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Original Research Article

Comparison of MV10B Stain and Haematoxylin &Eosin Stains on Central and Peripheral Nervous System Sections

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ABSTRACT

Introduction: A broad spectrum stain –Methyl Violet 10B (MV10B) was introduced lately. The present study attempts to compare the staining effects of routine haematoxylin and eosin (H & E) with that of MV10 B on nervous system tissues.

Material and Methods: Paraffin sections of cerebrum of the central nervous system and peripheral nerve (transverse section) dorsal root ganglia, autonomic ganglia belonging to the peripheral nervous system were used for this study. A solution of MV10 B was prepared by dissolving 0.25 grams of the dye in powder form with 100 ml of distilled water. The slides were taken to water through decreasing grades of alcohol after adequate dewaxing with xylene. The prepared stain was applied for 10 minutes and the slide was mounted in DPX after washing in 95% alcohol, dehydration and clearing. Similar paraffin sections were stained with H & E and the staining effect was compared with that of MV10 B.

Result: In the H & E stained sections the nucleus took up blue stain where as the fibers and cell cytoplasm was stained pink. With MV10B, the tissues were stained in varying grades of violet. MV10 B gives adequate contrast to the tissue for viewing under a microscope and also for photography.

Conclusion: As seen in the micrographs, the sections stained with H&E andMV10 B are comparable. MV10 B can be used as a quick, simple and cost effective staining method for paraffin embedded nervous system sections. This staining method can be used as an alternative method to stain nervous system tissues for routine classroom slides.

Key Words: central nervous system, peripheral nervous system, MV10 B stain, Haematoxylin and eosin stain, paraffin sections

INTRODUCTION

Haematoxylin and eosin (H & E) staining is considered the gold standard in routine staining. In case of nervous system tissues, although H& E is used, the presence of a large amount of lipid has been a deterrent in producing well stained sections. In the present study we compare paraffin embedded nervous tissue sections stained with methyl violet (MV10 B) stain with routine H & E. MV10B belongs to the group of triphenyl methane dyes. It is a blue anionic dye with quite a large dye structure and is water soluble and alcohol insoluble. MV10B has been used to stain spinal cord, cerebellum and longitudinal section of

peripheral nerve.[1-3] It was noticed that the sections took up the stain well, hence we decided to try out the stain on other nervous system sections and compare the staining effects with that of H& E.

MATERIALS AND METHODS

It has been determined from practice that when nervous tissue is to be stained, it essentially has to be fresh. The tissues were carefully removed from a well embalmed cadaver. The tissues - cerebrum, transverse sections (TS) of peripheral nerve, dorsal root ganglia and autonomic ganglia tissues were processed and embedded in paraffin. Sections were cut at 8 micron thickness and attached to egg albumin coated slides. The slides were kept at 37 degree in an incubator overnight. The tissues were then stained using routine MV10 B and compared with similar sections stained with H & Estain using the respective procedures.

The solutions for haematoxylin and eosin stains were prepared and the slides stained as per standardised routine procedure.[4]

Preparation of MV10 B stain:

0.25gms of MV10B dye in powder form was dissolved in 100 ml of autoclaved distilled water and filtered with Whatmann filter paper and stored in a brown bottle, away from sunlight at room temperature.

Staining procedure:

The sections were dewaxedin xylene and brought to water (hydration) through consecutive gradations of alcohol (100%, 90% and 70%). After hydration the slides are kept in distilled water for 2 to 3 minutes. Sections were stained with prepared solution of MV10 B for 10minutes. In the next step they were decolourised with 95% alcohol until the alcohol ran clear (about 10 to 15 seconds). It was then dehydrated in 100% alcohol, cleared in xylene and mounted with DPX.

RESULTS

Application of MV10 B in staining of paraffin embedded sections of cerebrum,TS of peripheral nerve, dorsal root ganglia and autonomic gangliayielded good results and can be compared with the corresponding sections stained with haematoxylin and eosin, as shown in the Figures 1a, 1b, 2a, 2b, 3a, 3b, 4a, 4b.

