APPLICATION OF THE MICROWAVE BEAM RADIATION IN DOUBLE SKELETON STAINING METHOD

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SUMMARY :

Purpose : *The fetal double skeleton staining method is combined with the microwave radiation method to reveal the developmental abnormalities in the skeletal system.* ***Methods :*** *In this study, the double skeleton staining method with the most extensively employed alizarin red-S and alcian blue, were successfully used in conjunction with the microwave beam radiation .* ***Results :*** *The fixation phase which priorly took 4-7 days was reduced to 2-2.5 minutes and the staining phase was reduced from 4 days to 23 minutes.* ***Conclusion :*** *Double skleton staining method took shorter and the cartilage and bone structures were more distinguishable.*

Key Words: *Microwave, Skeleton Staining, Alizarin Red-S, Alcian Blue.*

INTRODUCTION

Histologists and particularly the pathologists have been using the microwave radiation method in order to obtain high quality preparations in a short time.

Since 1970, many researchers have used the microwave radiation method during the fixation phase of the preparation process (1-8). During electron microscope studies and for dehydration and transparency formation phases of staining process, this method was successfully employed (3, 6, 9-20). In addition, the microwave radiation method was utilized in immunperoxidase, immunohistochemical, and immunocytochemical staining methods (7, 14, 21-26). The same method was used in decalcification of hard tissues, such as teeth and bones. Petrere and Schardern used this method for histological fixation of fetal samples (27).

In a study conducted by Inouye in 1976, double skeletal staining method was succesfully used for the purpose of showing the cartilage and bone structures separately (28).

In this study, the microwave radiation method of Inouye (1976) was used in the double fixation and staining phase of skeletons.

MATERIAL AND METHOD

In this study, 15 male and 15 female Swiss albino type mice were used. The nonpregnant female mice were left to copulate with the males for one night. Subsequently, the vaginal plugs of the females mice were examined. Those which had vaginal plugs were assumed to be pregnant and this time was taken to be the 0 (zero) day of the pregnancy. On the 18th day of the pregnancy (one day before delivery), the females were killed by cervical dislocation and the uteri were removed.

The skins of the fetuses were stipped off before the animals were plunged into fixation solution. The internal organs and subcutaneous adipose tissues were also removed. Before the fixation procedure, two separate groups were established. These are:

Group I (Control group) : For fixation, fetuses were left in 95% ethanol for 7 days. The samples which were taken from ethanol were kept in acetone for dehydration and cleaning from oil. Then, they were kept in the dye solution for 4 days at 37°C.

Preparation of the stain

* 300 mg alcian blue was solved within 100 ml 70 % ethanol.

* 199 mg alizarin red-S was solved in 100 ml 95 % ethanol.

* 1st and 2nd solutions were mixed and 100 ml acetic acid was added.

* 1700 ml 70 % ethanol was added to the mixture above.

After the staining process was completed, the fetuses were washed under tap water.

Method for development of transparency:

* 1 % potassium hydroxide (KOH) was applied for 1-2 days.

* 1 % (KOH) (80 cc) + Glycerine (20 cc) were applied for 5 days.

* 1 % (KOH) (50 cc) + Glycerine (50 cc) were applied for 5 days.

Thereafter, the samples were kept in 100 % glycerine and the samples that became transparent were photographed under an Olympus SZ-40 stereo microscope.

Group II : Both fixation and staining phases were made with microwave radiation.

For the experimental groups, Electrolux EME 2662 trademark kitchen type microwave oven was used. During microwave radiation, full power at (750 W, 2450 Mhz) was applied.

In order to prevent excessive rise in temperature, several cups of water were placed into the oven to absorb some of the energy. To avoid denaturation of the tissues, microwave beam radiation was made under conditions not allowing the solution temperature to exceed 50-55°C. Temperatures were measured after each radiation session. After 2-3 exposures, the solutions and cups of water were replaced.

The fetuses were exposed to microwave beam radiation for 2-2.5 min. The samples which were kept in acetone for one night underwent microwave radiation at 50-55°C in stain solution for a total of 23 min. Afterwards, the radiated samples were kept at 37°C and washed under tap water for 5 min. The following phases were exactly the same as those of the control group. The samples which became transparent were photographed.

