

Matrix Metalloproteinase-2 and its Relation with Incisional & Inguinal Hernia.

Vinod Jain*, Rohit Srivastava*, Nagendra Singh Rawat**, Samir Misra**, Shweta Jha**, DV Amla**

Department of General Surgery, *CSM Medical University, Lucknow &

**National Botanical Research Institute, Lucknow, Uttar Pradesh, India

Abstract: Abnormal collagen metabolism is thought to play an important role in the development of primary inguinal and ventral hernia. The detection of an impaired collagen balance both in the tissue as well as in cultured fibroblasts underlines the suspicion that the development of hernia is likely to be implemented primarily by a disturbance of the fibroblast function and their collagen genes. Based on these results we assume that the altered collagen synthesis in hernia patients can be regarded as a genetically linked deregulation serving as a basic initiating or promoting factor for the development of primary inguinal hernias. With the hypothesis that hernia is a local manifestation of a systemic disease manifested by increased expression of MMP-2, a study was planned with the objectives: 1) To establish a causal association between incisional & inguinal hernia and MMP-2; 2) To test the hypothesis that hernia is a local manifestation of a systemic disorder rather than being a mere local mechanical defect. A case control study was conducted on 10 cases of incisional hernia, 30 cases of indirect hernia & 30 cases of direct inguinal hernia with 30 controls. DAC ELISA test was used for analysis of serum (preoperative) and tissue samples (rectus sheath/fascia transversalis) in patients as well as controls. Statistically, Serum levels of MMP-2 were significantly increased in all the hernia patients as compared to controls. This maximum increment was seen in patients of direct hernia. MMP-2 was not detectable in any of tissue samples. Therefore, we can draw conclusion that hernia is a local manifestation of a systemic disease rather than being a mere local mechanical defect.

Keywords: MMP-2, Matrix Metalloproteinase-2, inguinal hernia, DAC-ELISA, collagen metabolism, PBST- Phosphate Buffer Saline Tween-20, Incisional hernia

INTRODUCTION

Usually an abdominal wall hernia is regarded as a mechanical problem with a local defect which has to be closed technically, either by sutures or, in modern time with meshes. In the long history of hernia repair, even the most experienced surgeon, irrespective of the utilized technique, has to face recurrences that have been treated by him and correspondingly have to be regarded as his personal technical failure. That's why it is obviously impossible in hernia surgery to make mechanical repair with success rates being expected similar to those for engineering^{1,2}.

The close causal relationship between one technical component and its failure is reflected by s-shaped survival curve. If the recurrence is considered just as a technical failure, this should occur either soon or with a certain delay, but in any case the outcome curve should reveal an s-shaped configuration. However, this contradicts the actual proportions. On the contrary, in incisional and inguinal hernia formation, the cumulative incidences show a linear rise over years without any s-shaped deformation^{3,4}. This course is in contradiction to any significant direct causal relationship between technique and recurrence. Instead, an underlying multifactor process has to be suggested. Furthermore, because most of the recurrences occur after 1 year within the linear rise of the cumulative incidences, a multifactor process seems to be far more important than any accusable factor of the early postoperative course.

There is a close association between inguinal hernia and collagen metabolism. A decreased collagen types I / III ratio is found in adult patients with groin hernia as well as in the scar of patients with recurrent hernia^{5,6}. Collagen type I is characteristic for mature scars or fascial tissue while the collagen type III represents the mechanically instable, less cross-linked collagen synthesized during the early days of wound healing. Correspondingly, in patients with recurrent hernias, there seems to be an impaired maturation of scar tissue which is not able to close the hernia gap or fix the mesh in place for long. Consequently, a recurrence may develop either through a scar or at the border of a synthetic mesh through its scary fixation.