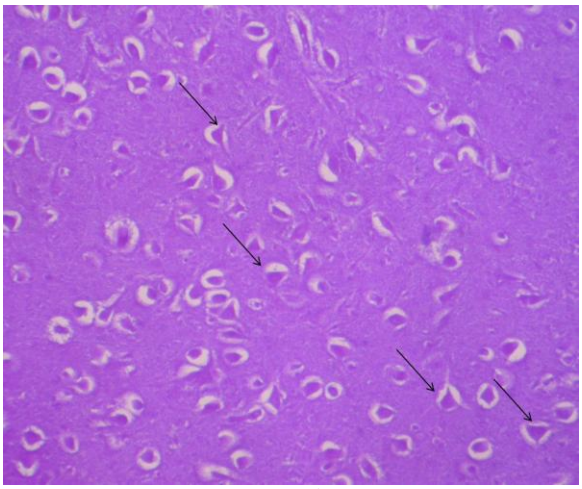

Figure 1a:MV10 B stained section of Cerebrum (x100).

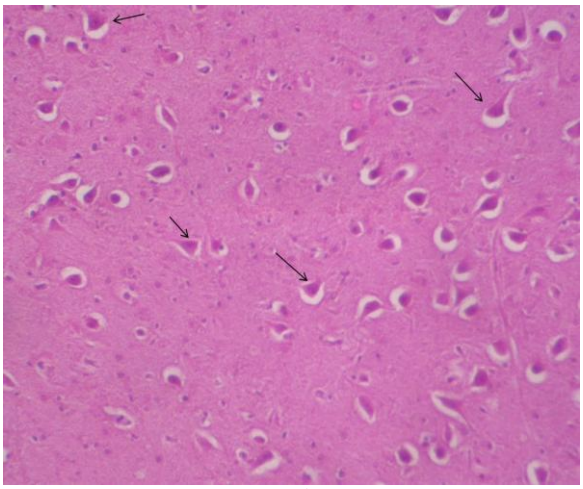

Figure 1b: H& E stained section of cerebrum (x100)Arrows pointing at large pyramidal cells.

The pyramidal cells of the cerebrum (Figure 1a and1b) were clearly seen in both H&E and MV10 B.

Figures 2a and 2b depict the TS of the peripheral nerve. The axon surrounded by myelin sheath and the nuclei of Schwann cells are well visualized in MV10 B as well as in H & E stain.

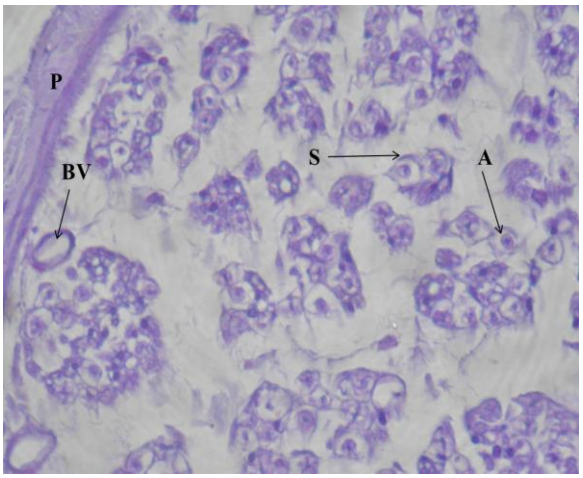

Figure 2a:MV10 B stained section of TS of peripheral nerve (x400).

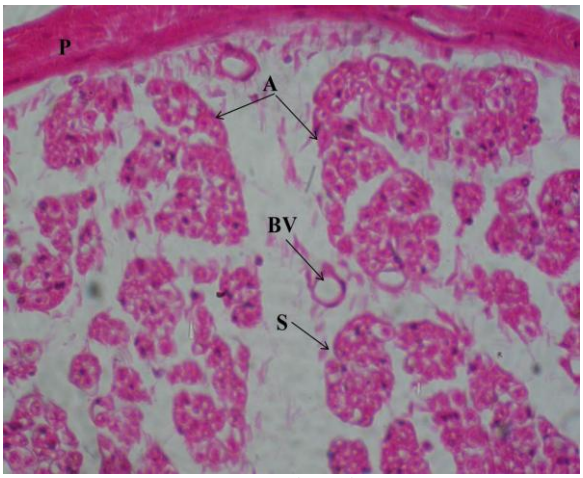

Figure 2b: H& E stained section of TS of peripheral nerve (x400) P- Perineurium, BV- Blood vessel, S- Schwann cell nucleus, A- Axon.

