Determination of reticular fibers in tissues fixed with sugarcane molasses

Research Article

ABSTRACT

Reticular fiber consists of one or more types of very thin and delicately woven type III collagen strands, which form a highly ordered cellular network and provide a supportive network. Since most of these collagen types are combined with carbohydrates, they react with silver stains and periodic acid-Schiff reagent, but are not shown by ordinary histological stains like those using hematoxylin. In this study, it was aimed to determine the reticular threads by using silver staining technique (Gordon Sweet –GS-) after routine histological tissue follow-up in tissues fixed with sugarcane molasses and formol. The reticular fibers in the tissues fixed with sugarcane molasses were compared with those in the tissues fixed with formol. According to the findings, the staining quality of the liver and spleen tissues fixed with sugarcane molasses showed similar characteristics with the tissues fixed with formol. Very weak staining was observed in stomach, kidney, skin and testicle tissues. The fact that the tissues fixed in the same fixation solution show different results in the Gordon Sweet (GS) staining method shows us that this issue needs to be supported by more detailed studies.

Keywords: Reticular fibers, fixing, sugarcane molasses, formol.

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INTRODUCTION

Reticular fibers are a type of connective tissue that is not observed in routine histological staining. They (reticular fibers) are abundant in the organism, but are scattered and mixed with other types of fibers (such as elastic fibers). These fibers are found in very high amounts according to their location in the organism (Alvarenga and Marti, 2014). Their reticular tissue has a high cellular content and is a special type of connective tissue that is predominantly observed in various places. Reticular fibers have a branched and web-like structure called the reticulum. These fibers are actually type III collagen fibers. Compared to type I collagen, which is abundant in the organism, type III fibers are narrower in diameter and have a high carbohydrate content. They consist of collagen molecules, each of which is a trimer of 3 alpha-1 chains. When examined with transmission electron microscopy (TEM), the fibers consist of aggregations of several transverse bands with a distance of 68 nm between two adjacent aggregations (Gelse, 2003; Kumar et al, 2015).

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Reticular fibers are found in other types of connective tissue as well as reticular connective tissue, but in very small amounts. In fact, the reticular fibers that develop first in the structure of each tissue containing these fibers serve as an important support for the tissue. They support the early synthesized extracellular matrix during wound healing, scar tissue formation and embryonic development. As the fibers mature, most of them are replaced by stronger type I collagen fibers (Pepin et al, 2000). To become histologically visible under the microscope, reticular fibers need special staining procedures as they are weakly stained with hematoxylin-eosin (H&E). These are: Periodic Acid-Schiff (PAS) reaction, Gomori and Wilder silver nitrate staining methods. Reticular fibers are found in connective tissue in endocrine glands, liver, bone marrow, and lymphoid organs. The structure of these tissues and organs all consist of densely branched septa and internal canals. The reticular fibers move deep into the tissue or organ along the path followed by these septa and canals. Therefore, the task of the skeletal structure formed by the reticular fibers is to support the cells and the small canals that circulate between the related tissues and organs. For example, it supports hepatocytes and sinusoids in the liver.

Histological techniques, which cover the processes such as fixation, tissue tracking and staining, basically aim to capture and visualize the relations between intracellular and extracellular tissue sections and various cells at a certain moment. The first stage of this is to fix the form closest to the living state of the tissue to be examined. It is assumed that the visual product obtained as a result of efforts starting from fixation and continuing with paraffin sections and histochemical or immunohistochemical staining, provides the best possible static image of living tissue. Thus, efforts during fixation and tissue processing must keep the changes following the separation of the tissue from the living organism to a minimum, in terms of structural and chemical integrity. The purpose of the fixation is to coagulate or precipitate proteins, lipids, carbohydrates and other substances in the protoplasm, making them resistant to the reagents they will encounter until the microscopic section is prepared. The fixation process protects the tissue from decomposition by autolysis, bacteria or fungi caused by cellular enzymes, inactivates or kills infectious agents, hardens the tissue, and stabilizes the tissue components. The agents used for fixation are chemicals, which are called fixatives. A good fixative should have lethal, penetrating and hardening qualities in the tissue. Fixation is an essential step in obtaining a quality section. However, fixation has also undesirable effects on tissues. These include changes in protein structure, dissolution of tissue components, shrinkage of tissue, and degradation of nucleic acids.

