibers, or a fascicular disruption associated with increased vacuolation.

Knowing the cyto-morphological aspects and the fibroblasts proliferative potential it might be possible to intervene in the future, through an appropriate targeted treatment according to the stage (type cells and their activity) of the development level of the node and also the mechanical activity inducing ultrastructural tissue modeling [55,56]

STUDY IMPORTANCE, PERSONAL CONTRIBUTIONS

The chronic disorders of the hand, with the palmar aponeurosis retraction, compromising the prehension functions and hindering normal daily activities, include as well Dupuytren's disease whose incidence has significantly increased in recent years, probably due to greater addressability to health services.

Research conducted so far have not been able to establish a unitary connection between the etiopathogenesis of Dupuytren's disease and the anatomical, ultrastructural and the physiopathological changes. There is still no etiological treatment in order to limit the disease

progression in coexistence conditions of genetic predisposition and other contributing factors (chronic ethylism , smoking, physical strain, chronic palmar microtrauma and other associated pathologies).

We have considered that in this context it is necessary physiopathological study to address the Dupuytren's disease by involving antioxidant mechanisms with protective role compared to local overproduction of free radicals and the microscopic study of fibroblasts intervention dictating the disease evolution and progression.

Seen as an chronic inflammatory disease Dupuytren's contracture, disease with genetic determinism, seems to have as critical factor in disease triggering both the overproduction of oxygen free radicals in the palmar fascia inducing local oxidative stress, as well as the clearly demonstrated deficit of antioxidant mechanisms (total antioxidant status) in these patients.

Quick proliferation of fibroblasts influencing the evolution of the disease is clearly the result of cytomorphological changes induced by the chronic inflammatory infiltrate, persistence of local activation of mast cells, macrophages, oxidative stress activation by hypoxia and release of cytokines (as fibroblast increase factors).

One aspect pursued within cyto-histological studies performed in test group patients operated on in Plastic Surgery and Reconstructive Microsurgery Clinic of Constanța Emergency Clinical County Hospital, was changing the ratio between the fibroblast cells number and collagen area. It was also made an assessment of cells size, aspect which can be related to the operating mode and respectively to the level and type of collagen secretion.

I consider that this study results complete the general knowledge concerning Dupuytren's disease, the novelty elements belonging to investigating oxidative stress and cytomorphologiccal analysis of fibroblasts at the palmar aponeurosis level, which could be applied in current medical practice.

CHAPTER VI

CONCLUSIONS

The present research aimed at highlighting epidemiologic and the etiopathogenic aspects in Dupuytren's disease including cytomorphological and oxidative stress studies with the antioxidant protective mechanisms analysis.

After interpreting this data study in this paper and in conjunction with the multiple literature data on Dupuytren's disease etiopathogenesis we can synthesize the following conclusions with demonstrated theoretical and clinical support:

- Etiopathogenic theories are numerous (traumatic theory, embryogenetic, malformation, toxic infections, endocrine, neurogenic, metabolic, autoimmune), but none has succeeded in proving the exact trigger factor and explain the physiopathological progressive evolution in outbursts of palmar aponeurosis retraction in Dupuytren's disease.
- The incidence of the disease both by age group and by gender varies so that in our study on 124 patients hospitalized in the Plastic Surgery and the Reconstructive Microsurgery Department of Constanta Emergency Clinical County Hospital were diagnosed with Dupuytren's disease 107 men and 17 women, aspect confirming the high frequency of this disease in males.
- Most of the patients diagnosed with Dupuytren's disease belong to the age group 60-69 years in both retrospective and prospective study, representing 54 patients.
- The disease cases most frequently encountered come from urban areas.
- Depending on the affected hand, the statistical study reveals damage to both hands without marking out a significantly increased frequency of this pathology on the right hand (aspect confirming the literature data excluding professional overstressing factor as the trigger factor of the disease). Only 7 subjects (18% of cases studied) show bilateral impairment.
- Dupuytren's disease viewed as an occupational disease for those with repeated micro traumatisms within the work process does not seem to have as triggering factor this local chronic traumatic action at the hand level, but this aspect should be seen as an aggravating risk factor of the disease only concerning subjects genetically predisposed (family medical history of Dupuytren's disease).
- The study regarding the correlation between these patients profession with the appearance of this disease shows there is not a directly proportional increase in the incidence of Dupuytren's disease in people with professions overstressing hands by manual labor, aspect suggesting the existence of genetic determinism in the appearance of this pathology and the importance of the professional factor as risk factor in the progression of this disease. The genetic factor involvement in the conducted studies reveals a number of 49 patients with a family medical history of Dupuytren's disease
- The pathology associated to Dupuytren's in test group patients shows a statistically significant increase of this disease association with the chronic toxic ethyl hepatopathy (chronic ethylism – 27 patients) and chronic pancreatitis. Literature data underlines the association of alcohol consumption with the Dupuytren's disease and the fact that the toxic

