Anil Aggrawal's Internet Journal of

Forensic Medicine and Toxicology

Volume 16, Number 1

January – June 2015

ISSN 0972-8074 (Online) · ISSN 0972-8066 (CD)
Editor-in-Chief: Professor Anil Aggrawal · www.anilaggrawal.com/ij/
Free to read. This file collects the whole issue; each item is also on the website.

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ANIL AGGRAWAL'S
Internet Journal of Forensic Medicine and Toxicology
The world's first online journal devoted entirely to forensic medicine and toxicology · Published 1 January and 1 July

VOL. 16, NO. 1 · JANUARY – JUNE 2015
Downloaded from www.anilaggrawal.com · ISSN 0972-8074 (Online) · https://www.anilaggrawal.com/ij/vol_016_no_001/others/editorial.html

Sexual Violence-An Emergent Epidemic

Uwom Eze[1*], Ndubuisi Eke[2]

[1]Consultant Forensic Pathologist, Department of Pathology, University College Hospital, Ibadan, Nigeria
[2]Urology Division, Department of Surgery, University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria

* Corresponding author: Dr Uwom Eze, Department of Pathology, University College Hospital (UCH), P.M.B 5116, Ibadan, Nigeria · uwomeze@gmail.com

CITE THIS ARTICLE

Eze U, Eke N. Sexual Violence-An Emergent Epidemic. *Anil Aggrawal's Internet Journal of Forensic Medicine and Toxicology* [serial online], 2015; Vol. 16, No. 1 (January – June 2015): []. Available from: https://www.anilaggrawal.com/ij/vol_016_no_001/others/editorial.html

Sexual violence, especially against women, has been a perennial problem that has lingered across ages and generations, including Biblical times [1]. It appears to have defied models of prevention or interventions [2]. From an isolated rural community in a developing country to a megacity in a developed economy, the basic ingredients of sexual violence remain constant. The principal targets are female adults and children, with girls being at greater risk [3]. Different levels of success have been achieved in different parts of the world in tackling this issue but such measures tend to address mainly punitive aspects of it. There is obviously increased reporting of incidents in the developed world unlike what obtains in developing countries where issues of basic rights of women and children are still debatable. In these developing countries, socio-cultural and religious factors influence orientation and practice. This makes it difficult to achieve any common template upon which a definition of sexual violence applies.

Sexual violence is defined as any sexual act, attempt to engage in a sexual act, unwanted sexual comments or advances, or acts to traffic, or otherwise directed, against a person's sexuality using coercion, by any person regardless of their relationship to the victim, in any setting, including but not limited to home and work [4]. There is no universally accepted definition of sexual violence. For example, some culture may not regard certain forms of sexual violence, like rape within marriage, as such. This creates inherent problems with definition and recognition of sexual violence across different cultures and religions. There is also paucity of research in this area as many victims of sexual violence may fail to report to appropriate authorities due to fear of stigmatization and further victimization [5].

To the public, the commonest and most widely publicised form of sexual violence is rape. Rape is defined as the forceful engagement of another person in a sexual activity. The definition of rape varies between countries. In the United States of America, the definition varies between the States in the Union. Recently, the definition has included all genders as potential victims [6,7]. It is acknowledged world wide as a crime. There are a few types of rape including date rape [8] and gang rape [9] . A new variant of rape, gang rape, when several individuals serially rape a victim, has been reported in India and Nigeria. It is widely believed that rape is under- reported. However, the perception of rape on the enormity scale varies between countries. In Saudi Arabia, Cuba, Taiwan, China, etc, it is punishable by death while in some developed countries the guilty may get away with community service.

In recent times, news reports have been awash with peculiarly daring and distressing episodes of rape, from India, through Kenya to West Africa. The prevalence has now reached a proportion at which it can be classified as an epidemic. Rape has been reported among all social classes from kings, professors, religious leaders, students, applicants and artisans [10]. Most perpetrators of rape are males on females. This is heterosexual rape. There can also be homosexual rape. A married person can be guilty of raping the spouse.

A devastating case occurs when the victim dies from physical complications of the criminal act.

In several countries, for religious and cultural reasons, prosecution of cases is encumbered with difficulties. Up to four male witnesses are required to prove a case of rape of a woman by a man in some cultural cum religious settings. Court processes discourage female victims from reporting cases and pressing charges. Smart lawyers often cite seduction of the male defendant by the female appellant. Embarrassing questions are thrown at the female victim.

