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Comparison between Giemsa and Van Geison stains in demonstration of collagen fibers (Kosti-2016)

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Trichrome stain (such as Van Geison) is usually used in histopathology laboratory for demonstration of collagenic fibers. Lack of selectivity and tendency of stain to fade makes van Gieson not ideal for collagen demonstration. This study was aimed to compare between Giemsa's and van Gieson's stains in collagen fibers demonstration. Twenty biopsies were obtained from rabbit's skin after anesthesia by chloroform and immediately fixed by 10% neutral buffered formalin for 48 h. Then samples were processed using tissue processing machine and sectioned by rotary microtome. Two hundred (200) tissue sections of 5 micron thickness were prepared. A 100 tissue sections was stained by Van Geison and another 100 tissue sections stained by Giemsa. The stained section was compared with illustrated photomicrographs in order to assess staining quality. Best collagen staining quality was obtained by Van Geison's 60 (60%) and 40 (40%), mean 1.40, followed by Giemsa's stain excellent 55 (55%) and good 45 (45%), mean 1.45. Conclusively, Van Geison's is superior but Giemsa stain is rapid, sensitive without fading tendency, easy to perform and low cost and can be used as special stain under optimized conditions.

Key words: Collagen, Giemsa, Van Geison.

INTRODUCTION

One of the most important vital roles which collagen fibers play is maintaining structural integrity. Also collagen determines tissue function (Whittaker and Canham, 1991); so many pathological conditions are closely associated with collagen degradation and collagen deformity. Such pathological conditions are: Infarct expansion after myocardial infarction, decrease in renal function due to increased fibrosis after kidney transplantation which finally leads to eventual graft failure (Diaz Encarnacion et al., 2003; Grimm et al., 2003). Hence, increase in the need of quantification of fibrosis for prediction of graft survival makes accurate identification of collagen fibers of great importance.

Stains such as Van Gieson and the various forms of trichrome have been used traditionally to detect collagen fibers in corresponding tissue sections. The mechanism of these stains is not completely understood but they bind different tissue components differentially. Such

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differentiation depends on various differences in different factors such as size of the dye molecules, differences in the tissue physical structure (for example, tightly versus loosely packed), and the amino acid composition of the elements of the tissue (Kiernan, 2002). Regarding these factors, in addition to the lack of selectivity; makes van Gieson not ideal for collagen demonstration (Kiernan, 2002; Whittaker et al., 1994). This confounding reason (poor staining of collagen fibers by Van Gieson's stain) and the tendency of the stain to fade, prompted colleagues (Sweat et al., 1964) to seek a better method. Picrosirius red F3BA was found to consistently stain thin collagen fibers, did not fade, and was suitable with polarized light microscopy.

A neutral stain is made from the interaction of acidic and basic dyes. Both cation and anion contain chromophoric groups and there is colored dye in both parts of the dye molecule. Owing to the combination of already large molecules, solutions of neutral stains are often colloidal.

Neutral dyes are soluble in alcohol only, rarely in water, whilst basic and acidic dyes are usually soluble in both. The Romanowsky dyes are the best known of the neutral stains and are formed by the interaction of polychrome methylene blue and eosin. The original Romanowsky stain was prepared by chance with an oxidized methylene blue and it is the oxidation of methylene blue into methylene azure that gives the stain its special selectivity; this oxidation is analogous to the 'ripening' of other stains, such as hematoxylin.

Basic stains color acidic tissue components such as nuclei. Acidic stains will combine with basic structures such as cytoplasm. Neutral dyes have, as expected, an affinity for acidophilic and basophilic elements in the cell, and certain tissue components also react with the compound neutral stain, thus giving a triple staining effect (Drury and Weilngton, 1980).

Giemsa stain is one of the Romanowsky dyes which was introduced early by Gustav Giemsa as stain for malaria parasite. Also Giemsa stain has a wide application in neuropathology as a stain for detection of mast cells (Woronzoff-Dashkoff, 1993). Giemsa is used in hematological patterns in differentiating leukocytes (Wittekind, 1983). The stain, which is classified under the neutral dyes, is requiring neutral pH (6.8- 7.5), which is carried out by using buffer solution.

MATERIALS AND METHODS

Twenty skin biopsies were taken from a rabbit after anesthesia. All biopsies were 2× 2× 0.3 cm in dimension. After the collection of specimens, all of them were immediately fixed in a wide suitable container by 10% neutral buffered formalin ten times the size of specimen for 48 h.

After fixation of specimens, the cut- up was done, specimens were put in cassettes then bearded the unique cases number. The specimen then passed into a tissue processing machine (Leica, 2000) for further treatment in Table 1.