Abnormal collagen metabolism is thought to play an important role in the development of primary inguinal hernia. This view is strengthened by detection of altered collagen metabolism and structural changes of the tissue in these patients. Several connective tissue diseases have been related to an abnormal collagen metabolism. Patients with an aortic Abdominal Aortic Aneurysm^{7,8}, Ehlers-Danlos Syndrome⁹, Polycystic Kidney Disease^{10,11} show an increased risk for inguinal herniation. Furthermore, previous studies on protein level indicate that patients with an inguinal hernia present a disturbed collagen proportion with a reduced ratio of type I and type III collagen as well as abnormal ultra-structural changes of the deposited collagen^{12,13}. A defective collagen metabolism contributes to a decreased tensile strength and mechanical stability of both the connective tissues and the induced scar tissue. Therefore these alterations in collagen formation should be of central relevance in the pathophysiology of hernias.

The altered ratio of the collagen subtypes can result either by a modified synthesis or by an imbalanced breakdown. The cleavage is regulated by the activity of the matrix metallo-proteinases (MMPs), proteins of a family of zinc-dependent endopeptidases. Among them MMP-1 and MMP-13 are the principal matrix enzymes cleaving fibrillar type I, II and III collagen. In particular, the alterations in MMP-1 and MMP-13 protein expressions could have been responsible for the changed ratio of type I to type III collagen on the protein level. Nevertheless, as firstly shown in investigations by Bellon *et al.* in 1997, cultured fibroblasts in fascia transversalis from patients with inguinal hernia showed no difference in the expression of matrix metallo-proteinase-1, whereas the same author later detected a MMP-2 over expression in these patients^{14,15}. These results on protein level appear to suggest that in comparison to MMP-1 and MMP-13, MMP-2 is an active part of degradation system of the extracellular matrix in hernia patients. Based on above facts, a hypothesis was generated that hernia is a local manifestation of a systemic disease which is manifested by increased expression of MMP-2. Thus a study was planned with research objectives to establish a causal association between hernia and mmp 2 and to test

the hypothesis that hernia is a local manifestation of a systemic disorder rather than being a mere local mechanical defect.

MATERIALS & METHODS

A Case Control Study was designed in which patients admitted in the department of General Surgery of CSM Medical University, Lucknow constituted the study and control group. In study group patients operated for direct, indirect inguinal and incisional hernia (n = 30 each for direct and indirect hernia & n=10 for incisional hernia) were included. Randomization was done according to Table of Random Number Method. Controls (n=30) were age and sex matched patients who were operated for abdominal trauma in emergency. Neither of the controls had any type of abdominal wall hernia. Patients suffering from any type of connective tissue disorder and with chronically raised intra abdominal pressure e.g. COPD, pregnancy, intra abdominal tumour etc were excluded from the study.

Sample Collection and Transportation

Serum Samples: Blood samples were taken preoperatively. Serum was separated from blood after allowing it to stay in a test tube for about 30 minutes followed by centrifugation at 3000 rpm for 10 minutes. Serum was stored in eppendorf vials till further processing. **Tissue samples:** In the study group, a section of about 1x1 cm of fascia transversalis tissue (inguinal hernia) & rectus sheath (incisional hernia) was taken while in the control group, section of rectus sheath was taken of same size. Tissue samples were kept in normal saline after washing with distilled water. Both serum and tissue samples were transported to the laboratory at National Botanical Research Institute, Lucknow in ice cooled boxes within 2 hours of extraction. These samples were kept at -70°C till commencement of the analysis.

Direct antigen coating ELISA (DAC-ELISA) test was done for detection of serum and tissue MMP-2 levels.

RESULTS

All the cases of direct hernia were males, while 8/10 (80%) cases of incisional hernia were females. Amongst indirect hernia, 1 out of 30 patients (3.33%) was female. Controls were aged between 22 to 57 years; the mean age of control group subjects was 35.90±10.67 years; patients of indirect hernia were aged between 22-42 years with a mean age of 31.03±5.60 years and patients of direct hernia were aged between 33-60 years with a mean age of 49.33±7.21 years. The patients of incisional hernia were aged between 30-52 years with a mean age of 43.00±6.98 years.

