

Validation of age-related changes in contusions by gross examination and objective analyses

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Abstract

The determination of age of injuries has been a longstanding issue in Forensic Medicine. There is paucity of work in this field and standardized methodology. Estimation of age of wounds by visual inspection alone is subjective and susceptible to variation in perception. This study intends to record, document and interpret the age of wounds from available history, gross examination by naked eye and results of objective analyses by magnified digital photograph, examination under Wood's lamp and histological evaluation, to devise a method for retrospective evaluation of the age of contusions. This is an autopsy based prospective study for a period of 1 year, involving 50 consecutive cases of contusions, conducted on dead bodies brought to the Department of Forensic Medicine. The data obtained was analyzed by SPSS v18. Comparison of different components, significance of association, level of correlation between various variables were determined, and sensitivity and specificity of various methods of analysis in determining the age of wounds was established. On gross examination, contusions were predominantly red when <24 hours old, bluish black on day 2, a greenish colour appeared at the earliest on day 3, and yellow on day 7. There was co-existence of yellow and green colours on 8-9 days and all contusions on day 10 were yellowish. There was positive correlation between the period of survival with histopathological findings and also with colour by magnification of digital photograph which increased till 5-6 days. The association between colour of contusion could be established precisely when examined under Wood's lamp illumination and survival period reached maximum on 5-6 days. Histology of contusions <24 hours showed red blood cells, day 2 showed neutrophils, lymphocytes on day 3, macrophages from day 4, pigments from day 5, collagen fibres from 6 days, complete re-epithelisation from day 7, fibroblasts from day 8, which increased in density on day 9 and 10. The age of contusions was determined, and sensitivity and specificity of various methods were assessed. It was concluded that an array of subjective and objective analyses can be used to establish the age of wound.

Keywords

Wound age; Contusions; Gross examination; Digital photography; Histopathology; Ultraviolet; Wood's-lamp-illumination

Introduction

The evaluation of any tissue injury is an essential component in the practice of Forensic Medicine whereby furnishing a final word on the age of injuries in cases such as assault, abuse etc. and the interpretation has significant medicolegal implications which may include the incrimination or exclusion of a suspect as the perpetrator of a crime, time of occurrence of the event or crime and if possible discriminate if all injuries found on the body may not have been inflicted by the same assailant or even at the same time. Estimation of age of wound by visual inspection alone is subjective and susceptible to variation in perception, but previous studies have shown that it may be possible to determine the age of wound by complementing direct observation with other objective analyses.

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Materials and Methods

The objectives of the study were to estimate the time of contusions by gross examination, Wood's lamp examination, magnification of digital photography and histopathological changes and to validate the age of contusion obtained by above methods with the age of injury as per available history.

The current study is an autopsy based prospective study conducted on dead bodies brought to the department of Forensic Medicine, over a period of 1 year spanning from February 2015 to February 2016. The study involves 50 consecutive cases of contusions of known age.

The inclusion criteria was injuries with known age as recorded in the inquest and the exclusion criteria was dead bodies in a state of decomposition and cases where there is no definite history regarding the age of the injury or its time of infliction.

A diagnostic evaluation of contusion on the dead bodies in which the time of sustaining the injury is known through inquest details and treatment records and correlating the time since injury to the findings from different methods of analyses was done.

The gross changes of the surface injuries, namely contusions, were observed and documented. The injuries were then

photographed by digital camera and magnified 3times and details were evaluated. Conventional images are sometimes impaired by spurious light reflectance from skin caused by electronic flash. So, examination under Wood's lamp illumination was done.

The apparatus used was Gadget's 60X Magnifying Loupe with LED and UV-A light No:9592 which uses UV-A light of wavelength 320-400nm(~365nm). Wood's lamp is UV-A lamp which helps in clearly visualizing the skin lesion and injuries thereby helps in deciphering the unclear injuries and the margins. The peripheral portion from the margin of the contusion was excised in full depth and subjected to histopathology examination to assess the age of the wound.

The data obtained were analysed using SPSS v18. The frequency of occurrence of different variables were obtained and classified into groups. The cross tabulations were prepared for comparison of different variables to be assessed simultaneously. Comparison of the different components were done by statistical methods and interpreted. The significance of association(p value ≤ 0.05) was estimated for all by Chi square test and Kappa agreement test was applied to determine the level of correlation between various variables under study. Sensitivity and specificity of various methods of analyses to determine the age of wounds were established.

