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ORIGINAL ARTICLE

Advanced glycation end products as a biomarker for incisional hernia

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Abstract
Background Incisional hernia is one of the most frequent complications after abdominal surgery, with incidences up to 30%. A reliable biomarker for the prediction of this complication is lacking. Advanced glycosylation end products (AGEs), also known as non-enzymatic collagen crosslinks, are correlated with aging, smoking, hyperglycemia, hyperlipidemia and oxidative stress. In this study the accumulation of AGEs and the relation between AGEs and incisional hernia were investigated.
Materials and methods In an exploratory case–control study, 23 patients with incisional hernia after midline incision were compared with 17 patients without clinical or radiological signs of incisional hernia after midline incision, AGEs were measured using a Skin Auto Fluorescence (SAF)-reader.
Results Twenty-three patients with a clinically significant incisional hernia and 17 control patients were included. The study groups had significant differences in mean BMI. There was a significant difference between mean AGEs in patients with and without incisional hernia after midline incision (3.00 ± 0.15 vs. 2.56 ± 0.11, T test $p = 0.03$).
Conclusion AGE accumulation measured in the skin indirectly with autofluorescence might be associated with incisional hernia. Prospective larger trials should confirm this finding.

Keywords Incisional hernia · Collagen cross links · Biomarker · Advanced glycation endproducts

Introduction

Incisional hernia is the most frequent complication of abdominal surgery requiring a reoperation. The reported incidence of incisional hernia following abdominal surgery ranges from 2 to 30% [1–6]. The cause of this frequent complication of abdominal surgery is unclear. Several

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factors may play a role, like suboptimal operative technique, postoperative complications such as wound infections, increased abdominal wall tension or disturbed metabolism in the extracellular matrix [7].

A strong indication that the latter may play a role is the increased incidence of abdominal wall hernias in patients with aortic aneurysmatic disease. Other patient related factors like age, smoking, type 2 diabetes mellitus (DM), obesity, connective tissue diseases (i.e., Marfan's disease) and renal failure, are also known to affect both the metabolism in the extracellular matrix thereby promoting incisional hernia formation. [2, 7–10].

In other connective tissue diseases like prolapse of the posterior vaginal wall, a significant higher level of Advanced Glycolysation End products (AGEs) was found in tissue samples of the posterior vaginal wall [11, 12]. AGEs are essential biomarkers of metabolic and glycemic stress. AGEs form cross-links with matrix proteins like collagen, laminin and elastin, undermining their flexibility. [13, 14] AGEs have been implicated as causative factors in the progression of age-related diseases, such as atherosclerosis, diabetes and renal failure. AGEs accumulate in the human body with age [15].

Due to the auto-fluorescent properties of AGEs, measurements can be carried out on the skin using auto-fluorescent readers (AF readers) [16]. The levels of accumulated AGEs measured in the skin correlate with systemic AGE levels [17].

This study aimed to explore the relationship between AGE levels, (measured with autofluorescence) in incisional hernia patients in comparison to control patients with a history of abdominal surgery without an incisional hernia after surgery.

Materials and methods

Study population

The local medical ethical committee approved the study protocol and signed informed consent was obtained from all participants. Forty patients with a history of open abdominal surgery through a midline incision were included. The patients were grouped in two categories. The first group (the cases) were patients with a clinically relevant incisional hernia or history of incisional hernia after a median laparotomy at least 1 year before the study. The second group consisted of a control group of patients, who had undergone a median laparotomy at least 1 year before the study, without development of an incisional hernia, neither on CT-scan nor during physical examination. Patients with a follow up shorter than 2 years have had a CT-scan for diagnosis of "non-incisional" hernia. Patients with a parastomal hernia and patients with a darker skin tone were excluded, as the AGE-reader has not been validated in patients with this skin type.

AGE measurements

Skin AGE measurements were performed using a non-invasive AGE-reader (www.diagnoptics.com, DiagnOptics BV, Groningen, the Netherlands) [18]. The AGE-reader illuminates skin surfaces of approximately 4 cm^2, with a wavelength between 300 and 420 nm (peak excitation 370 nm). Light reflections of the skin are measured with a spectrometer in the 300- to 600-nm range, using 200-µm glass fiber. Skin-auto fluorescence (SAF) is measured on the volar side of the lower arm, 10–15 cm below the elbow fold. Three consecutive measurements were performed on each patient. Hereafter, the mean SAF was calculated for each patient, to rule out local disruptions of auto fluorescence by imperfections of skin.

Statistics

Differences on baseline characteristics between cases and controls were tested using Chi-square tests (categorical variables: indication for surgery, ASA, comorbidity, smoking, sex) or Student's *t* test (continuous variables: age, BMI). If distributional assumption of normality was violated, Mann-Whitney (MW) test was used. Mean SAF was calculated. The difference of mean SAF between cases and controls was tested using Student's *t* test. The distribution between mean SAF and AGE was visualized in a graph. ROC curve analysis was performed to evaluate the diagnostic value of SAF for incisional hernia formation. Statistical analysis was conducted using SPSS 20.0 for MAC. A $p < 0.05$ was considered to be statistically significant.