Rape is a criminal and antisocial act. It should be prevented and deterred. Potential victims should adopt preventive life styles. Individuals should avoid being alone in dark environments. Wards should be protected by their parents and guardians. Just as paedophiles are registered, people found guilty of rape should be logged.

Stemming the tide

The first major assignment is to ensure that practitioners and community speak the same language about the meaning and understanding of sexual violence. There is significant discrepancy between what physicians, the police, the justice system and the community understand about what constitutes sexual violence. It will be merely an academic exercise to reel out a textbook meaning or understanding of sexual violence when such meaning is alien to the law existing in a community and where members of the community share a different ethos and orientation. Researchers worldwide may need to focus on documenting views of distinct communities on the meaning of sexual violence across the various strata of the society. Incidentally, sex topics are generally repressed in many cultures and it is almost impossible to determine with any reasonable degree of certainty the innuendoes of sexual dynamics behind the scene. There may be only a thin line between “wanted” and “unwanted” sexual activity in a particular context?

Second, there is need to rethink the way and manner sex topics or subjects are conveyed from one culture to another. There will be little or no progress made in addressing the problems of sexual violence if practitioners fail to learn specific languages or peculiar expressions acceptable to a particular community or culture in discussing the issue. In the western world there are quite some liberal expressions due to prevailing culture of individualism and assertiveness. In Africa, Asia and

other developing regions, the concept of personhood is in the sense of a strong relationship with family and the community. In essence people belong to one another and to the community, and not to individual self. It is therefore difficult or impossible to transpose Western orientation on sexual matters unto other cultures. Cultures that promote and condone forced marriages obviously create the environment in which rape may prevail. Most of the texts and researches on sexual violence are from the West and this obviously poses some significant problems in applying certain principles, processes and procedures to the rest of the world. Rethinking how these topics are conveyed in a foreign culture and different religions will go a long way in facilitating acceptance of prescribed measures for the prevention or management of sexual violence.

Third, there is need to match increasing expertise and innovations in the field of forensic management of sexual violence with enabling laws in various jurisdictions. It is rather frustrating that despite all the knowledge and expertise available, evidence may not be acceptable in court due to restrictions imposed by existing provisions in law in a particular jurisdiction. An example is the Evidence Act which may place stringent conditions on what is admissible in court as evidence and how such evidence can be tested to be true. Potentially, this discourages victims of sexual violence from reporting cases and thus diminishes the role of investigators who may really be working hard in this field. The heterogeneous mix in most of our societies and competing interest groups, usually laced with background factors, makes changes in existing law a difficult venture. This calls for more active and wide ranging engagements by stakeholders. Concerned forensic professionals, civil society groups, Human Rights activists and relevant nongovernmental organizations (NGOs) will need to work on one the hand for the improvement of expert response to cases of sexual violence and on the other hand, advocate for relevant changes in the legislature to accommodate the implementation of such advancement in expert care. This has been a limiting gap in the fight against sexual violence especially in the developing world where a gulf still exists between the provisions of the laws and contemporary practice in the forensic management of sexual violence [11].

Fourth, there is need for training and retraining of persons involved in prevention and management of sexual violence. Lots of emphases need to be put into the multidisciplinary nature of this field of practice. Currently, there is a lot of disconnect among NGOs, social welfare agencies, medical practitioners, the police and other concerned groups on this issue. This situation has therefore created a loophole in the management of sexual violence as victims are deprived of critical care and attention that would have come with a coordinated multidisciplinary approach that is accepted as best practice in this field. In addition to impacting knowledge and skills, roles and responsibilities should be streamlined and boundaries clearly defined for each of the agencies as they work together to prevent incidents, protect victims and manage cases with professionalism. The ultimate goal is justice.

Fifth and finally, the notion of victims moving from one police station to some distant hospital for medical evaluation and to yet another centre for some other form of triaging is outdated. It is a disincentive for victims and others working in this field. ***One Stop Centre*** has emerged as a veritable means of providing a comprehensive service to victims in one location. A purpose built service centre will facilitate a timely, empathic, acceptable and professional service to survivors of sexual violence. Huge success has been reported in jurisdictions where there has been the application of this concept in the management of sexual violence [12].

Victims or survivors of sexual violence including rape should find it easy to report cases and press charges. There are societies and agencies in several countries that make it easy for victims to seek redress. Society must enact penalties that are deterrent. Sentences that appear to pat the perpetrator on the back should be rejected. Rapes that result in the death of the victim should be treated as first degree murder.