Results

Survival period frequency has been depicted in Table 1. Table 2 shows the comparison of contusion colours observed using different methodologies. On gross examination, 75% of contusions with survival period <24hours were red in colour. All the 2-day old and 83.3% 3-day old contusions were bluish or bluish black in colour. 66.7% of 4days old, 100% of 5days old, 75% and 66.7% of 6days and 7days old contusions were greenish. The earliest appearance of greenish colour was on day3 and of yellowish colour was on day7 after sustaining injury. 8days old contusions showed bluish, greenish and yellowish discoloration (33.3% each) on gross examination. 9days old contusions were greenish (50%) and yellowish (50%) in colour and 100% of contusions with period of survival of 10days or more were yellowish

Correlating colour of magnified digital photograph and survival period, 75% of the contusions with survival period of 1day were reddish and all 2days old contusions showed bluish black colour. 33.3% of 3days old, 66.6% of 4days old and 80% of 5days old contusions were greenish. 83.3% of 7days old contusions were greenish and 16.7% were yellowish in colour. 66.7% of 8days old contusions were greenish. 75% contusions on day9 and 100% of contusions with 10days or more survival period showed yellowish colour. The earliest appreciation of greenish colour in a contusion was on day2, and yellow was on day7. The agreement between the colour of the wound by magnification of digital



Figure 1: Red Contusion



Figure 2: Bluish black contusion



Figure 3: Greenish contusion

photograph with that of survival period was maximum (88.9%) at 5-6days then declined. Kappa agreement test showed a moderate agreement (0.527). In the current study, the exact colour of the contusion could be precisely made out by Wood's lamp examination where 2% and 4% of greenish contusions were bluish black and yellowish respectively. The correlation between colour of wound by Wood's lamp examination and survival

period was 100% at 5-6days and then declined. Kappa agreement test showed good agreement(0.627).

Histological analysis of contusions for correlation with survival period showed that 87.5% of contusions with survival <24hours

