Research Article

Effect of Microwave Irradiation on staining methods of Connective Tissue Fibers (collagen Fiber)

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***Abstract*.**

Background: This study was conducted at Elsheikh Abdallah Elbadri University in barber to assess the effect of the microwave irradiation on staining procedures for connective tissue fiber especially collagen fiber.

Rationale: Most of histochemical staining procedures require long time to be performed. Reduction of staining time with improvement of staining quality will lead to more rapid and accurate diagnosis

Objective: To compare between ordinary and microwave irradiation methods for Masson-Trichrome staining in order to reduction time of staining and increase the staining quality.

Methodology: 50 samples was collected from appendix and skin, in order to stain collagen fiber by Masson Trichrome with both technique by using microwave irradiation and by ordinary method, to compare the quality of stain and time consumption in these two methods.

Results: The study showed that 17(68%) of samples give high quality result, 5(20%) give midd quality and 3(12%) give poor quality stain from total 25 samples done by microwave in three minutes. While 7(28%) of samples give high quality, 17(68%) give middle quality and 1(14%) give poor quality from total 25 sample done by ordinary method in thirty minutes.

Conclusion: from this study that applying microwave in staining collagen fiber by Masson Trichrome stain give high quality result and little time compare to ordinary method.

Keywords: Connective tissue, Masson Trichrome, Microwave irradiation Collagen fiber

I. Introduction:

Microwaves are waves of energy, heatless. The amount of microwave energy absorbed by a given specimen (or "load") based on several factors, the important one is the load size, its location to receive the waves, and the dielectric and thermal characteristics of the material. (1)

Staining of tissues is depend on two factors; distribution of the dye into the cells and dye binding to the substrate. Microwaves accelerate this diffusion and binding as well as reducing the time of staining. (2)

Ordinary staining methods that usually take several minutes, might be done in a microwave oven in seconds. There for other ordinary staining methods which take hours could be done in minutes (3).

II. Connective tissue:

Connective tissue is one of the four tissue types found between other tissues everywhere throughout the body. It develops from the mesoderm. The term 'connect' comes from the Latin word 'connecter' meaning 'to bind'. The essential function is connecting and provides support to other tissues of the body. The connective tissue generally consists of a cellular portion in and surrounded framework of a non-cellular substance. The cell types of connective tissue can contain entities such as fibroblasts, mast cells, histiocytes, adipose cells, reticular cells, osteoblasts and osteocytes, chondroblasts and chondrocytes, blood cells, and blood-forming cells. (4)

III. Masson Trichrome:

Using acid base chemistry three dyes are employed to selectively stain muscle, collagen fiber, fibrin and erythrocytes on formalin fixed section. The sections are treated with bouin's solution to intensify the final coloration. Nuclei will be stained black (by Wiegert's iron Heamatoxylin) and the background stained red (by biebrich scarlet acid fuchsine) after treatment with Phosphotungstic or Phosphomolybdic acid. Collagen is demonstrated by staining with aniline blue, rinsing in acetic acid after staining renders the shades of color more delicate and transparent. (4)

IV. Methodology

a) Study design:

Retrospective, comparative study.

b) Study area:

Department of Histopathology, Laboratory of Elsheikh Abdallah Elbadri University, Barbar, Sudan. December 2019.

c) Samples size:

Fifty blocks were obtained from Atbara educational hospital and histopathology lab of Elsheikh Abdullah Elbadri University blocks were taken from appendicular tissue and skin. The 25 sections were stained with ordinary method and the other 25 sections were stained with microwave method.

d) Gross examination:

Fixed specimen was received in the lab where it was examined macroscopically then the required part of specimen was determined, cut off small pieces of the

tissue and then were placed into labeled cassettes.

e) Sample processing:

Fifty blocks were placed in automated tissue-processing machine, processed of the blocks, which includes dehydration, clearing, and wax impregnation with 12 steps. The dehydration was done by immersing the basket containing the plastic cassettes in 70% alcohol for 2 hours, then 90% alcohol for another 2 hours, and 3 changes of absolute alcohol for 1 hour in each of the first two and two hours in the last one. After that clearing was done to remove alcohol from the tissue by immersing the basket in three changes of Xylene for one hour in the first one, and two hours in each of the other two changes. Finally, the wax impregnation was done by immersing the basket in three wax baths containing melted paraffin wax for one hour in the first bath, and two hours in each of the last two baths. Blocks of impregnated biopsy surrounded by paraffin wax was made using a mold of metal. Then fifty paraffin blocks were cutted by microtome at 3–4 μm, the ribbons were held by forceps, , then were placed in placed in water bath tap water, and adjusted to the proper temperature, then transported to slides and let air dry, finally dewaxation of sections by placed in hot air oven at 60-65°C Overnight. (4)

The 25 sections were deparaffinized with Xylene and hydrated in alcohols to Tap water, then slides were Placed in Wiegerts' Heamatoxylin Stain (Mixed equal parts Wiegert's "A" & "B"(just before use) For10 minutes. Slides were rinsed in water, and then differentiated by 1% Acid Alcohol 2-5seconds, rinsed in distilled water to stop reaction of differentiation and to remove access stain put in ruining tap water. Sections were placed in Acid Fuchsine for 5minutes, Rinsed in Distilled water. Then placed in Phosphomolybdic Acid for 5minutes, rinsed in distilled water, then slides were placed in Aniline Blue Stain for 1-5 minutes, and rinsed slide in Distilled water. Then differentiated with 1% Acetic Acid for 3-5 minutes. Dehydrated, clearing, mounting and finally covered by cover slide using a permanent mounting media. (4)

The other 25 Sections were hydrated like steps of ordinary staining methods. 800Wattmicrowave was adjusted at mid-low power. Then slides were placed in microwave oven with Wiegert hematoxylin's for 5-10 seconds, Incubated for 5-7secondsat room temperature, washed by distilled water, Then differentiation by acid alcohol for one second, Then stained in microwave with acid fuchsine for 5-10 seconds, incubated for 5-7 seconds at room temperature and washed by distilled water, treated in microwave with Phosphomolybdic Acid solution for 5-10 seconds, incubated for 5 seconds at room temperature and washed by distilled water, stained with Methylene blue 5 seconds in microwave oven, incubated for 5-7secondsat room temperature, washed, dehydrated, cleared, and mounted with mounting media. (5)

V. Results

The study showed that, there were 25 specimens. *Masson Trichrome staining with ordinary technique* showed 7(28%) specimens strongly positive (three positive marks +++), 17(68%) specimens moderately positive (++), and 1(4%) specimens weakly positive (+).

Masson Trichrome staining with microwave technique showed 17 (68%) specimens strongly positive, 5(20%) specimens moderately positive and 3 (12%) specimens' weakly positive.

The study results in Masson Trichrome for both techniques did not show negative results and they showed 24 specimens strongly positive.

The time requires for stain by microwave irradiation is 3 minute compared by ordinary which is takes 30 minutes.