RESULTS

Group I : In the cranial bones of the control group, the calcified regions that should be stained by alizarin red-S were only apparent in the calcification focuses. Alcian blue was dominant at these regions (Fig. 1). The upper and lower jaw bones, while they had to be stained to red, were in fact stained by alcian blue (Fig. 2).

In the vertebral corpuses of the control group, calcification focuses could not be differentiated due to the excessive dominance of alcian blue stain (Fig. 3).

In the control group and at the upper extremity, although humerus diaphyses were expected to be stained to red, they appeared blue due to the dominance of alcian blue color. Although calcification centers were apparent in ulna and radius, these were also marked by the blue color

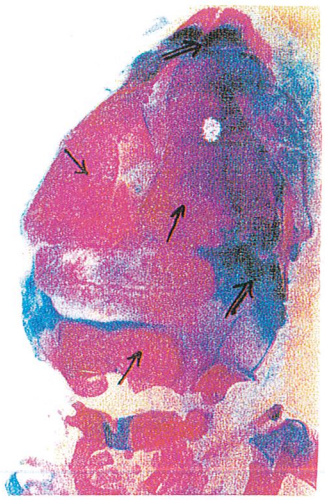

Fig - 1 : Control group. The bones (arrows) and cartilages (double arrows) of cranium were not distinguishable. Alizarin red-S, alcian blue X 10.

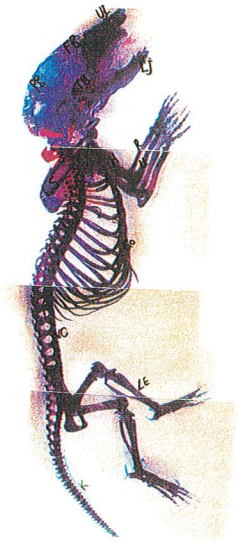

Fig - 2 : Calcification regions in cranium were stained by alcian blue. The lower jaw (LJ) and upper jaw (UJ) bones, frontal (F), parietal (P) and temporal (T) bones were stained alcian blue. Basis of cranium was colored in red. In the vertebral corpuses (VC), calcification focuses were not differentiated due to the excessive dominance of the alcian blue stain. At the upper extremity, although humerus (H), scapula (S), radius (R), ulna (U) diaphyses were expected to be stained in red, alizarin red-S. At the lower extremity (LE) and costae the dominance of alcian blue was striking. Alizarin red-S, alcian blue x 6,7.

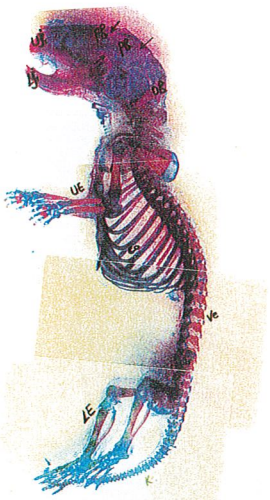

Fig - 4 : When the whole skeletal system was examined in general, it appeared that the cartilage and bone regions were stained rather in a stable way. Calcification regions in cranium were dominant stained by alizarin red-S (arrows). Frontal (F), parietal (P) and occipital (O) bones were also stained to red by alizarin red - S due to their calcification. Lower and upper jaw bones (LJ) (UJ) were extensively colored. Vertebrae were better stained than the other groups (V). In the first part of the tail, the ossification centers could be determined (T) but the last part of the tail was found in cartilage form. Alizarin red-S, alcian blue X 6,7.

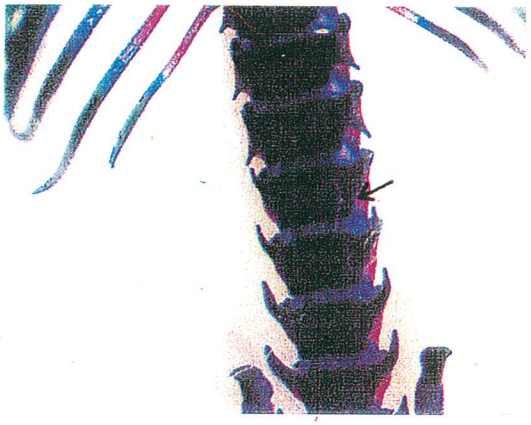

Fig - 3 : Control group. Alizarin red-S, alcian blue x10.