action at microvascular level and through peripheral neuropathy favors the progression of this disease.

- The diagnosis of Dupuytren's disease is a clinical diagnosis relatively simple, based on the fundamental characteristics of this condition, namely the presence of nodules and clamps. Risk factors favoring the occurrence of these pathognomonic changes in Dupuytren's disease are: gender, family medical history, alcohol, smoking, geographical area, old age, physical strain, palmar microtrauma history and dyslipidemia.
- The present study revealed on the test group patients the oxidative stress mechanisms involvement in Dupuytren's disease. Determination of total antioxidant status (with essential role as protective mechanism against free radical mediated tissue lesions) in patients with Dupuytren's disease and chronic ethyl hepatopathy shows a statistically significant decrease in TAS values (pT <0.0001) compared to the values obtained in the control group, aspect indicating **a pronounced impairment of antioxidant mechanisms in these patients**.
- Local overproduction of free radicals and their effect on fibroblast proliferation may play a significant role in the development and progression of Dupuytren's disease. With regard to cytomorphological evaluation at the palmar aponeurosis level (histological study of extirpation parts following the aponeurectomy in test group patients hospitalized in the Plastic Surgery and Reconstructive Microsurgery Department of Constanța Emergency Clinical County Hospital) it is noted an important polymorphism of cellular elements characteristic to proliferation and involution phases.
- Through optical microscopy studies we have tried to identify changes cytomorphological induced at the palmar aponeurosis level. The literature presents little information about how fibrosis modifies fibroblasts structure. One aspect followed within the cyto-histological studies conducted on test group patients operated on within the Plastic Surgery Clinic of Contsnața Emergency Clinical County Hospital was changing the ratio of the number of fibroblast cells and the collagen area. It was also made an assessment of cells size, aspect which can be related to the operating mode and respectively to the level and type of collagen secretion. Following this assessment were identified several architectural types, depending on the type and abundance of fibroblast cells: **the sector of active, proliferating, oval, elongated, with abundant cytoplasm and large nuclei fibroblasts; the sector with few fibroblasts arranged in islands among the collagen structures; the sector with disorganized collagen structures and lacunar areas** associated with neovascular elements and cells with irregular shape and non-uniform nuclei, possibly in apoptotic phases

- With regard to fibroblasts length variation (colored with the HE), measurements indicate variations in cell diameter between 40-112μm for proliferative cells and the 71-162μm for active secretory cells. The average differences are of about 40μm with a relatively balanced distribution around the average value. Observations from optical microscopy studies by the classical staining with hematoxylin-eosin method and Masson' trichrome stain, allow us to assert that there is a functional cytomorphological differentiation that can be correlated with the severity of Dupuytren's disease progression. Most literature data are focused on studies in vitro molecular cytology and immunohistochemistry, few of them taking into account the fibroblasts morphological appearance which makes our study to add its contributions to the general knowledge concerning Dupuytren's disease.
- Knowing the cyto-morphological aspects and the fibroblasts proliferative potential it might be possible to intervene in the future, through an appropriate targeted treatment according to the stage (type cells and their activity) of the development level of the node and also the mechanical activity inducing ultrastructural tissue modeling.
- The present study opens new perspectives on the etiopathogenesis of Dupuytren's disease, which may have implications which can cause radical changes in the future treatment.