Sexual violence is a complex subject. This is more so in the developing world where peculiar factors make it difficult to adopt some “universal” or simple measures. It is therefore important that policy makers, practitioners, governmental and non governmental agencies as well as other stakeholders appreciate the challenges in understanding, preventing and managing sexual violence in the developing world. This may well be a veritable step in stemming the tide of this emergent epidemic.

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ANIL AGGRAWAL'S
Internet Journal of Forensic Medicine and Toxicology
The world's first online journal devoted entirely to forensic medicine and toxicology · Published 1 January and 1 July

VOL. 16, NO. 1 · JANUARY – JUNE 2015
Downloaded from www.anilaggrawal.com · ISSN 0972-8074 (Online) · https://www.anilaggrawal.com/ij/vol_016_no_001/papers/paper001.html

Amitraz - A New Poison With Unusual Neurotoxic Effects.

Swati C. Aundhakar[1*], Sanket K. Mahajan[2], Makarand B. Mane[3], Shirish M. Agrawal[4]

[1]Professor & Head, Department of Medicine, Krishna Institute of Medical Sciences University, Karad, Maharashtra-415 110, India
[2]Resident Doctor, Department of Medicine, Krishna Institute of Medical Sciences University, Karad, Maharashtra-415 110, India
[3]Lecturer, Department of Medicine, Krishna Institute of Medical Sciences University, Karad, Maharashtra-415 110, India
[4]Resident Doctor, Department of Medicine, Krishna Institute of Medical Sciences University, Karad, Maharashtra-415 110, India

Contact: Dr. Aundhakar · dr.swatiaundhakar@yahoo.co.in

CITE THIS ARTICLE

Aundhakar SC, Mahajan SK, Mane MB, Agrawal SM. Amitraz - A New Poison With Unusual Neurotoxic Effects.. *Anil Aggrawal's Internet Journal of Forensic Medicine and Toxicology* [serial online], 2015; Vol. 16, No. 1 (January – June 2015): [about 6 p]. Available from: https://www.anilaggrawal.com/ij/vol_016_no_001/papers/paper001.html

ABSTRACT

Amitraz is an insecticide/acaricide of formamidine pesticides used worldwide to control ectoparasites in animals. Its effects in humans may mimic organophosphate poisoning. Amitraz poisoning is a rare disorder characterized by central nervous system (CNS) and respiratory depression, bradycardia, hypotension, hypothermia, hyperglycemia, nausea and vomiting. Poisoning may occur either by oral inhalation and dermal route. Here we will discuss one such patient of amitraz poisoning who presented to us in a comatose state with respiratory depression, bradycardia and miosis thus mimicking organophosphate poisoning. He recovered completely within 5 days. The importance of this case report is highlighted by the increasing use of this compound, the life-threatening presentation, the excellent prognosis with early recognition and supportive management and the limited human toxicological data.

Keywords: Amitraz, Insecticide, α-adrenergic agonists

Introduction

Amitraz, 1,5 di-(2,4-dimethylphenyl)-3- methyl-1,3,5-tri-aza-penta-1,4 diene is a member of the formamidine pesticides.[1] It is an acaricide and insecticide indicated for the treatment of

generalized demodicosis in dogs, for control of ticks and mites on cattle and sheep, and to control psylla infestations of pears.[2] Amitraz is a pharmacologically active compound which has α2-agonist actions. The stimulation effect of α2-receptors are in part responsible for neurotoxic and preconvulsant effects.[1] Adverse reactions and side effects have been reported in animals exposed to the product but only a limited number of human intoxication cases have been published in the literature. Cases of human poisoning with amitraz are extremely rare in the literature. The reported effects include CNS depression, hypothermia, bradycardia, hypotension, hyperglycemia, glycosuria, vomiting and respiratory failure.[3] Mortality in humans due to amitraz poisoning has been reported in some research studies (Bonsall and Turnbull 1983; Abu-Al Ragheb et al. 1986). Sporadic fatal cases due to accidental industrial inhalation or ingestion of this product have also been reported (Abu-Al Ragheb et al. 1986). Here, we would like to emphasise that although Amitraz poisoning has a life-threatening presentation, most cases result in complete recovery if appropriate supportive treatment is instituted in a timely fashion. Also, very few cases of amitraz poisoning have been reported in India so our case report will significantly contribute to the limited pool of data available about this poison in India, thereby allowing timely recognition and treatment.