In this study, it was aimed to determine the reticular fibers in tissues fixed with sugarcane molasses and formol using silver staining technique (Gordon Sweet –GS-). The reticular fibers in the tissues fixed with sugarcane molasses were compared with those in the tissues fixed with formol.

MATERIAL and METHOD

Experimental Procedure

In this study, the experimental animals were obtained from Selcuk University Experimental Medicine Application and Research Center (This study was carried out by Selcuk University Experimental Medicine Application and Research Center with the approval of the ethics committee numbered 2017-14). Laboratory studies were carried out in Aksaray University Central Research Laboratory. Liver, spleen, kidney and testicle tissue samples from 4 rats of both genders were used as material in the study. Tissue samples were divided into two parts and fixed in 30% sugarcane molasses

(Group A) and 10% buffered formal-saline (Group B) for 24 hours at room temperature.

Preparation of Fixative Solutions

Group A- 30% sugarcane molasses – the sugarcane molasses purchased was prepared by diluting with distilled water so that the final sugarcane molasses concentration was 30%.

Group B- 10% buffered formaldehyde - 10% buffered formol solution with pH 7 was prepared with 0.1M phosphate buffer. After 24 hours of fixation, tissue samples were blocked in paraffin after washing, dehydration and polishing with known histological techniques. Gordon Sweet (GS) staining method was applied to 5 µm thick sections taken from the blocks. After staining, the preparations covered with entellan were evaluated under the light microscope.

RESULTS

Sugarcane Molasses Solution (30%)

Liver Well stained reticular fibers were observed around the sinusoids and major blood vessels (Figure 1a).

Spleen: Well stained reticular fibers were observed in the capsule and intercellular space (Figure 2a).

Kidney: Palely stained reticular fibers were observed between the capsule, corpusculum renis and tubules (Figure 3a).

Testicle: Very thin and pale staining was observed around the tubulus seminiferus contours (Figure 4a).

Formol Solution (10%)

Liver: Well stained reticular fibers were observed around the sinusoids (Figure 1b).

Spleen: Well stained reticular fibers were observed in the capsule and intercellular space (Figure 2b).

Kidney: Well stained reticular fibers were observed between the capsule, corpusculum renis and tubules (Figure 3b).

Testicle: Well stained reticular fibers were observed around the tubulus seminiferus contours (Figure 4b).

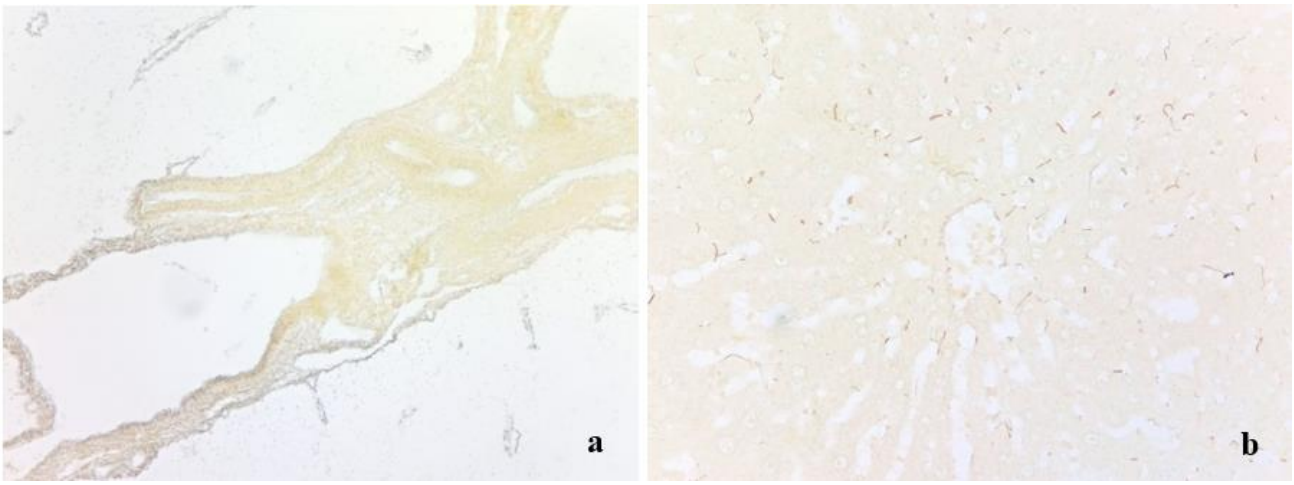

Figure 1. Liver, Gordon Sweet (GS) staining method. a) Tissue fixed with sugarcane molasses solution (30%), b) Tissue fixed with formol solution (10%).