Surgical treatment and surgery time are determined by the anatomoclinical shape, evolutionary stage of disease, age, asscoaited defficiencies and unilateral or bilateral localization. Regular follow up of patients allows assessment of individual evolution as well as identifying evolutionary stagnation period, which is the optimal time for intervention. The surgical procedure recommended is subtotal aponeurectomy with radical tendency, adapted to each case. Immediate results are conditioned by the correct indication and the existence of postoperative complications. Postoperatively, all test group patients had favorable evolution with the functional deficit recovery through active mobilization and the physical therapy, with quick social and professional reintegration.

Selective bibliography

1. **Richard L. Drake, A. Waine Vogl, Adam W.M. Mitchell,** - Gray's Anatomy pentru studenți - Ediția a- II- a, București, Prior&Books, 2010, pg. 751-774.
2. **Mincă Dana Galieta, Marcu M.G.,** - Sănătate publică și management sanitar - Ed. Universitară „Carol Davila", București, 2005, pg.102-106.
3. **Enăchescu D., Marcu M.G.,** - Sănătate publică și management sanitar - Ed. All, București, 1998.
4. **Landrion G., Delahaye F.,** - Cercetare clinică – de la idee la publicare - Ed. Dan, 2001.
5. **Moineagu C., și colab.,** - Statistică- concepte, principii, metode - Ed. Științifică și Enciclopedică, București, 1976.
6. **Synevo,**- Ghidul serviciilor medicale al Laboratoarelor Synevo - Ediția a II- a, 2010, pg. 322-325, 357-358, 407-411, 463-464.
7. **Bătăiosu Monica, Catrinoiu Liliana, Jan van E., -** Diabetul zaharat tip 2.- Ghid de practică pentru medicii de familie - Ed. Infomedica, 2005.
8. **Synevo,** - Referințele specifice tehnologiei de lucru utilizate 2010 - Ref. Type: Catalog.
9. **Mogoantă L., Popescu Carmen Florina, Georgescu Claudia Valentina, Comănescu Violeta, Pirici D.,** - Ghid de tehnici de histologie, citologie și imunohistochimie- Ed. Medicală Universitară, Craiova, 2007, pg. 368.
10. **Cotrutz C.,** - Manual de lucrări practice de biologie celulară - Ed. Tehnică Chișinău, 1994, pg. 247.
11. **Dimofte Iuliana și colab,** - Lucrări practice - Biologie celulară și moleculară - Ed. Cartea Universitară, Constanța, 2003, pg. 101.
12. **Meșter R., Scripcaru D.,** - Manual de lucrări practice de biologie celulară - Ed. Univ. București, 1976, pg. 267.
13. **Litarczek G., -** Tratat de patologie chirurgicală - vol.II Ed. Medicală, București ,1998 , pg. 30-39, 374-388, 667-668,696-698.
14. **Skoog T.,** - Dupuytren's contracture; pathogenesis and surgical treatment in Dupuytren's disease, in -„Dupuytren's disease"- red. J.T. Hueston, R. Tubiana, Churchill - Livingstone, Edinburgh, 1974, pg. 109.
15. **Skoog T.,** - Dupuytren's contracture; pathogenesis and surgical treatment in Dupuytren's disease, in - „Dupuytren's disease" - red. J.T. Hueston, R. Tubiana, Churchill - Livingstone, Edinburgh, 1985, pg. 184.
16. **McCash C.R.,** - The open palm technique in Dupuytren's contracture - Br. J. Plast. Surg., 1964, 17:271.
17. **McCash C.R., -** The open palm technique in Dupuytren's disease, in „Dupuytren's disease" - red. J.T. Hueston și R. Tubiana, Churchill - Livingstone, N.Y., 1985, pg. 136.
18. **Boyes, J.H., Hammer M.,** - Dupuytren's disease: A study in pathogenesis in „Clinical trends in othopedics" - Stranbond Wilson, N.Y., 1982, pg. 49.
19. **Bojsen - Moller P., Schmidt L.,** - The palmar aponevrosis and the central spaces of the hand - J. Arext, 1974, 117:55.
20. **Beitran J.E.,** -Dupuytren's disease: the results of the open palm and digit technique, in „Dupuytren's disease" - red. J.T, Hueston și R. Tubiana, Churchill - Livingstone, Edinburgh, 1985, pg. 142.
21. **Kaplan E.B.,** - Functional and Surgical Anatomy of the Hand - J.B. Lippincott Co., Philadelphia, 1965, pg. 25.

