Original Research

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# The influence of GSTT/GSTM null genotypes in scarring

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## Abstract

**Background and aims.** The process of scarring is a common denominator of interest for the medical field. From general medicine to dentistry, pathological scar tissue represents a challenge in providing optimal care to a patient. The present study aims to investigate whether a systemically reduced antioxidant potential, revealed by null isoforms of glutathione S transferase, affects the process of scarring in a group of female patients.

**Methods.** The study is based on a group of 54 patients with physiological scars after a 6-month observation period, as well as 18 patients with hypertrophic or atrophic scars. Peripheral venous blood was collected, from which DNA was extracted using a commercial kit. Genotyping followed a Multiplex PCR protocol for GSTT1/GSTM1.

**Results.** In a dominant model, the combination of wild type (heterozygous or homozygous) GSTT1 and GSTM1 was negatively associated with pathological scarring, with the wild type (heterozygous or homozygous) GSTM1 genotype being potentially responsible for this effect. Other factors affecting pathological scarring were investigated: family history, phototype, as well as scores on the POSAS and SCAR scales.

**Conclusions.** The presence of GSTT1 and GSTM1 alleles brings forward an increased antioxidant capacity, serving as a protective factor for patients during scar formation.

**Keywords:** glutathione S-transferase M1, glutathione S-transferase T1, genotype, scarring

## Background and aims

Scarring defines the phenomenon whereby a skin lesion reaching the dermis is repaired, leading to a supple, light, thin strip of skin - this constitutes the physiological response to skin injury. However, pathological responses to skin injury are common: some authors estimate that 30 up to 90% of patients may suffer from pathological scarring [1]. This brings forward a psychological and functional burden for the individual, negatively affecting their quality of life [2,3].

Thus, it would be useful to pinpoint an individual's predisposition to pathological scarring, in order to optimize surgical planning and prophylactic measures. Pathological scars have been investigated with regards to the local modifications which contribute to their formation; however, little research is carried out on the intrinsic factors which predispose a patient towards developing pathological scars.

Glutathione S transferase is a phase-two enzyme involved in detoxification; its many isoforms constitute protective factors against oxidative stress, inflammation, mutagenicity and genotoxicity [4]. Polymorphic variants of these genes lead to an imbalance between anti- and pro-oxidant factors, having been associated with a plethora of conditions, ranging from type 2 diabetes mellitus [5a considerably large number of genes and polymorphic gene variants are screened and linked with the complex pathogenesis of type 2 diabetes (DM] to psoriasis [6].

In the following study, we investigated whether null alleles of

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the isoforms GSTT1 and GSTM1 are involved in the development of pathological scars.

## Methods

The present study follows a case-control design, with 54 controls with physiological scars, 13 patients with hypertrophic scars and 5 patients with atrophic scars. The patients were recruited following caesarean section at the 1st Gynaecology Clinic in Cluj-Napoca.

Inclusion criteria were as follows:

- over 18 years of age
- able and willing to offer informed consent to the procedure and follow-up
- patients undergoing planned caesarean section with no postoperative complications

Exclusion criteria were as follows:

- patients unwilling or unable to maintain contact with the investigator for follow-up
- incision overlapping with previous surgeries or trauma

After signing the informed consent, a peripheral blood sample was drawn in a purple cap K3EDTA vacutainer and stored at 4˚C until processing. Patient history was collected, as well as an objective evaluation of the patients' skin. POSAS and SCAR scales were used. Photographs were taken of the scars.

The scarring process was evaluated at 3 and 6 months, by completing SCAR and POSAS scores by phone interview and using photographs taken by the patients and transmitted to the investigator.

The Ethics Committee of Iuliu Hatieganu University of Medicine and Pharmacy approved the design of the study and the collection of data (approval of the study no. 299, 26.07.2018), in accordance to the Declaration of Helsinki.

After collection of the patient samples, the genomic DNA was extracted using a commercially available kit (Wizard Genomic DNA Purification Kit, Promega, Madison, Wisconsin, USA). The resulting DNA was stored at -20˚C.