Results

Twenty-three patients with a clinically significant incisional hernia and 17 control patients were included. Twelve out of 17 (71%) controls have had a CT-scan or ultrasound to confirm the non-incisional hernia. The other 5 patients had non-incisional hernia diagnosed by clinical examination at least 2 years after operation. The study groups had significant differences in mean BMI. There was no difference between groups with regard to indication for operation, age, sex, American Society of Anesthesiologists score (ASA), cardiovascular disease, renal failure smoking, and presence of COPD (Table 1).

Skin auto fluorescence

There was a significant difference between mean SAF in patients with and without incisional hernia after midline

Table 1 Patient characteristics

Basic characteristics	Incisional hernia $n = 23$	No incisional hernia $n = 17$	p value
Indication, n (%)			
Cancer	6 (26)	10 (58)	NS*
AAA	3 (13)	1 (6)	
Other	14 (61)	6 (35)	
ASA-classification (%)			
ASA 1	4 (24)	5 (29)	NS*
ASA 2	10 (40)	9 (53)	
ASA 3	8 (32)	3 (18)	
ASA 4	1 (4)	0	
Comorbidity, n (%)			
COPD	9 (36)	2 (12)	NS*
Diabetes mellitus 2	6 (24)	2 (12)	NS*
Renal failure	1 (4)	2 (12)	NS*
Cardiovascular disease	13 (52)	9 (53)	NS*
Smoking (%)	3 (12)	7 (36)	NS*
Gender			
Male	16 (64)	6 (35)	NS*
Female	7 (36)	11 (65)	
Age (range)			
Years	67 (54–82)	71 (55–89)	NS**
BMI (mean)			
Kg/m^2	30 (21–45)	25 (18–32)	0,005**

* Chi-square

** t test

incision (3.00 ± 0.15 vs. 2.56 ± 0.11, T test $p = 0.03$). Figure 1 shows the relation between SAF and age in years.

ROC curve analysis

ROC curve analysis was performed to evaluate the diagnostic value of SAF for incisional hernia formation (Fig. 2). The area under the curve (AUC) value for mean SAF was 0.71 (95% CI 0.55–0.87) The mean SAF level, at cut-off value of $\geq$2.5 or higher had 83% sensitivity and 53% specificity.

Discussion

This is the first study investigating the relationship between AGEs and incisional hernia, the most frequent long-term complication in general surgery. The non-invasive measurement of SAF has a potential to select patients who are suitable for major surgical procedures. In this study we found a difference in SAF between patients with an incisional hernia and controls. In our study there were some younger incisional hernia patients with relatively higher levels of SAF and older patients without incisional hernia with relatively lower levels of SAF. The ROC curve showed a moderate sensitive diagnostic value of SAF for incisional hernia formation, but low specificity.

Age, smoking, type 2 diabetes mellitus (DM), obesity, aorta aneurism, and renal failure are known individual risk factors for incisional hernia [10]. AGEs are elevated in DM, cardiovascular disease, smoking and renal failure patients especially in patients with a severe form and longer duration [15]. Measuring AGE accumulation with SAF may differentiate between high and lower risk groups within these known risk groups. For example, an SAF level of above $\geq$2.70 is an independent risk factor for microvascular and macrovascular complications in DM and an independent predictor in 5-year mortality and cardiovascular events in patients with peripheral arterial disease [19, 20].

It is still unclear, what is the role of AGEs in connective tissue metabolism and particularly in wound healing. Increased AGEs are correlated to inhibited expression of collagen type I and type III in human gingival fibroblasts [21]. Several studies have shown that patients with incisional hernia have a decreased collagen type I/III ratio in the scar of the abdominal wall, the skin and in the uninjured fascia laterally to the scar postoperatively [8, 22–24].