Case History

A 35 years old male was brought to emergency department of Krishna hospital with history of consumption of about 30 ml of amitraz compound followed by one episode of vomiting half an hour before being brought to emergency department. He consumed the poison due to some financial problem at his home. Subsequently, he started becoming drowsy and breathless. He was immediately shifted to ICU. In ICU, he underwent endotracheal intubation with mechanical ventilator support (Intermittent Positive Pressure Ventilation Mode) and was given a gastric lavage, activated charcoal and intravenous crystalloids. Atropine was started owing to history of compound consumption and bradycardia. Over the next couple of hours, his sensorium deteriorated and he went into deep coma. There was no history suggestive of seizures. The patient did not have any known premorbid medical condition and had no history of prescription drug usage.

On admission, His pulse rate was 42 beats per minute and blood pressure was 130/80mm Hg. He had a shallow respiration with a respiratory rate of 10/min. Oxygen saturation at presentation was 68% on room air, which improved to 97% with mechanical ventilator support. On examination of the CNS, he was deeply comatose with a Glasgow Coma Scale (GCS) of 4/15, his pupils were bilaterally constricted (2mm) and not reacting to light, all four limbs had hypotonia with absent reflexes and bilateral plantar response was not elicitable. Bowel sounds were sluggish. Other systemic examinations were normal. There were no excessive oral secretions or any fasciculations. Baseline line blood investigations (complete blood count, renal function test, serum electrolytes, liver function test), cholinesterase level, electrocardiogram, chest X-ray, routine urine testing were normal.

He was started on atropine infusion of 16mg/hour, titrated according to the heart rate to maintain the heart rate of 110/min for first 24 hours. He was adequately hydrated and the urine output was strictly monitored. After 48 hours, his BP started falling and dropped down to 70/50 mmHg. He was started on dopamine infusion which was continued for 24 hours and then tapered off slowly and completely after patient's BP was stabilized. Gradually, over a period of 4 days, he was weaned off the ventilator and the atropine infusion was tapered and omitted over next 4 days. He was late on

discharged after 6 days of stay in hospital in a haemodynamically stable condition, after a counselling session by a psychiatrist.

Discussion

Formamidines show toxic effects on both humans and animals and studies presented until now have reported the reversible nature of these effects. The EPA classifies Amitraz as Class III – slightly toxic.[4] Although the ingested dose of Amitraz can not be determined because it is diluted at 1 part in 500 before usage, the acute oral medical lethal dose (LD50) for the rats is 800mg/kg body weight.[5] Amitraz is a potent hepatotoxic drug acting by decreasing hepatic glutathione activity. [5]

Amitraz stimulates alpha 2 receptors in the CNS and alpha 2 and alpha 1 receptors in the periphery. The clinical manifestations due to this pharmacodynamic property include CNS depression (drowsiness, coma, convulsion), respiratory depression, hypotension, bradycardia, hypothermia, miosis (presynaptic effect at low doses) or rarely mydriasis (postsynaptic effect at higher doses), polyuria (inhibition of antidiuretic hormone and renin)[9] and intestinal distension. CNS depression developing within 30 to 180 minutes was seen as one of the most common manifestation in other studies.[9]

Seizures have been reported by Yilmaz et al and Ertekin et al and responded to treatment with injectable diazepam or lorazepam. The solvent, xylene, may additionally cause acute toxic signs like CNS depression, ataxia, nystagmus, stupor, coma and episodes of neuroirritability. Co-existence of bradycardia and miosis may suggest organophosphorus Poisoning.[6] In our case, bradycardia was present along with miosis. At the same time, there was no other finding of Organophosphorus Poisoning.

Garnier et al reported plasma amitraz concentration of 100 ng / ml , 2 hours after ingestion in an asymptomatic patient and of 500 ng / ml after 2 hours in a patient with drowsiness. Plasma levels are generally unlikely to be of clinical use because of their limited availability. [7]

No antidote is available for this poisoning. Only two cases of death due to this drug have been reported in the literature.[9] The main approach while treating the patients of Amitraz intoxication includes hemodynamic stabilization by proper hydration, maintaining airway, oxygen administration, reduce absorption of poisonous material and measure to improve elimination of the toxin from the body..[8] In most studies, the CNS depression recovered within 4-28 hours unlike in our patient. Most cases completely recover with aggressive supportive treatment, just like our patient.