After tissue processing was completed the specimens placed in an embedding centre where they were removed from their cassettes and placed in wax-filled molds that best correspond to the size of the tissue. At this stage specimens were carefully orientated. The cassette in which the tissue has been processed was then placed on top of the mold and attached by adding further wax. The specimens "blocks" were allowed to solidify on a cold surface and when set the molds were removed. The cassette, already filled with wax and forming part of the block, provided a stable base for clamping in the microtome. The block containing the specimen was thereafter subjected to section cutting (Edriss, 2015). 20 blocks were prepared. The blocks were cooled to solidify to turn out their moulds and were then cut by rotary microtome (Diapath Galileo, fully automatic microtome Galileo, 2012). 10 sections of 5 µm thickness were sectioned from each block and kept in incubator with a temperature of 5 to 6ºC above the melting point of wax, that is, at 60ºC for 40 min.

Table 1. Tissue processing schedule.

10% buffered formalin	2
70 percent alcohol	3
90 percent alcohol	3
Absolute alcohol	1
Absolute alcohol	1
Absolute alcohol	2
Absolute alcohol	2
Xylene	2
Xylene	2
Wax bath	3
Wax bath	3

Staining

All sections were de-waxed by xylene for 10 min and rehydrated in descending alcohol concentrations of 100% through 90 and 70% to distilled water for 3 min in each stage. Each section was stained separately.

Verhöeff's Van Geison's method

1. Verhöeff's solution (freshly prepared) for 20 minutes.
2. Rinse in water.
3. Differentiate in 2% aqueous ferric chloride until elastic tissue fibers appear black on a gray background.
4. Rinse in water.
5. Rinse in 95% alcohol to remove any staining due to iodine alone.
6. Counter stain in van gieson for 3 to 5 min.
7. Blot to remove excess stain.
8. Dehydrate rapidly through ascending grades of alcohol.
9. Clear in xylene and mount in DPX (Verhöeff's 1908).

Giemsa staining method

1. Rinse in stock solution of acetic acid.
2. Stain in giemsa working solution in coplin jar for 10 minutes.
3. Wash with buffer.
4. Differentiate in 0.5% acetic acid three dips.
5. Rinse in 100% alcohol.
6. Clear in xylene and mount in permanent mounting medium.

Table 2. Microscopic evaluation of staining quality.

Stain	Excellent	Good	Bad	Total
Geimsa	55 (55%)	45 (45%)	0	100
Van Geison's	60 (60%)	40 (40%)	0	100

Table 3. Giemsa staining.

Entities	Color
Collagen	Pink
Nucleus	Blue

Table 4. Van Gieson staining.

Entities	Color
Collagen	Red
Nucleus	Black

Table 5. The Report of the Giemsa stain.

Std. Deviation	N	Mean	Giemsa
0.00000	55	1.0000	Excellent
0.31782	45	1.8889	Good
0.49237	100	1.4000	Total

Table 6. The correlations.

Correlations		Giemsa results	Van gieson results
Result Giemsa	Pearson Correlation	1	0.903**
	Sig. (2-tailed)		0.000
	N	100	100
Result Van gieson	Pearson Correlation	0.903**	1
	Sig. (2-tailed)	0.000	
	N	100	100

**, Correlation is significant at the 0.01 level (2-tailed).

RESULTS

All quality control measures were adopted throughout the study procedures. Sections were examined by light microscope (LABOMED, LaboAmerica, inc 2013) for the assessment of histomorphological appearance. The characteristics were compared with illustrated micro-photographs (Gartner and James, 2005).

Mean count for each procedure was calculated from 100 sections. Giemsa's stain collagen fibers exhibited excellent 55 (55%) and good 45 (45%) mean 1.5, while Van Geison's gave 60 (60%) and 40 (40%) excellent and good histomorphology respectively mean 1.6 (Table 2 to 6, Figure 1).

DISCUSSION

The routine stain in histopathology is hematoxylin and eosin stain. Any stain used to bring about histological