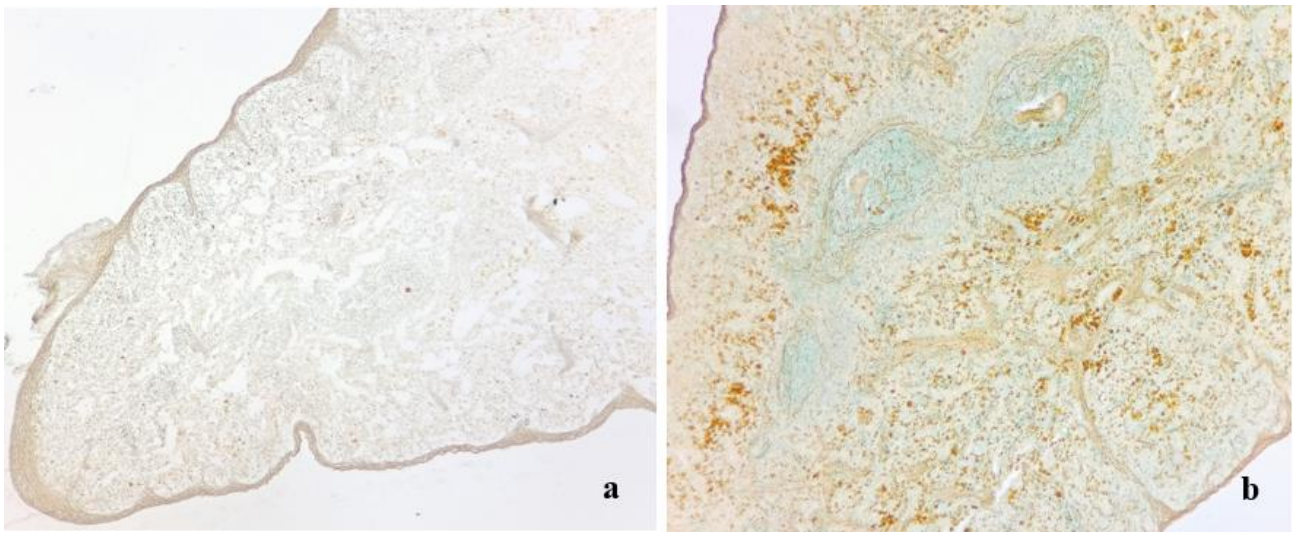

Figure 2. Spleen, Gordon Sweet (GS) staining method. a) Tissue fixed with sugarcane molasses solution (30%), b) Tissue fixed with formol solution (10%).