22. **Kaplan E.B.,** - Operation for Dupuytren's contracture based on anathomy of palmar fascia - Bull. Press. Med. Soc. N.Y., 1939, pg. 78.
23. **Moermans, J.P.,** - Place of segmental aponeurectomy in the treatment of Dupuytren's disease - PhD Thesis 1997/ http:// www chirurgiemain.eu/.
24. **Chiotan N., Florescu I., Matusz P**., - Considerații critice asupra tratamentului chirurgical al maladiei Dupuytren - J. Român de Chir. Plast., 2/1993.
25. **Moermans, J.P.,** - Recurrence after surgery for Dupuytren's disease – Eur. J. Plast. Surg., 1997, 20:240-245.
26. **Enescu D.M., Bordeianu I.**,- Manual de chirurgie plastică - Ed. Ovidius University Press, Constanța, 2001, pg. 327-334.
27. **Hindocha S., John S., Stanley J.K., Watson S.J., Bayat A., -** The heribility of Dupuytren's disease: familial aggregation and its clinical significance – J. Hand Surg. Am.2006; 31:204-10.
28. **McFarlane R.M.**, - Dupuytren's contracture, in „Operative Hand Surgery"- red. D.P. Green, Churchill - Livingstone, N.Y., 1985, pg. 553.
29. **McFarlane R.M.,** - Progress in Dupuytren's disease - Rom. J. Plast. Surg., Cluj,1998,VI, l.
30. **Mikkelsen O.A.**, - Dupuytren's Disease. The influence of occupation and previous hand injuries. The hand, 1978, 10:1.
31. **Burch P.R.J.,** - Dupuytren's contracture: an autoimmune disease ? - J.Bone Joint Surg., 1966. 48B:312.
32. **Carantino A.M.**,- Considerații morfologice cu valoare clinică și chirurgicală privind aponevroza palmară - Teză de doctorat, Universitatea „Ovidius" Constanța, 2006, pg.105-106, 110.
33. **Early P.F.,** - Population studies in Dupuytren's contracture - J. Bone Joint Surg.,1962,44-B:602.
34. **Burge P., Hoy G., Regan P., Milne R., -** Smooking, alcohol and the risk of Dupuytren contracture – J. Bone Jont Surg. Br. 1977;79:206-10.
35. **Burke F.D, Proud G, Lawson I.J, McGeoch K.L, Miles J.N., -** An assessment of the effects of exposure to vibration, smoking, alcohol and diabetes on the prevalence of Dupuytren's disease in 97,537 miners – J. Hand. Surg. Eur. vol 2007;32: 400-6. Epub. 2007 May 25.
36. **Skoog T.**, - Dupuytren's contraction with special reference to etiology and improved surgical treatment: its occurrence in epileptics; note on knuckle pads - Acta Chir. Scand., 1948, 96, Suppi, 139.
37. **Niculescu T., Toma I., Pavel Anca,** - Medicina Muncii, Vol. I - Ed. Medmun, București, 1999, pg. 200.
38. **Pasupathi P., Rao Y.Y., Farook J., Saravanan G., Bakthavathsalam G.,** - Oxidative Stress and Cardiac Biomarkers in Patients with Myocardial Infarction - European Journal of Scientific Research - 27 (2):275-285 ; 2009.
39. **Salvemini D., Cuzzocrea S.,** - Therapeutic potential of superoxide dismutase mimetics as therapeutic agents in critical care medicine - Crit. Care Med., 31(1 Suppl): S29-S38; 2003.
40. **Kaul N., Siveski-Iliskovic N., Hill M., Slezak J., Singal P.K.,** - Free radicals and the heart - J. Pharmacol. Toxicol. Methods. 30(2): 55-67; 1993.
41. **Murrell G.A, Francis M.J, Bromley L.**, - Radicalii liberi și contractura Dupuytren – Br.J. Med. (Clin Res Ed). 28 noiembrie 1987, 295 (6610): 1373-5.