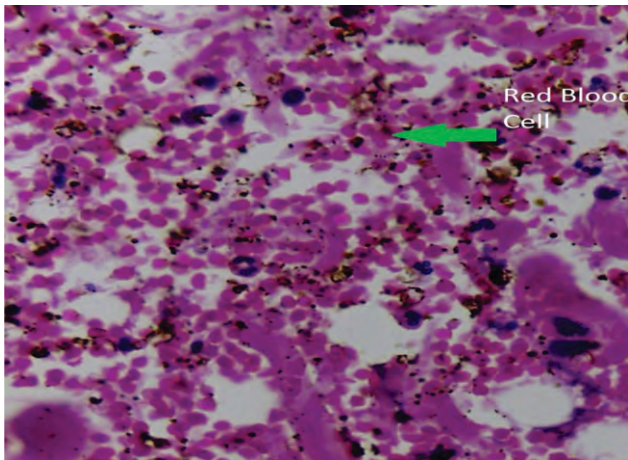


Figure 4: Infiltration of red blood cells

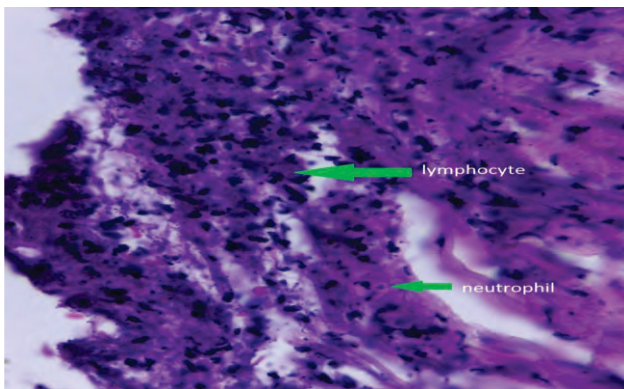


Figure 5: Infiltration of neutrophils and lymphocytes

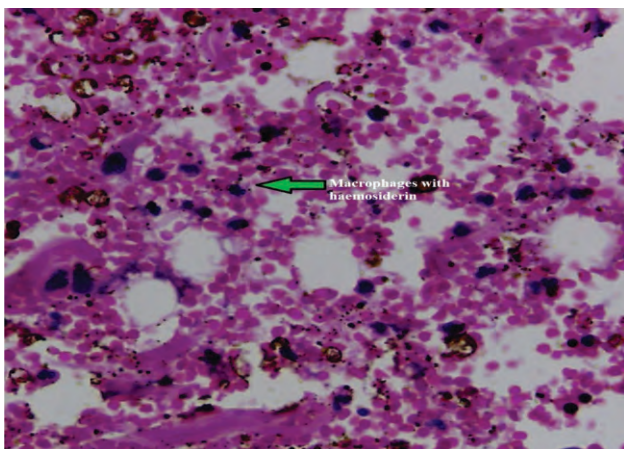


Figure 6: Macrophages with haemosiderin

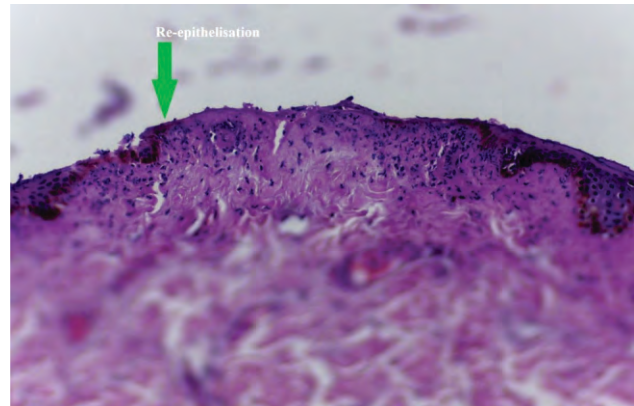


Figure 7: Re-epithelialisation

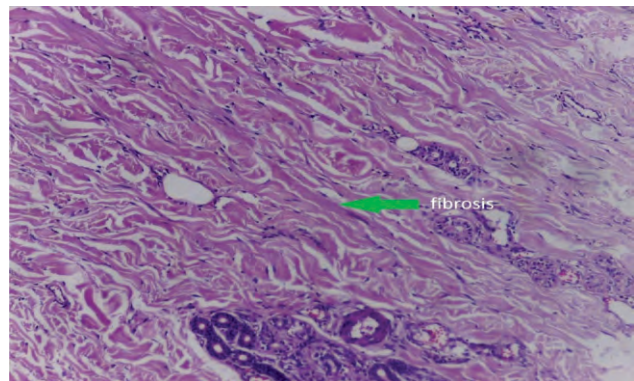


Figure 8: Fibrosis

showed red blood cells infiltration. 100% of 2days old contusions showed neutrophilic predominance. 50% of 3days old contusions showed lymphocytic infiltration. 100% 4days old contusions showed macrophages. By 5days, 80% showed characteristic presence of pigments, and 75% of 6days old contusions showed collagen fibres. Evidence of complete re-epithelialisation was seen by 7days(33.3%) and fibroblasts were noted in 16.7% by 8days. 50% of contusions showed varying degrees of increasing density of fibroblasts since day9 and high density fibroblasts in contusions with survival period 10days or more (Table 3).

Table 4 shows the difference in stage of healing as seen by gross examination and histopathology. On evaluating the period of survival by gross examination and histology,12.5% of injuries <24 hours showed histologically advanced healing stage by 1day, whereas,87.5% showed no difference in the stage of healing. All injuries with survival period of 2days showed exact correlation of survival period by gross examination and histopathology. In 3days old injuries, 50% showed no difference, and 50% showed a histological delay by 1day. In all 4days old contusions and 80% of 5days old contusions, the estimated period of survival was same by both methods whereas 20% of the cases in the latter group showed delay of 2days or more

histologically. The survival period was the same by both methods in 75% of 6days old contusions while 25% showed a histopathological lag of 2days or more. 16.7% of 7days old contusions showed a histological age of 2days when gross examination showed survival period of 1day and 33.3% showed concordance by both methods. 50% of the subjects showed a delay in histological age by 2days and more. Among the persons who survived for 8days, 16.7% and 50% showed a lag of 1day and 2days or more respectively histologically. 50% of 9days old contusions showed same survival period by both methods. All the subjects, with period of survival 10days or more, showed similar age of wounds by both the methods of evaluation. Figures 1-3 show the different colours of contusions. Figures 4-8 represent histological changes.