Genotyping followed a Multiplex PCR protocol, using the following primers:

- GSTM1: 5′-GAACTCCCTGAAAAGCTAAAGC-3′ and 5′-GTTGGGCTCAAATATAGGGTGG-3′
- GSTT1: 5′-TTCCTTACTGGTCCTCACATCTC-3′ and 5′-TCACCGGATCATGGCCAGCA-3′
- βglobin: 5′-CAACTTCATCCACGTTCACC-3′ and 5′-GAAGAGCCAAGGACAGGTAC-3′

βglobin was used as an internal marker to validate amplification.

The samples amplified under the following conditions:

- initial denaturation at 94˚C for 5 min
- 35 cycles of
  - denaturation at 94˚C for 1min
  - annealing at 58˚C for 1 min
  - polymerization at 72˚C for 1 min
- final elongation at 72˚C for 1 min

The amplification product was migrated through 2% agarose gel (MetaPhor Agarose, Lonza, Rockland, ME, USA stained with RedSafe Nucleic Acid Staining Solution, Intron Biotechnology), resulting 3 possible fragments:

- 219 bp fragment, corresponding to GSTM1
- 480bp fragment, corresponding to GSTT1
- 299bp fragment, corresponding to βglobin

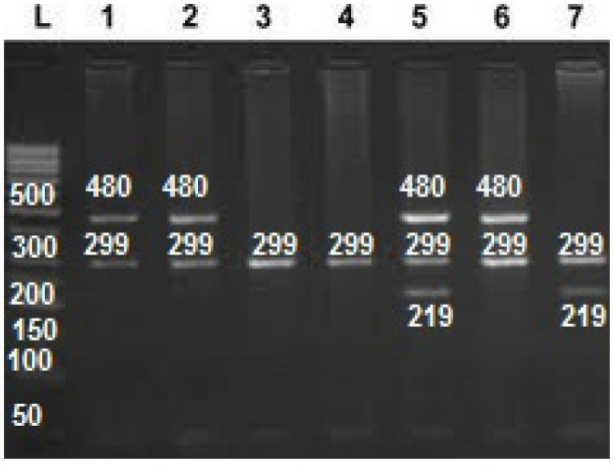


**Figure 1.** Electrophoresis gel depicting the possible outcomes of the protocol used. Column L is a 50bp ladder, Columns 1, 2, 6 are GSTT1 wild type (heterozygous or homozygous) - GSTM1 null genotypes, Columns 3, 4 are GSTT1 and GSTM1 null genotypes, Column 5 is GSTT1 - GSTM1 wild type (heterozygous or homozygous), Column 7 is GSTM1 wild type (heterozygous or homozygous) - GSTT1 null genotype.

The obtained data were transferred into a Microsoft Excel Spreadsheet. The statistical analysis was performed by using IBM SPSS 21 package. The chi-square ($\chi 2$) test was used to evaluate the Hardy-Weinberg (HW) equilibrium. Genotypic dependence between groups was calculated by the use of $\chi 2$ test or Fisher's exact test, and odds ratio (OR) was determined from 2x2 contingency tables. In addition, Spearman's rho test and Mann Whitney U test were performed to analyze the correlation of age, gender, scarring type and genotype for each study group. We measured the association between the SCAR and POSAS scoring system and genotypes using Spearman correlation. A p-value of 0.05 (two-sided) was considered the limit of significance.

## Results

The clinical aspects and demographic data of participants are presented in Table I. Differences in age of cases and controls were not corrected in all statistical analyses. Control samples were under HW equilibrium ($\chi 2 = 4.55$, $P = 0.045$), but patient samples did not confirm the HW expectation ($\chi 2 = 0.08$, $P = 0.79$).