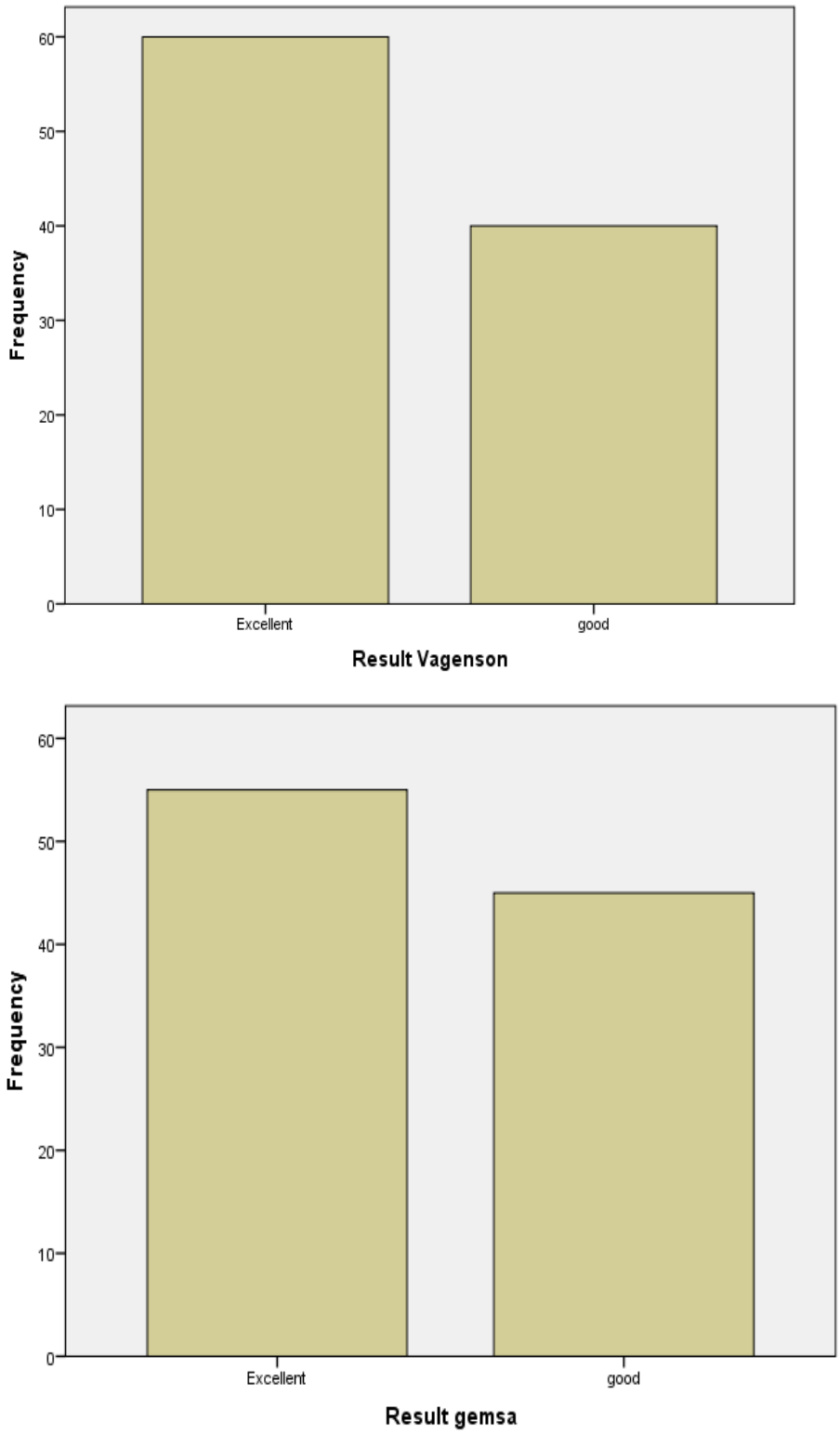

Figure 1. Van Gieson and Giemsa staining quality.

structure in tissue section rather than hematoxylin and eosin stain is termed "special stain". Immunohistochemical and *in situ* hybridization stains were included in this term. There are two broad areas of application: Research and

diagnosis utilize special stains. In research, special stains are used for identifying normal and abnormal cells in tissue section.

Although the Giemsa dye has been shown to work well with a wide variety of procedures, it does not gain wide acceptance (Iniguez et al., 1985). The colors are different from those seen in blood films fixed in alcohol. When Giemsa is used for staining bacteria in tissue section fixed by formaldehyde, the organism stains purple and pink cytoplasm will be seen (Kiernan, 2008).

Wittekind et al. (1991) found that Giemsa stain seems suitable to replace the Gomori-type trichrome stains under appropriate staining conditions. The staining result depends on many factors such as pH and differentiation and this is in line with current study (Wolf-Dieter, 2006).

In conclusion, though the results of van Geison's were superior, Giemsa stain has several properties, such as being rapid; sensitive without fading tendency; easy to perform and low cost, and when used for detection of collagen fibers, there is no need for counter stain (the nucleus takes up methylene blue thus stains blue); so the study recommends the use of Giemsa's as special stain under optimized conditions for skin biopsies in case of collagen demonstration and when infection is suspected.

Conflict of Interests

The authors have not declared any conflict of interests.

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