Discussion

On gross examination, the predominant colour of contusions with survival period <24hours was red, 2-3days were bluish black, greenish colour was seen at the earliest on day3 and yellowish colour on day7. All the contusions with period of survival of 10days or more were yellowish. These were in complete

Table 1: Survival period frequency in contusion (N=50)

Time	n	%
< 24 hours	8	16.0
24 - 48 hours	6	12.0
48 - 72 hours	6	12.0
4 days	3	6.0
5 days	5	10.0
6 days	4	8.0
7 days	6	12.0
8 -10 days	6	12.0
10 -15 days	4	8.0
> 15 days	2	4.0

Table 2: Frequency of various colours of the contusions observed using different methodologies

Colour	Gross observation		Digital photography		Wood's lamp examination	
	n	%	n	%	n	%
Red	6	12	6	12	6	12
Bluish black	18	36	15	30	16	32
Green	19	38	22	44	19	38
Yellow	7	14	7	14	9	18

Table 3: Evaluation of contusion by histopathology and frequency of findings (N=50)

Histopathology	N	%
RBCs	7	14.0
Neutrophils	10	20.0
Lymphocytes	5	10.0
Macrophages	4	8.0
Pigments	10	20.0
Collagen fibres	3	6.0
Re- epithelisation	4	8.0
Fibroblasts	2	4.0
Dense fibroblasts	3	6.0
High density fibroblasts	2	4.0

Table 4: Difference in estimated survival period by gross and histopathology evaluation

Survival period	Count	Difference				
		-1day	0 day	1day	2days	3 days
1 day	n	1	7	0	0	0
	%	12.5	87.5	0	0	0
2 days	n	0	6	0	0	0
	%	0	100	0	0	0
3 days	n	0	3	3	0	0
	%	0	50	50	0	0
4 days	n	0	3	0	0	0
	%	0	100	0	0	0
5 days	n	0	4	0	1	0
	%	0	80	0	20	0
6 days	n	0	3	0	1	0
	%	0	75	0	25	0
7 days	n	1	2	0	2	0
	%	16.7	33.33	0	33.33	0
8 days	n	1	1	1	0	3
	%	16.7	16.7	16.7	0	50
9 days	n	0	2	0	1	0
	%	0	50	0	50	0
≥ 10 days	n	0	2	0	0	0
	%	0	100	0	0	0
Total	n	3	33	4	5	3
	%	6	66	8	10	6

agreement with the findings obtained by Adelson¹ and also by Camps.² Knight³ observed that contusions appeared red on day1, blue or bluish black in 2-4days, greenish by 5-6days and yellowish by 7-12days of infliction. Observations of Dimitrova and Georgieva et al.⁴ and Glaister⁵ were concordant.

Moritz⁶ found brown discoloration in contusions older than 24hours⁶, whereas Spitz and Fisher⁷ noticed brown colour by the end of the first week⁷, unlike in the current study where a specific brown discoloration was not found in the contusion at

any stage of healing. On magnification of digital photograph of contusion with period of survival <24 hours, the predominant colour obtained was red, whereas on days 2 and 3 it was bluish black. The earliest appreciation of greenish colour was on day 3 and of yellow on day 7, which then coexisted till only shades of yellow were visible from day 10.

Stephenson and Bialas⁸ noted red colour in the photograph of an injury <1 week old, and they found that shades of green and yellow suggest injury is at least 24 to 48 hours old⁸, whereas in the current study it was noted that red colour was seen predominantly in contusions <24 hours old, bluish black on days 2 and 3. Carpenter⁹ found yellow colour after 48 hours of sustaining the injury.

There was increasing correlation between colour of the contusion by magnified digital photograph and survival period till 5-6 days and decreases thereafter for which there was moderate agreement. Barciak et al., Stephenson and Bialas⁸ found that any colour could be present in fresh, intermediate and old bruises. Barciak et al.¹⁰ stated that the accuracy of ageing of a bruise to within 24 hours of its occurrence is less than 50%.