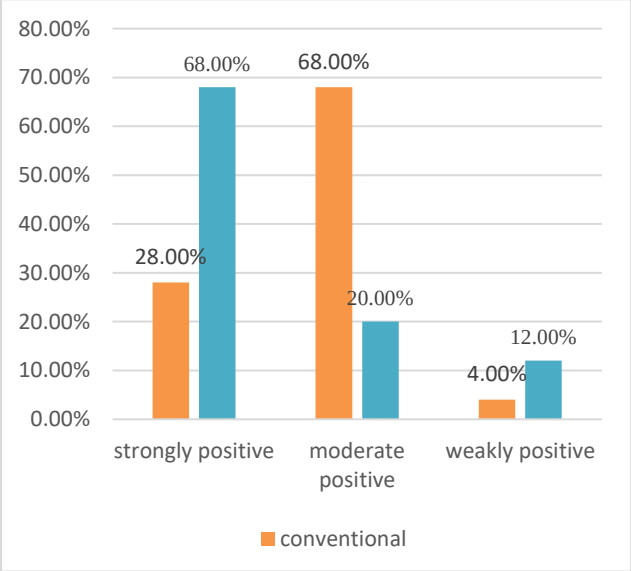
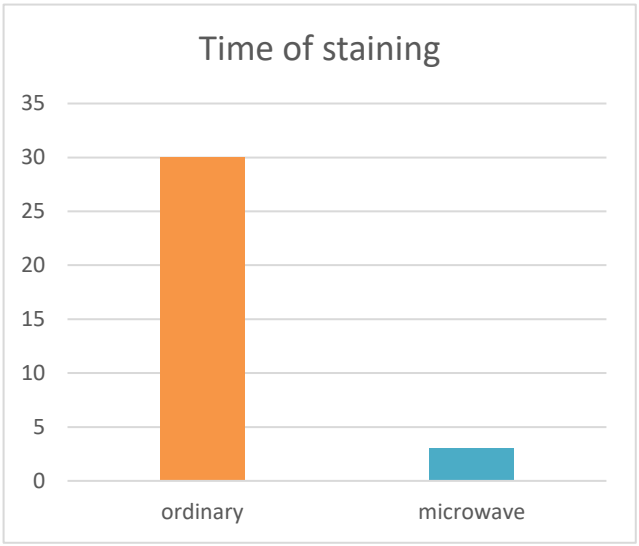


Figure1. Bar chart explain results of Collagen using Masson Trichrome compared between ordinary & microwave methods.

Figure2. Time consumption to stain Masson Trichrome with using ordinary and microwave methods



VI. Data Analysis

The result was presents by percentage as tables and graph, the difference in values of the different methods was determined using the chi squares test and students *t* test. All the statistical analyses were done using the SPSS, version 17.0 and p-values less than0.05 were considered sign. The statistical result P-value in chi squares to test the difference between two methods was (p-value $\leq$ 0.01) that means highly significates difference in matter of positivity of the results. In the comparison of time consuming of the two methods, the mean of time in the two group were tested by *t*-test and the result was (p-value $\leq$ 0.0001) which is very highly significant.

VII. Discussion

A study done by Garvey et al used a domestic microwave oven to speed up the following staining procedures; Heamatoxylin-Eosin stain on frozen sections, and Romanowesky-Giemsa ,

Ziehl-Neelson, Papanicolaou, Feulgen and Grocott stains on buffered formalin fixed sections or cytological smears. They found that the microwave-assisted staining procedures are equal to or even superior to those of the standard methods. Staining times can be reduced to 2%-10% of the conventional staining procedures, So that results was agree with this study, beside that this study was enhanced quality of staining.(6)

Regarding Masson Trichrome staining in this study, there were 24 out of 25 specimens, which are strongly positive in both techniques. In microwave technique 5 out of twenty five specimens were moderately positive compared to 17 specimens with ordinary technique. These results indicate that the quality of staining obtained by using microwave technique was better than the ordinary technique, because the microwave accelerates the diffusion and binding of the stain. This was observed in the short time that had been consumed and in intensity of staining. These findings consent with (Klump Vincent), who believed that; staining of tissues is based on two essential factors, the first on is penetration and diffusion of the dye into the cells, and the second is binding of the dye to the substrate founded inside cell structures. (7)

VIII. Conclusion & recommendation

According to this study, the use of microwave irradiation is regarded as an important tool for staining of collagen with Masson Trichrome staining method. It reduces the time required for staining; as well as slightly increases the staining quality. The technique is useful in routine histopathological diagnosis but detachment of section can occur if use high or median power or long staining time.

The study recommended that to use a microwave irradiation method in other special stain for demonstration of connective tissue.

The researchers also recommended that farther studies and methods required to promote the positivity of the microwave method.

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