**Table I.** Baseline characteristics of studied/control patients.

| N = 72 | Age (years) Mean SD | Current weight (kg) Mean SD | Height (m) Mean SD | Preconception BMI Mean SD | Weight gain during pregnancy (kg) Mean SD | Pre-conception weight (kg) Mean SD | After birth BMI Mean SD |
|---|---|---|---|---|---|---|---|
| normal scarring | 30.96 | 80.06 | 1.64 | 23.95 | 15.26 | 64.80 | *29.59* |
| 54 | 5.29 | 14.57 | 0.06 | 5.17 | 5.32 | 14.69 | *5.01* |
| hypertrophic scarring | 30.77 | 73.15 | 1.64 | 21.88 | 14.15 | 59.00 | *27.16* |
| 13 | 4.48 | 14.50 | 0.08 | 3.50 | 5.77 | 12.28 | *4.25* |
| atrophic scarring | 31.20 | 85.40 | 1.61 | 27.76 | 13.20 | 72.20 | *32.91* |
| 5 | 4.82 | 21.78 | 0.04 | 8.72 | 4.44 | 24.83 | *7.58* |

**Table II.** Odds Ratios (OR) of GST and Scarring in both groups combined.

| | | Normal scarring | Hypertrophic scarring | Atrophic scarring | OR (95% CI) | P-value |
|---|---|---|---|---|---|---|
| GSTT | Wild type (heterozygous or homozygous) | 52 | 12 | 4 | 0.462 (0.039-5.518) | 0.2 |
| | Null | 2 | 1 | 1 | 0.308 (0.040-2.363) | 0.12 |
| GSTM | Wild type (heterozygous or homozygous) | 26 | 3 | 2 | 0.323 (0.080-1.305) | 0.127 |
| | Null | 28 | 10 | 3 | 0.414 (0.130-1.323) | 0.1 |

*Fisher's exact test $p<0.05$*

Age was not associated with the predisposition for scarring development. The difference in weight measurements before and after birth were statistically significant (p=0.03) in all examined groups. There was a difference between normal and hypertrophic scarring individuals with regards to BMI (p=0.01). Hypertrophic scarring was positively correlated with weight, BMI and age (p=0.04)

Distinct frequencies of isolated and combined GSTM and GSTT genotypes were seen in patients and controls (Table II). Individuals with distinct genotypes were not under similar risks for the pathological scarring.

The GSTT1 wild type (heterozygous or homozygous) genotype was significantly more frequent in normal and hypertrophic scarring patients than the null GSTT (96.29% versus 3.7%, 92.3% versus 7.69%) (p = 0.04), while GSTM null genotype was more frequent in normal and hypertrophic scarring than the GSTM1 wild type (heterozygous or homozygous) (51.855% versus 48.14%, 76.92% versus 23.07%) (p = 0.05). Patients with atrophic scarring were not statistically different in terms of GSTM1 or GSTT1 genotypes.

The risk evaluation using Fisher's exact test did not prove any statistical significance between scar development predisposition in association with the GSTT and GSTM genotypes individually analyzed (OR= 0.154, 0.011-2.086 CI, p=0.1; OR= 0.718, 0.111-4.645 CI, p=0.54 similarly).

In Table III the frequency distributions of the double combination between GSTM and GSTT in cases and controls by way of Fisher's exact test are presented. The combination of two genotypes showed no increased risk of developing pathological scarring in pregnant women, not even the combination of two high-risk genotypes, GSTM null and GSTT null. We found a decreased risk for pathological scarring for the combination between GSTM wild type (heterozygous or homozygous) and GSTT wild type (heterozygous or homozygous) in normal scarring compared to hypertrophic scarring. No statistically significant observations were found regarding atrophic scarring due to the low number of subjects.