Conclusion

Amitraz is a deadly poison – with mainly central nervous system and respiratory system depression, without any antidote. Early and aggressive management helps in excellent patient outcome in the form of complete recovery. Also, a lot of public awareness is required to prevent poisoning by such a deadly compound, especially among the illiterate farmers who use such compounds, day-in and day-out, even without knowing its hazards.

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ANIL AGGRAWAL'S
Internet Journal of Forensic Medicine and Toxicology
The world's first online journal devoted entirely to forensic medicine and toxicology · Published 1 January and 1 July

VOL. 16, NO. 1 · JANUARY – JUNE 2015
Downloaded from www.anilaggrawal.com · ISSN 0972-8074 (Online) · https://www.anilaggrawal.com/ij/vol_016_no_001/papers/paper002.html

An Autopsy Case of Hemopericardium Caused by Ascending Aortic Rupture Associated with Idiopathic Aortitis.

Hiromitsu Kurata[a, b, c], Akiko Ishigami[a, b], Itsuo Tokunaga[a], Yasushi Nagasaki[b], Akiyoshi Nishimura[a, b, *]

[a]Department of Forensic Medicine, Institute of Health Biosciences, The University of Tokushima Graduate School, 3-18-15 Kuramotocho Tokushima 770-8503, Japan
[b]Hyogo Medical Examiner's Office, 2-1-31 Aratacho Hyogoku Kobe 652-0032, Japan
[c]Department of Neurosurgery, Mima Hospital, 497 Jogeshima Kamojimacho Yoshinogawa Tokushima 776-0013, Japan

Contact: A. Nishimura · ncc1701abcde@basic.med.tokushima-u.ac.jp

CITE THIS ARTICLE

Kurata H, Ishigami A, Tokunaga I, Nagasaki Y, Nishimura A. An Autopsy Case of Hemopericardium Caused by Ascending Aortic Rupture Associated with Idiopathic Aortitis.. *Anil Aggrawal's Internet Journal of Forensic Medicine and Toxicology* [serial online], 2015; Vol. 16, No. 1 (January – June 2015): [about 12 p]. Available from: https://www.anilaggrawal.com/ij/vol_016_no_001/papers/paper002.html

ABSTRACT

We report the case of a 92-year-old Japanese woman who suddenly died of rupture of the ascending aorta following hemopericardium due to idiopathic aortitis. Rupture of the ascending aorta is usually caused by trauma, an aortic aneurysm, aortic dissection, infection, or a neoplasm, but no such etiologies were found in this case. The pathogenesis of the rupture of the aorta in the present case might have involved the destruction of elastic and collagen fiber due to lymphoplasmacytic infiltration through idiopathic aortitis and increased vulnerability of aortic wall following rupture. Aortitis is usually the cause of aortic aneurysm or dissection, however, aortic perforation is very rare. The frequency of sudden death due to this condition might be much higher than has been reported because cases in which no autopsy was performed would have been classified as unknown acute cardiac death. In cases involving sudden death, we have to keep in mind that the aorta can rupture in the absence of trauma or other typical etiologies.

Keywords: Forensic pathology, Idiopathic aortitis, Hemopericardium, Spontaneous rupture, Ascending aorta

Introduction

Hemopericardium due to rupturing of the ascending aorta is usually caused by trauma, an aortic aneurysm, aortic dissection, infection of the aortic wall, or tumoral invasion of the aortic wall. We describe a very rare case of hemopericardium caused by rupture through idiopathic aortitis of the ascending aorta and discuss its pathogenesis.

Case report

A 92-year-old Japanese woman was found dead on her bed by her son. She had a history of hypertension, atrial fibrillation, and angina pectoris and had been taking aspirin and warfarin. She had no other clinical symptoms and had not required nursing care during her daily life.

Autopsy findings

The subject was 145.0cm in height and weighed 57.2kg. Moderate rigor mortis was observed in all joints, and severe livor mortis was also present although it was easily displaced by digital pressure. Her rectal temperature was 31.0°C (ambient temperature: 22°C). The bilateral bulbar conjunctivas were hyperemic, and many petechiae were observed. The bilateral corneas were mildly turbid. No injuries were observed.