The mean serum concentration of MMP 2 in control group was 745.37±30.05 ng/ml with a range from 667 to 803 ng/ml; mean serum concentration in incisional hernia group was found to be 1091.00±286.73 ng/ml (range - 761-1573 ng/ml). while that in direct hernia group was 1473.37±118.95 ng/ml with a range of 1244-1678 ng/ml. Mean value of serum MMP 2 in indirect hernia group was 1076.07±80.06 ng/ml with values ranging from 967-1247 ng/ml, thereby showing a statistically significant difference among groups. (table-1 & diag-1)

Intergroup comparison revealed a statistically significant difference between indirect hernia and direct hernia (p<0.001) but no statistically significant difference between indirect hernia and incisional hernia groups (p=0.986). As compared to control group, the indirect hernia, the direct hernia group and incisional hernia group had a difference of 330.70±30.76; 728.00±30.76 and 345.633±43.499 ng/ml which was significant statistically. Thus all the study groups had higher mean serum concentration values as compared to control group (p<0.001).

Table 1: Mean serum concentration of MMP2 in different groups

	N	Mean	SD	Minimum	Maximum
Control	30	745.37	30.05	667	803
Ind Hernia	30	1076.07	80.06	967	1247
DIR Hernia	30	1473.37	118.95	1244	1678
INC Hernia	10	1091.00	286.73	761	1573
Total	100	1097.54	307.0675	667	1678

F=187.264; P<0.001 (ANOVA)

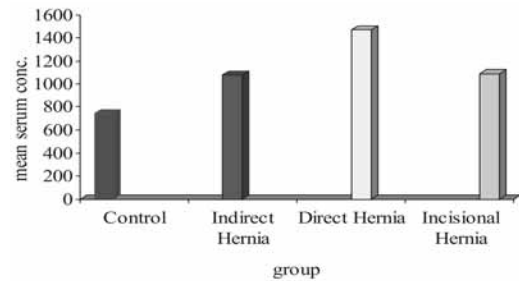


Diagram-1: Barred diagram Showing mean serum concentration of MMP2

Intergroup comparison showed significantly higher values in direct hernia in comparison to all the other groups (p<0.001).

On the basis of above observations the serum MMP 2 concentration could be shown as:

Control < Indirect Hernia ≈ Incisional Hernia < Direct Hernia

Analysis of Tissue samples: We were not able to find any detectable amount of MMP 2 in tissue samples (neither in controls nor in test samples)

DISCUSSION

In a study conducted by J. Smigielski, K. Kolomecki et al from the department of Endocrinological and General Surgery, Medical University of Lodz, Poland in 2007¹⁶, significant increase in serum mmp 2 levels of indirect, direct and recurrent inguinal hernia as compared to controls was found. There was also significant difference with respect to age. The levels were higher in subgroup of younger patients as compared to subgroup of older patients (highest levels were found in young patients with direct hernia). In our study the patients of direct hernia showed highest levels of serum mmp2 but no significant difference was found with respect to age. Juan M. Bellon, Ana Bajo, Natalio G et al¹⁷ performed a cell culture of fibroblasts from fascia transversalis of young patients with direct inguinal hernia and observed significant expression of active mmp2. These findings were confirmed by immunosorbent assay, immunoblotting, immunocytochemistry and zymography in the culture media. In our study, we directly used the tissue samples after crushing them in liquid nitrogen. Probably cell culture would have been beneficial for amplification of mmp2 expression. Immunocytochemistry can be more sensitive but it requires long duration of time for sectioning, staining etc. We adopted a simple procedure which was easy to perform and required a shorter time span.