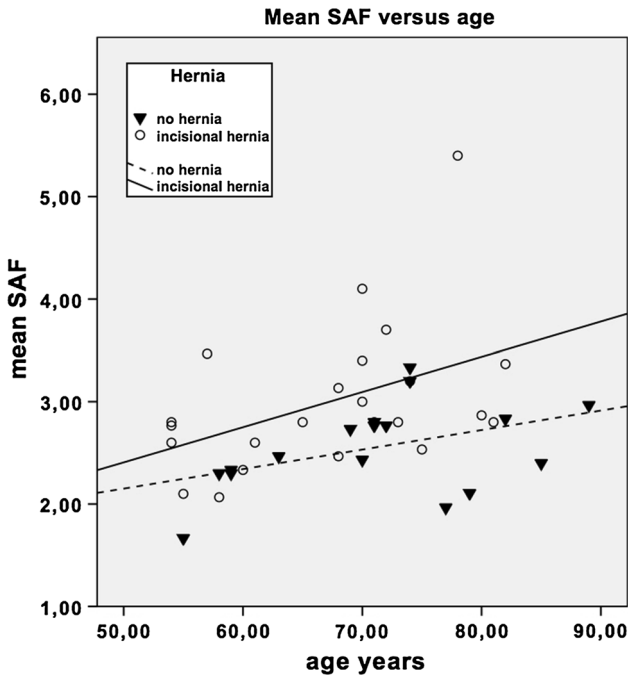

Fig. 1 Relation between age in years and mean SAF

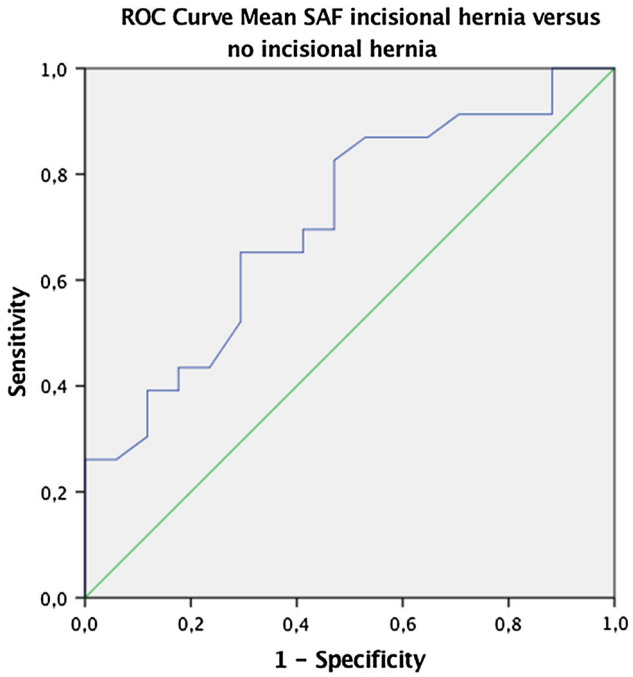

Fig. 2 ROC curve Mean SAF incisional hernia versus no incisional hernia

A decreased collagen type I/III ratio has been associated with increased fibrotic tissue, resulting in an impaired strength of connective tissue. Also Matrix Metallo Proteinases (MMPs) 1, 2, 9 and 13 are up-regulated in incisional hernia patients. The balance between MMPs and their inhibitors, the tissue inhibitors of metalloproteinases (TIMPs), is one of the responsible factors for the remodeling of tissues [23, 25, 26].

This study has several limitations. A limitation of this exploratory study is the small number of patients that was included. No previous studies have evaluated the correlation between SAF and hernia disease, and, therefore, no sample size calculation could be performed. For this pilot study we included clinical significant incisional hernia's which we compared with patients without clinical incisional hernia by the majority (71%) controlled by radiological examination. The majority of patients were examined several years after surgery. Unfortunately there is a small risk that later on incisional hernia might occur in the non-hernia groups. AGEs can also be elevated after a period of oxidative stress. A period of critical illness in the hospital or even intensive care may also influence SAF. The optimal set up for a future trial would be a large prospective (registry) trial with SAF measurements before surgery with standardized small bites closure technique and incisional hernia occurrence at least 3 years after surgery with both physical and radiological examination as a primary outcome.

A preoperative screening tool or biomarker for postoperative surgical complications may be helpful to select patients who are fit for surgery. This could lead to prevention of major complications like wound complications or anastomotic leakage. High-risk patients may need a prophylactic mesh or a protective ileostomy. Larger prospective studies are needed to investigate this preoperative screening marker.

Conclusion

AGE accumulation measured in the skin indirectly with autofluorescence might be associated with incisional hernia. Large prospective trials with a standardized closure technique and radiological outcome measurement should confirm this data and investigate other extracellular matrix components to select high-risk patients for a tailor made approach.

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Author contributions JJH: literature search, study design, data collection, data interpretation, data analysis, writing; HHE: study design, data interpretation, writing; KAV: data collection, data interpretation, writing; MCC: data collection, data interpretation, writing; ACH: data collection, data interpretation, writing; JJ: study design, data interpretation, writing; JFL: study design, data interpretation, writing.

Compliance with ethical standards

Conflict of interest J.J. Harlaar, declares no conflict of interest H.H. Eker declares no conflict of interest K.A. Vakalopoulos declares no conflict of interest M Castro Cabezas declares no conflict of interest

A.C. van der Ham declares no conflict of interest W.W. Vrijland declares no conflict of interest J. Jeekel declares no conflict of interest J.F. Lange declares no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Human and animal rights Compliance with ethical standards was adhered to Institutional Review and Board approval.

Informed consent Informed consent was obtained from all individual participants included in the study.

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