42. **Patra R.C., Swarup D., Dwivedi S.K.,** - Antioxidant effects of alpha tocopherol, ascorbic acid and L-methionine on lead induced oxidative stress to the liver, kidney and brain in rats - Toxicology 11; 162(2): 81-88; 2001.
43. **Muzakova V., Kandar R., Vojtisek P., Skalicky J., Vankova R., Cegan A., Cervinkova Z.,** - Antioxidant vitamin levels and glutathione peroxidase activity during ischemia/reperfusion in myocardial infarction - Physiol. Res., 50: 389-396; 2001.
44. **Chabrier P.E., -** The role of endothelin in the vessel wall - Baillieres Clin Haematol., 1993; 6: 577-591.
45. **Liu J., Zeo H.C., Overvik-Douki E., Hagen T., Doniger S.J., Chu D.W., Brooks G.A., Ames B.N., -** Chronically and acutely exercised rats biomarkers of oxidative stress and endogenous antioxidants – J. Appl. Physiol; 2000, 89: 21-28.
46. **Bryson J.M., Coy P.E., Gottlob G., Hay N., Robey R.B.,** - Either Ectopic or Endogenous Origin, Protects Renal Epithelial Cells against Acute Oxidant - induced Cell Death - J. Biol. Chem.; 277(13), 11392-11400; 2002.
47. **Bordeianu I., Iordache I.V., Caraban B.M.,** - Chirurgie plastică şi microchirurgie reconstructivă - Ed. Muntenia, Constanţa, 2010, pg. 128-134.
48. **Guyton A.C., Hall J.E**., – Tratat de fiziologie a omului - ediţia a 11-a , editori: Dr. Cuculici Gh., Dr. Anca Gheorghiu, Ed. Medicală Callisto, Bucureşti, 2007, pg. 149 - 159, pg. 268-276.
49. **Yi IS, Johnson G., Moneim M.S.**, - Etiology of Dupytren's disease - Hand Clin, 1999 Feb; 15 (1):43-51,vi.
50. **Forsman M., Kallioinen L., Kallioinen M., Ryhänen J.,** - Dupuytren's contracture; increased cellularity – proliferation, is there equality? - Scandinavian Journal of Surgery 94, 2005, 71–75.
51. **Angello J.C, Pendergrass W.R, Norwood T.H, Prothero J.** - Proliferative potential of human fibroblasts: an inverse dependence on cell size – J. Cell. Physiol. Jul. 1987;132(1):125-30.
52. **Ivarsson M, McWhirter A, Borg T.K, Rubin K**. -Type I collagen synthesis in cultured human fibroblasts: regulation by cell spreading, platelet - derived growth factor and interactions with collagen fibers - Matrix Biol. Feb. 1998;16(7):409-25.
53. **Matthew J. Dalby1, Mathis O. Riehlel, Duncan S. Sutherland, Hossein Agheli , Adam S.G. Curtis**, - Morphological and Microarray Analysis of Human Fibroblasts Cultured on Nanocolumns Produced by Colloidal Lithography - European Cell and Materials Vol. 9,2005, pg 1– 8.
54. **Florescu I., Chiotan N., Apostolescu Ioana, Carantino A.M., Giuglea C.,** - Maladia Dupuytren - Ed. Naţional, Bucureşti 1999, pg. 88-106, 132-170.
55. **Angello J.C., Pendergrass W.R., Norwood T.H., Prothero J.,** - Cell enlargement: one possible mechanism underlying cellular senescence – J. Cell Physiol. Aug 1989; 140(2):288-94.
56. **Tomasek J.J., Gabbiani G., Hinz B., Chaponnier Christine, Brown R.A.,** - Myofibroblasts and mechanoregulation of connective tissue remodelling - Molecular Cell Biology, Volume 3 , May 2002, pg. 349-363.