R. Rosch, et al in 2005¹⁸ aimed to investigate the MMP-2 expression in patients with recurrent incisional hernias with and without mesh-materials. In primary fibroblast cultures obtained from skin scars of patients with and without recurrent incisional hernias, MMP-2 synthesis and gene expression were investigated. Furthermore, MMP-2 synthesis and gene expression of fibroblasts were compared after incubation with two different mesh materials: polypropylene and absorbable polyglactin filaments. MMP-2 enzyme activity was determined by semiquantitative zymography and mRNA synthesis by quantitative RT-PCR. Both

MMP-2 enzyme activity and mRNA expression were similar in hernia and control fibroblasts in vitro. In control fibroblasts mesh incubation did not significantly affect MMP-2 expression, whereas polypropylene mesh contact of fibroblasts from patients with recurrent incisional hernias led to a major decrease of MMP-2 activity and of mRNA expression. In the absence of biomaterials fibroblasts from recurrent hernia, patients have no alterations of their MMP-2 synthesis compared to control, whereas a specific response was found after biomaterial contact indicating the differences in fibroblast phenotype.

Based on international research and our own results, we found that increase in MMP-2 activity could be considered to play a significant role in the etiology of inguinal hernias. The increased activity may lead to the dysfunction of collagen fibers, which are responsible for forming fascial structures, and as a result it can weaken their durability.

CONCLUSIONS

Because in present study, there is statistically significant increase in serum MMP 2 levels in patients of indirect and direct hernia both as compared to controls, we can conclude that hernia is not a mere local defect but a local manifestation of a systemic disease. This is more apparent in patients of direct hernia group, as mean serum concentration of serum mmp 2 is highest in these patients. Development of incisional hernia may also be regarded partly as manifestation of systemic effect. However, further study with more Meta analysis is needed to derive a definite conclusion.

REFERENCES

1. *Trupka AW, Hallfeldt KK, Schmidbauer S, Schweiberer L. Management of complicated incisional hernias with underlay-technique implanted polypropylene mesh. An effective technique in French hernia surgery. Chirurg 1998;69:766-72.*

2. *Langer C, Liersch T, Kley C, Flosman M, Suss M, Siemer A, et al. Twenty five years of experience in incisional hernia surgery. A comparative retrospective study of 432 incisional hernia repairs. Chirurg 2003;74:638-45.*
 3. *Schumpelick V, Klinge U, Welty G, Klosterhalfen B. Meshes within the abdominal wall. Chirurg 1999;70:876-87.*
 4. *Flum DR, Fisher N, Thompson J, Marcus-Smith M, Florence M, Pellegrini CA. Washington State's approach to variability in surgical processes/outcomes: Surgical Clinical Outcomes Assessment Program (SCOAP). Surgery 2005;138:821-8.*
 5. *Junge K, Klinge U, Rosch R, Mertens PR, Kirch J, Klosterhalfen B, et al. Decreased collagen type I/III ratio in patients with recurring hernia after implantation of alloplastic prostheses. Langenbecks Arch Surg 2004; 389: 17-22.*
 6. *Rosch R, Junge K, Knops M, Lynen P, Klinge U, Schumpelick V. Analysis of collagen-interacting proteins in patients with incisional hernias. Langenbecks Arch Surg 2003; 387: 427-32.*
 7. *Menashi S, Campa JS, Greenhalgh RM, Powell JT. Collagen in abdominal aortic aneurysm: typing, content, and degradation. J Vasc Surg. 1987;6:578-582.*
 8. *Lehnert B, Wadouh F. High coincidence of inguinal hernias and abdominal aortic aneurysms. Ann Vasc Surg. 1992;6:134-137.*
 9. *Liem MS, van der GY, Beemer FA, van Vroonhoven TJ. Increased risk for inguinal hernia in patients with Ehlers-Danlos syndrome. Surgery. 1997; 122: 114-115.*
 10. *Morris-Stiff G, Coles G, Moore R, Jurewicz A, Lord R. Abdominal wall hernia in autosomal dominant polycystic kidney disease. Br J Surg. 1997; 84: 615-617*
 11. *Morris-Stiff G, Coles G, Moore R, Jurewicz A, Lord R. Abdominal wall hernia in autosomal dominant polycystic kidney disease. Br J Surg. 1997; 84: 615-617*
 12. *Friedman DW, Boyd CD, Norton P, Greco RS, Boyarsky AH, Mackenzie JW, et al. Increases in type III collagen gene expression and protein synthesis in patients with inguinal hernias. Ann Surg. 1993; 218: 754-760.*
 13. *Nikolov S, Beltschev B. [Several ultrastructural peculiarities of the fascia transversalis in direct inguinal hernias of senile men]. Anat ANZ. 1990; 170: 265-272.*
 14. *Bellon JM, Bujan J, Honduvilla NG, Jurado F, Gimeno MJ, Turnay J, Olmo N, Lizarbe MA. Study of biochemical substrate and role of metalloproteinases in fascia transversalis from hernial processes. Eur J Clin Invest. 1997; 27: 510-516.*
 15. *Bellon JM, Bajo A, Ga-Honduvilla N, Gimeno MJ, Pascual G, Guerrero A, Bujan J. Fibroblasts from the transversalis fascia of young patients with direct inguinal hernias show constitutive MMP-2 overexpression. Ann Surg. 2001; 233: 287-291.*
 16. *J. Smigielski, K. Kolomecki et al. European Surgical Research 2009; 42: 118-121.*
 17. *Juan M Bellen, Anna Bajo et al. Annals of Surgery, Vol. 233, No. 2, 287-291.*
 18. *R. Rosch, P. Lynen-Jansen, K. Junge, M. Knops, B. Klosterhalfen, U. Klinge, P. R. Mertens and V. Schumpelick. Springer Paris, vol 10, number2, April 2006; 125-130.*