Figures 3a and 3b are of the dorsal root ganglia. The neurons when compared to sympathetic ganglia are larger. They are pseudounipolar and hence appear more rounded. The neurons are arranged in groups with a clear circle of satellite cells around and a centrally placed nucleus. Nucleolus was also noted.

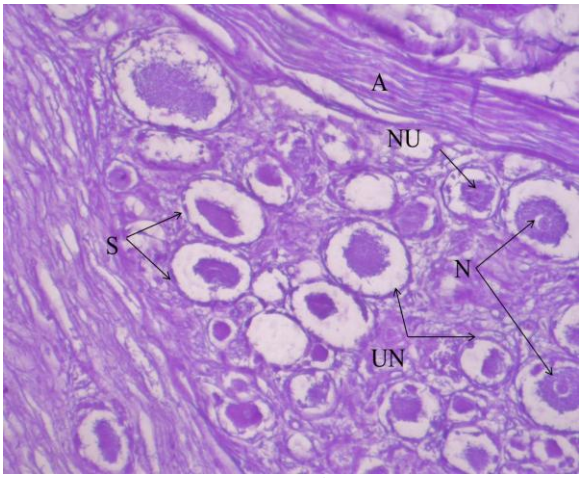

Figure 3a: MV10 B stained section of dorsal root ganglia (x400).

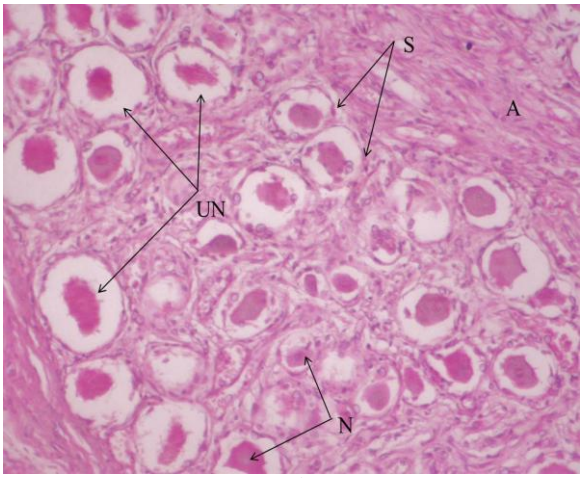

Figure 3b: H& E stained section of dorsal root ganglia (x400) UN- unipolar neurons, N- nucleus, Nu- Nucleolus, A-bundle of sensory axons, S-Satellite cells.

The dorsal root ganglia can be compared with Figure 4a and 4b of autonomic ganglia. Note that the neuron cell bodies here are not clustered as in dorsal root ganglia, but are dispersed. The cells are also smaller. The eccentric nucleus is a prominent feature. Binucleated cells may also be seen.

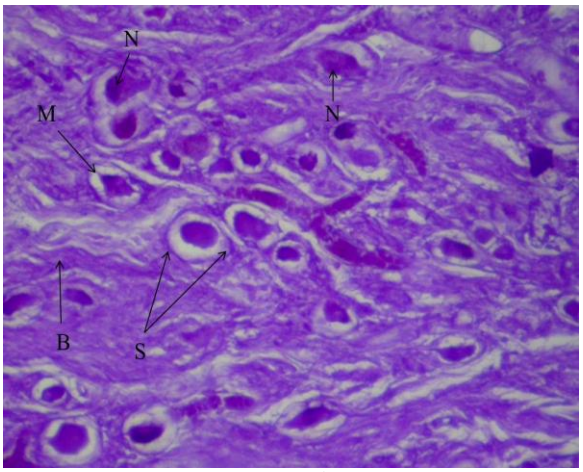

Figure 4a: MV10 B stained section of autonomic (sympathetic) Ganglia(x400).