Fig - 5 : Both fixation and stain have been done by means of microwave radiation. The bones (arrows) and cartilages (double arrows) of cranium were distinguishable. Alizarin red-S, alcian blue X 1.

(Fig. 2).

In the lower extremity, the dominance of alcian blue was even more striking. The calcification focuses in pelvis were not apparent. Alcian blue was also held in the costas (Fig. 2).

Group II : When the whole skeletal system was examined in general, it appeared that the cartilage and bone regions were stained in a stable way. The upper and lower jaw bones were stained to red by alizarin red-S and there were occasional blue colored cartilage parts between them (Fig. 4).

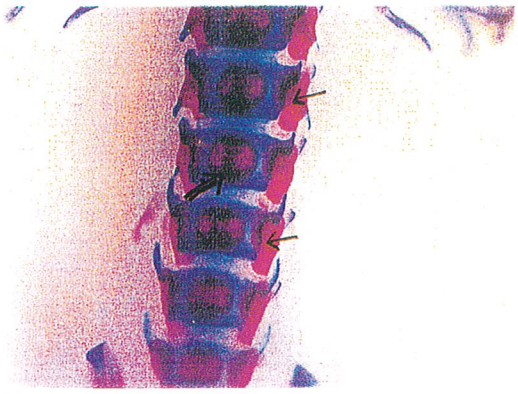

Fig - 6 : A view of the fetal skeletal vertebral colon fixed and stained by means of microwave radiation. Cartilage regions (double arrows) and bone regions (arrows). Alizarin red-S, alcian blue x10.

Occipital and parietal bones were also stained to red by alizarin red-S due to their calcification (Fig. 5).

In the vertebral corpuses, the calcification focuses were easily distinguishable in contrast with the control group (Fig. 6).

In the upper and lower extremities, all the bones and cartilage regions were stained in a stable and distinguishable way (Fig. 4).

DISCUSSION

As the microwaves provide non-ionised heat, it is a type of energy that is complementary or auxiliary to the other heat exchange systems. Its wave length is between 1 mm to 30 cm, which is between infrared and radio waves. The microwave which is used in ovens has wave length of 12.5 cm and a frequency of 2450 Mhz (29).

The radiant energy that is formed by microwave ovens provides kinetic (motion) energy to dipolar molecules like water and thereby leads to heat formation. This heat, which is created through the inter-molecular friction, is distributed homogeneously from inward to outward of the mass.

In 1970, the microwaves were adopted to histotechnique field in several applications of histopathological methods. The advantages of fixation through microwave radiation were reported. In electronmicroscopic examinations cellular details were apparent. Microwave beam radiation provided significant advantage in the immunoflorescent staining that originally took long time; and it was also used successfully in immunohistochemical, immunocytochemical, and immunoflorescent staining (30).

It has been reported that the tissues of cerebrum, cerebellum and m. spinalis which are difficult to fix could be fixed through microwave beam radiation in a much easier and faster way (28, 31, 32). Despite the classical methods, it has been reported that the use of microwave radiation provides fixation of more tissues in less time, by using fewer chemical substances, with less pollution of the environment and less risk to the human health (33).

Minore Inouye's alizarin red-S and alcian blue fetal double skeletal staining method is used extensively for disclosing the developmental disorders or deformities in the skeletal system (28). The alizarin red-S which has high affinity to calcium ions stains the bones into red, and alcian blue which is special to the mucopolysaccarides into blue (32, 34, 35).

The double skeleton staining method of Inouye takes considerable time. Therefore, it was the objective to shorten the time requirement and to fixate the developing structures in detail through employing microwaves. The fixation phase which took 4-7 days was reduced to 2-2.5 min and compared with the control group. The staining phase, which took 4 days in Inouye' s method, was completed within 23 min. The cartilage and bone structures were more distinguishable in the experimental group than in the control group.

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