DTF
MICRO LABS
SWEET LIFE. DELIVERED

<p>Tripride TM 1 LP / 2 LP</p> <p>Glimepiride 1 mg / 2 mg + Metformin 500 mg SR + Pioglitazone 15 mg tablets</p>	<p>Melmet [®] 500 / 1000 SR</p> <p>Metformin 500 mg / 1000 mg extended release tablets</p>	<p>PREGATOR TM</p> <p>Pregabalin 75 mg + Methylcobalamin 1500 mcg + Alpha Lipoic Acid 200 mg + Pyridoxine 3 mg + Folic Acid 1.5 mg capsules</p>
<p>Tripride TM 1 Forte / 2 Forte</p> <p>Glimepiride 1 mg / 2 mg + Metformin 1000 mg SR + Pioglitazone 15 mg tablets</p>	<p>Dibizide-M [®]</p> <p>Glipizide 5 mg + Metformin 500 mg tablets</p>	<p>Eparel-50 TM</p> <p>Epalrestat 50mg tablets</p>
<p>Diapride [®] 1 2 4</p> <p>Glimepiride 1 mg / 2 mg / 4 mg tablets</p>	<p>Glutowin TM Forte</p> <p>Glibendamide 5 mg + Metformin 500 mg SR tablets</p>	<p>Meconerv Plus TM</p> <p>Methylcobalamin 750 mcg + Pyridoxine 3 mg + Folic Acid 1.5 mg + Alpha Lipoic Acid 100 mg softgels</p>
<p>Diapride-M1 Diapride-M2</p> <p>Glimepiride 1 mg + Metformin 500 mg SR tablets Glimepiride 2 mg + Metformin 500 mg SR tablets</p>	<p>Diavas TM 1 / 2</p> <p>Glimepiride 1 mg / 2 mg + Metformin 500 mg SR + Atorvastatin 10 mg tablets</p>	<p>Melcovit TM Gold</p> <p>Lycopene, Alpha Lipoic Acid, β-carotene, Inositol, Vitamins and Minerals capsules</p>
<p>Diapride-M1 Forte Diapride-M2 Forte</p> <p>Glimepiride 1 mg + Metformin 1000 mg SR tablets Glimepiride 2 mg + Metformin 1000 mg SR tablets</p>	<p>Obitrol-120</p> <p>Orlistat 120 mg capsules</p>	<p>Diarich TM</p> <p>Chocolate flavour Soy protein, Dietary fibres & other Antioxidant Vitamins & Minerals powder</p>

For the use only of a registered medical practitioner, hospital or laboratory ML / MKTG / Wow - 3 / 04-11 / 13k