After analysing relevant literature over 30 years, it was concluded by Spector and Willoughby¹¹, that a bruise cannot be accurately aged from clinical assessment in vivo or on a photograph. Though it was stated by Hughes and Langlois et al. that alternative light source was unable to assist in determining the age of bruise in their study¹², in the current study it was found to delineate the subtle colour changes in the contusions.

2% and 4% of greenish contusions were found to be bluish black and yellowish respectively when examined under Wood's lamp, which was in accordance with observations of Vogeley, Pierce and Bertocci, who stated that the margins of the wound could be better visualized by this method.¹³ In this study, the association between colour of contusion by Wood's lamp examination and survival period increased from day 1 till 5-6 days, then declined.

In histopathological analysis of contusions with survival period <24 hours, the predominant feature was infiltration of red blood cells, and, there was neutrophilic predominance by day 2, lymphocyte infiltration by day 3 and macrophages were observed by day 4. Pigments were noted at the earliest by day 5, collagen fibres from 6 days, complete re-epithelisation from day 7 and the earliest fibroblasts by day 8. Increasing density of fibroblasts was noted since day 9 and a level of high-density fibroblasts were seen from day 10.

Jayson and Payne found that neutrophils, if found, denote post infliction interval of approximately 15 hours upto several months¹⁴ and macrophages were noted from 3 hours.

Virchow found iron containing pigments in older injuries, but specific age could not be ascertained. The earliest evidence of leukocyte reaction was noted by Walcher by 20 to 30 minutes, polymorphonuclear lymphocytes after 4 hours, macrophages after

15 hours and haemosiderin laden macrophages 24 hours after infliction of trauma.

Raekellio noted the presence of polymorph leucocytes from 4 hours¹⁵, macrophages from 8 hours, increasing in number after 16 hours and reaches a maximum by 48 hours. Fibroblasts were seen from 12 hours and peaked on day 14. Vascularisation was seen from day 3 and collagen fibres from day 4.

Ishida et al.¹⁶ had similar findings. They found that more than 10 fibrocytes indicated wound age between 9-14 days.

Nerlich et al.¹⁷ found no typical granulation tissue with fibroblasts in contusions less than 5 days, fibroblasts being detected earliest on day 5.

Betz¹⁸ detected neutrophilic granulocytes at the earliest at about 20-30 minutes after wounding. Pigments were demonstrated at the earliest on day 2-3 and spot like lymphocytic infiltrates in granulation tissue by 1 week or more. There was excellent correlation between the period of survival and histopathological findings though there was increasing histopathological delay in healing of contusions with a survival period of 6 days or more.

Conclusion

The predominant colour of contusion with survival period <24 hours was red. Most contusions with period of survival 2 to 3 days were bluish black. The appearance of greenish colour in the contusion occur at the earliest on day 3 after sustaining the injury. The earliest evidence of yellowish colour in a contusion was seen on day 7. There was co-existence of yellowish and greenish colour till 8-9 days. All the contusions with period of survival 10 days were yellowish on gross examination. On magnification of digital photograph of contusions <24 hours, the predominant colour obtained was red, whereas on days 2 and 3 it was bluish black. The earliest appreciation of greenish colour was on day 3 and yellow on day 7. Coexistence of both yellow and green colour in contusions noted on day 8 and 9 though the predominant colour on day 9 was yellow and only shades of yellow was visible on day 10. Histopathological analysis of contusions <24 hours old showed infiltration of predominantly red blood cells into the wound, neutrophilic predominance by 2 days, lymphocyte infiltration by day 3 and by 4 days presence of macrophages were noted. Pigments were noted at the earliest in contusions with 5 days survival period. Presence of collagen fibres were detected from 6 days onwards. Complete re-epithelisation was noted from day 7. The earliest evidence of appearance of fibroblasts was noted by 8th day. Increasing density of fibroblasts was noted since day 9 and a level of high density fibroblasts was attained by day 10. There was excellent correlation between the period of survival and histopathological findings. There was increasing

histopathological delay in healing of contusions subjects with survival period 6 days or more. There was increasing correlation between colour of the contusion assessed by magnification of digital photography with that of survival period till 5-6 days, when it reaches maximum and then decreases thereafter. By Woods lamp examination, the exact colour of the contusion could be precisely made out. The association between colour of contusion when examined under Woods lamp illumination and survival period increased from day 1 to reach maximum on 5-6 days.

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