**Table III.** Inferential analysis on genotype combinations (GSTT1/GSTM1 null or wild type (heterozygous or homozygous) genotypes).

| Variables | Fisher'exact test | |
|---|---|---|
| | OR (95%) | p-value |
| GSTTw/GSTMw | 0.449 (0.126-0.784) | 0.036 |
| GSTTw/GSTMn | 0.76 (0.219-1.098) | 0.065 |
| GSTTn/GSTMw | 0.556 (0.168-0.982) | 0.066 |
| GSTTn/GSTMn | 1.127 (0.905-1.42) | 0.100 |

Prenatal normal BMI seems to be associated with physiological scarring, having a mild POSAS and SCAR scoring (p = 0.045). Weight gain over 12 kilograms is associated with hypertrophic scarring (OR = 0.118, 0.708-1.414 CI, p = 0.04). A negative history for skin diseases

is mildly suggestive for a better outcome in scarring process (OR = 0.563, 0.0201-736 CI, p = 0.04). Individuals carrying GSTT wild type (heterozygous or homozygous) genotype and having the type 4 of Fitzpatrick scale are associated with a borderline decreased risk for developing pathological scarring (OR = 0.963, 0.736-1.174 CI, p = 0.048). The GSTT evaluation regarding the outcomes using POSAS score at 3 and 6 months revealed a lower score in patients with hypertrophic scarring (p-0.04). The SCAR score was used to evaluate the outcomes at 3 and 6 months and revealed no statistical differences between normal and pathological scarring in relationship with the genotypes investigated.

## Discussion

A combination of wild type (heterozygous or homozygous) genotypes of GSTT1 and GSTM1 seem to have a protective effect regarding the development of pathological scars in the population studied. This effect seems to rely somewhat on GSTT1, considering the lower POSAS scores noticed in patients with wild type (heterozygous or homozygous) GSTT1 genotypes (a low POSAS score is indicative of a scar leaning towards the physiological aspect). This evidence supports the findings of a GWAS which pinpointed that glutathione-s-transferase is under-expressed in keloid tissue samples [7].

Successful scarring involves complex interactions between growth factors and growth inhibitors, collagen production and destruction, fibroblast proliferation, angiogenesis. The most severe pathological scars, keloid scars, have been likened to an in situ malignant tumor [8], with overexpression of angiogenesis factors, decrease of fibroblast apoptosis [9]. With GST null genotypes being associated with malignancies such as lung cancer [10randomized, case control study for the evaluation of the frequency of A1AT (MS, MZ], it stands to reason that a preserved antioxidant capacity, represented by wild type (heterozygous or homozygous) GSTT1 and GSTM1 alleles, would provide a positive medium for physiological scarring to occur.

Secondary findings show that a prenatal BMI ranging between 18 and 25 is a predictive factor for physiological scarring, while pregnancy weight gain of over 12 kgs is negatively associated with the development of hypertrophic scars. Regarding phototype, patients with a Fitzpatrick phototype 4 seem to benefit from a protective influence of wild type (heterozygous or homozygous) GSTT1 genotype with regards to pathological scarring.

To our knowledge, the present study is the first investigating germline mutations in the glutathione-s-transferase system with regards to scarring. Another strong point of the study design is the uniformity of the intervention: all patients went through a scheduled caesarian section, in the same clinic using the same operative protocols, thus ruling out technique differences.

The weak points of our study reside in the genotyping protocol's inability to determine whether patients with a positive GST isoform are heterozygous or homozygous carriers of the allele. Furthermore, the patients were enrolled in the study during a period of 3 months, and followed up for 6 months: this accounts for the uneven distribution of cases and controls throughout our groups.

In order to strengthen certain results, more patients are needed, specifically in the pathological scarring group, and genotyping should be carried out using two separate RFLP-PCR protocols, thus creating a more accurate genotype representation.

While in its initial state, research into germline mutations related to pathological scarring is valuable when considering the perspective of personalized medicine. Pinpointing the mutations which predispose a patient to develop pathological scars offers the possibility of pre-operative planning and primary prophylaxis, thus sparing the need for reintervention or costly and time-consuming treatments ahead. Furthermore, understanding the molecular basis of scarring is the first step in creating molecular treatment options for keloid scars, as seen with TGF-1 promoter silencing therapy [11].

## Conclusions

GSTT1 and GSTM1 wild type (heterozygous or homozygous) genotypes seem to be associated with physiological scarring in a group of Caucasian patients. A normal prenatal BMI seems to be associated with physiological scarring after caesarean section.

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