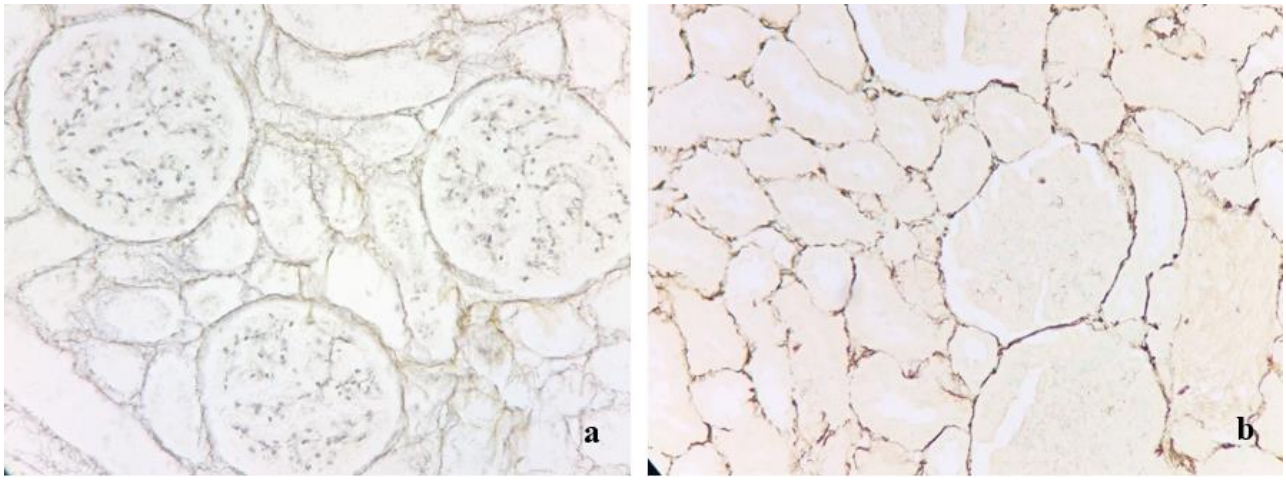

Figure 3. Kidney, Gordon Sweet (GS) staining method. a) Tissue fixed with sugarcane molasses solution (30%), b) Tissue fixed with formol solution (10%).

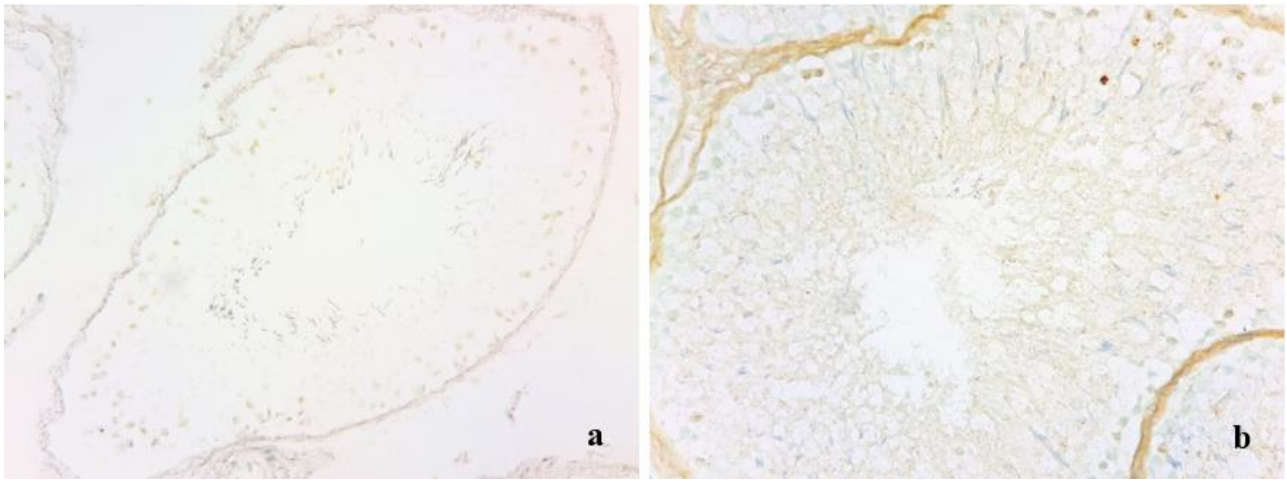

Figure 4. Testicle, Gordon Sweet (GS) staining method. a) Tissue fixed with sugarcane molasses solution (30%), b) Tissue fixed with formol solution (10%).

DISCUSSION

Fixation is an initial and important step in preparing tissue for microscopic examination. The main purpose of fixation is to keep the tissues in the closest form to their living state, to prevent bacterial destruction, to prevent autolysis, and to increase the index of better examination of the tissue. In this study, the staining qualities of reticular fibers in the tissues fixed with sugarcane molasses were compared with those in the tissues fixed with formol by using Gordon Sweet (GS) staining method.

CONCLUSION

According to the findings, the staining quality of the liver and spleen tissues fixed with sugarcane molasses showed similar characteristics with the tissues fixed with formol. Very weak staining was observed in kidney and testicle tissues. The fact that the tissues fixed in the same fixation solution show different results in the Gordon Sweet (GS) staining method shows us that this issue needs to be supported by more detailed studies.

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Ethical approval:

This study was carried out by Selcuk University Experimental Medicine Application and Research Center with the approval of the ethics committee numbered 2017-14.

Conflict of interest: The authors declare that there is no conflict of interest.

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