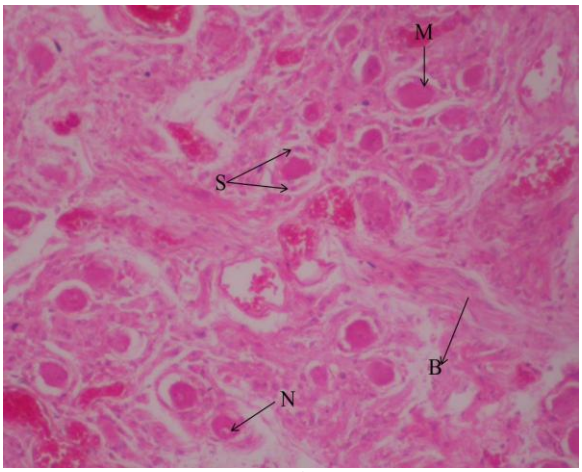

Figure 4b: H &E stained section of autonomic (sympathetic) ganglia(x400) M- Multipolar neuron cell body, N – Nucleus (eccentric in position), S – Satellite cell nucleus, B – Bundle of nerve fibers.

DISCUSSION

Neural tissue staining has been in focus for a long time now. Special staining methods are needed to characterize different types of neurons and to reveal such elements as dendrites, axons, myelin sheaths and the cytoplasmic processes of glial cells. Some of the traditional techniques, especially for glia, have largely been replaced by immunohistochemistry, but there is still a need for dye-based staining of neuronal cell bodies and myelin sheaths in neuropathology, research in neuroscience and the preparation of teaching materials for human and animal neuroanatomy.[5] The search for a simple stain for neural tissues has been on for a long time now. There are specific stains for nissl granules, myelin fibers, neuron and neurofibrils. Nissl granules can be stained using Cresyl violet, neutral red and toluidine blue, whereas myelin can be stained using Pal Weigert's method. Kluver Barrera stain can be used to depict both nerve cells and myelin. It actually includes both cresyl violet and luxol fast blue.[6] The Bielschowsky method is used for staining nerve fibers and neurofibrils. Silver nitrate is the main component in this stain. It stains them brown to black on a yellow to brown background.[7] Margoli's and Gato's method involves the use of triple stains- luxol Fast blue, Periodic Acid Schiff and Harris Haematoxylin.[8, 9] A modification of the Pal Weigert method by Tolivia has also been described. Here the myelinatedfibers appear dark blue and the nerve cell bodies are stained red and the cell nucleoli dark blue due to the use of pyronine as counter stain.[10]

The above stains are useful in neuropathological cases. For undergraduate level of understanding neural tissue structure a single stain that depicts the important constituents would be more than adequate in the opinion of the authors. The different stains mentioned above are not easy to prepare and requires a higher level of technical skill and knowledge. Further they are not cost effective and are time consuming. The H & E stain, although relatively easier than the above still requires technical skill for stain preparation. The preparation of the stock solutions of haematoxylin involves the use of an oxidising agent and a mordant. Good results are dependent on experience and training of personnel involved. On comparison MV10B

staining procedure is complete within 10 to 15 minutes and the section is ready for viewing immediately. Preparation of the stain is very simple as has been mentioned earlier. The MV10 B stain that has been used is a single stain that gives adequate results. The dye - methyl violet is readily available in the market at a reasonably cheap price. Methyl violet was the first synthetic dye used to demonstrate amyloid as long ago as 1875 by Cornil. The chemical name for MV10 B is *hexamethyl pararosaniline chloride*. [11]

In the micrographs comparing MV10B and H & E stained sections, it can be noted that structures visible in routine H & E are also clearly visualised in MV10 B albeit in different shades of violet. The advantage in H & E is the presence of two colours, but as can be noted in MV10 B stains, the varying shades of violet also depict the constituents of the tissue clearly.

The micrographs of the stained sections of both CNS and PNS tissues have shown that MV10B can be regarded as a stain that is as good as if not better than H &E. The preparation of the stain and the staining technique is simple enough to be done without much training. It can even be used by postgraduate students as a learning tool for tissue staining before proceeding to more complex stains.

CONCLUSION

MV10 B stain can be used as simpler alternative to H & E staining for paraffin embedded central and peripheral nervous systems that gives consistent results

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