Her heart weighed 342g (normal weight [1]: 327.0+/-49.3g) and contained 200ml of dark red fluid blood. In addition, 190ml blood containing soft clots had accumulated in the pericardial space (Fig. 1A). No perforations or necrotic changes were found in the cardiac wall. The ascending aorta contained a perforation at 2cm distal from the aortic valve together with a subadventitial hematoma that measured 1.3cm in length (Fig. 1B). The aorta did not display any dissection or aneurysms, but did exhibit macroscopic atherosclerotic changes without ulceration or plaque at perforating site. All of the coronary arteries showed moderate atherosclerotic changes but remained patent. No myocardial degeneration or scar formation was found. Her other organs were congested, but did not display any remarkable findings.



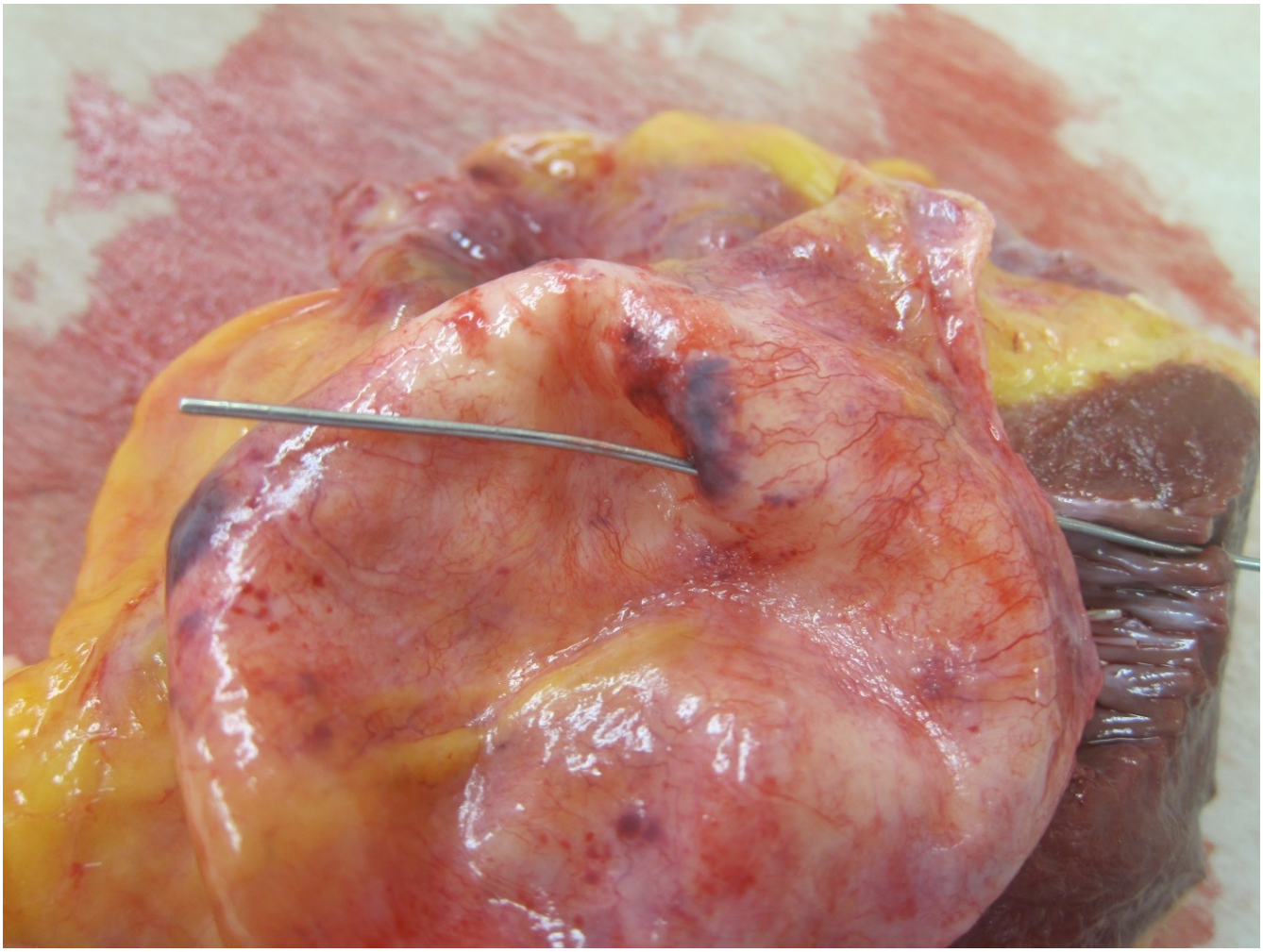
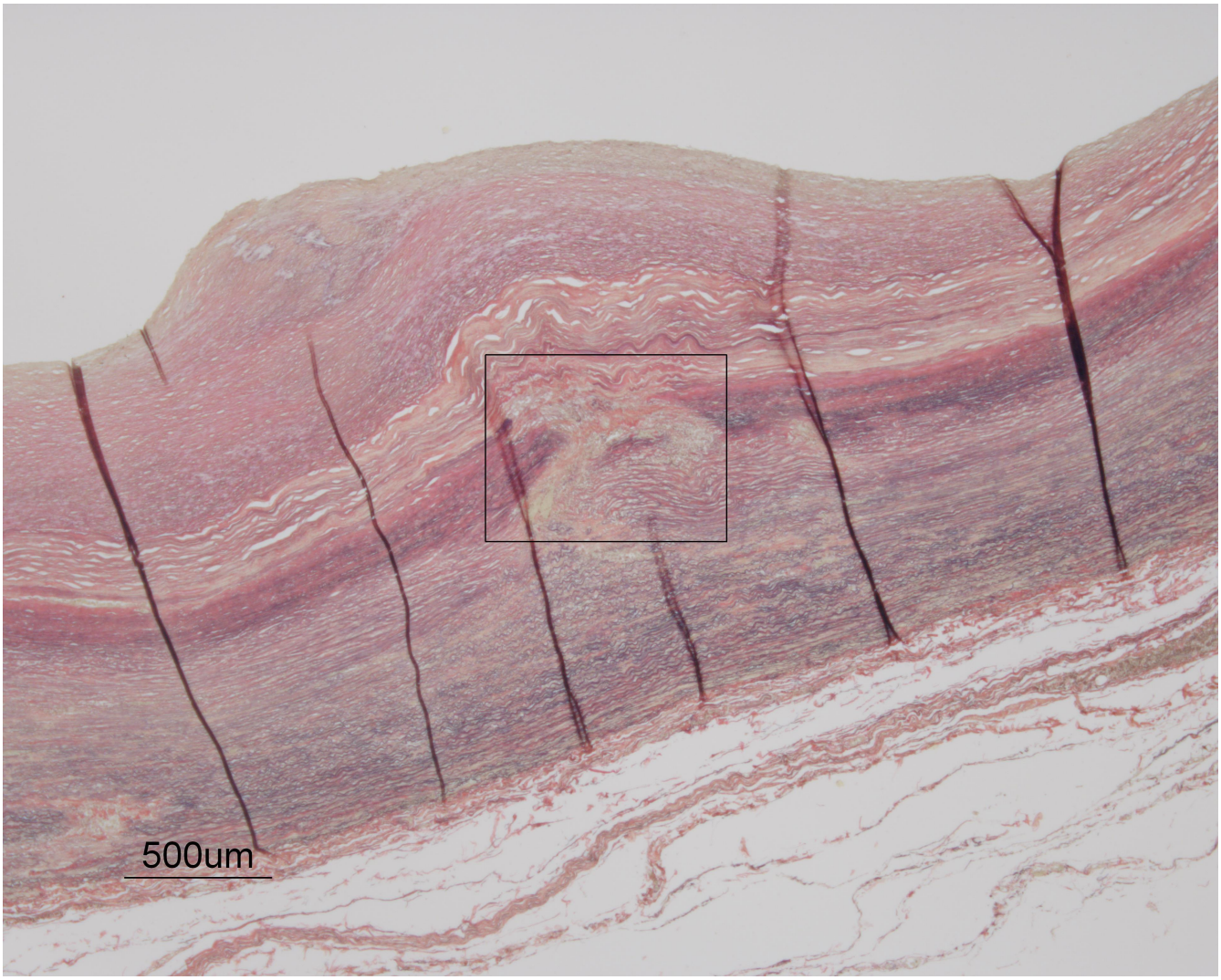


Fig. 1 Hemopericardium containing fluid blood mixed with soft clots is visible within the pericardial cavity (A). Perforation accompanied by intramural hematoma formation visualized on the aortic wall 2 cm distal to the aortic valve (B).

Histological findings

Histological examination of sections stained with Elastica van Gieson (EVG) showed rupture of the elastic fibers and collagen fibers constituting the internal elastic lamina of the aortic wall (Fig. 2A). Examination of HE-stained sections revealed sporadic infiltration by lymphocytes and plasmocytes at the sites of rupture of the elastic fibers, partially accompanied by bleeding, although polynucleated giant cells were absent (Fig. 2B). On the other hand, examination of EVG-stained sections did not reveal lymphocyte or plasmocyte infiltration at the site of rupture of the elastic fibers of the internal elastic lamina, although partial scarring was noted. The tunica intima was thickened, showing some atherosclerotic changes (e.g., hyalinization). There was no evidence of vasculitis in any other organs.


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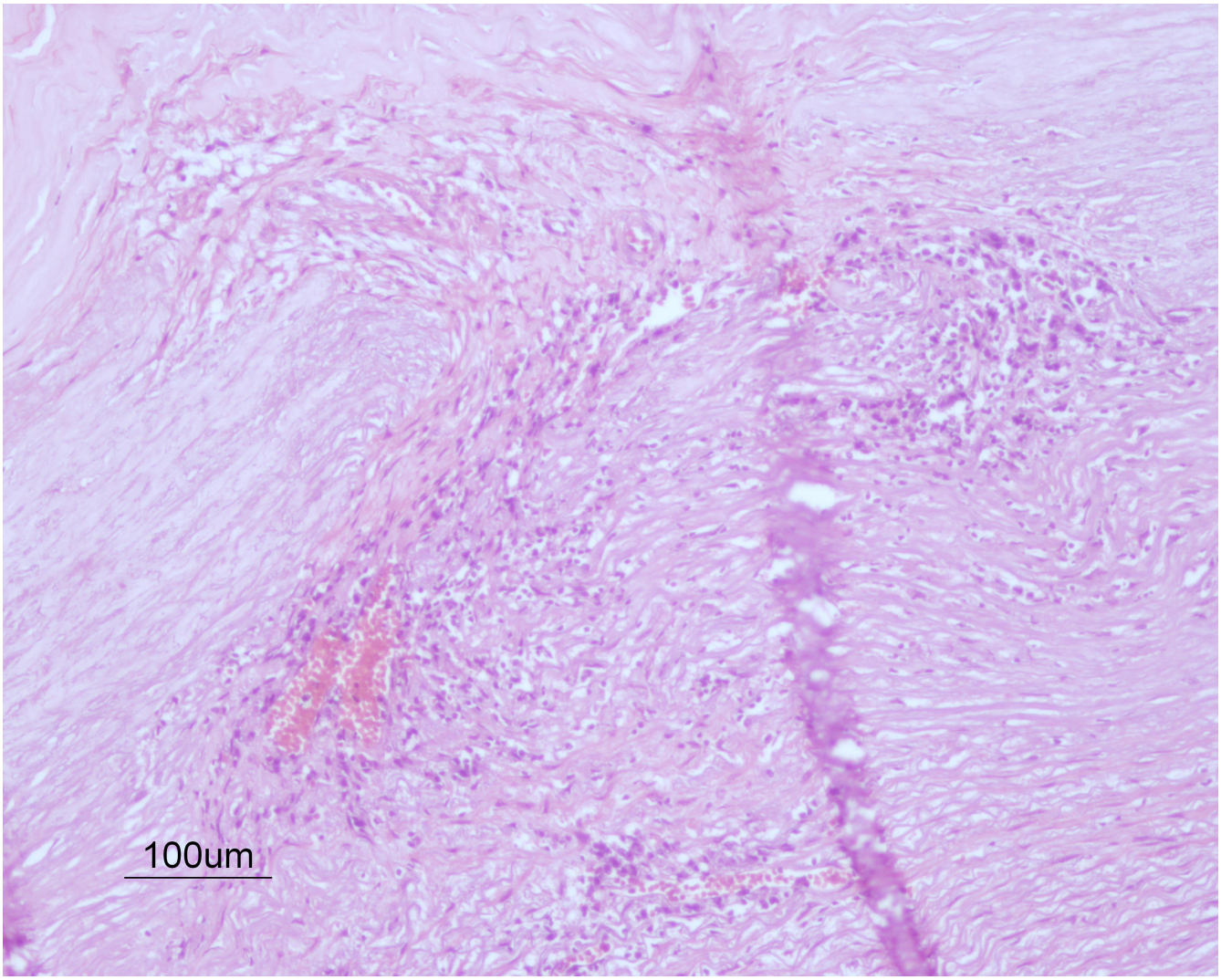


Fig. 2 Rupture of the internal elastic lamina of the aorta revealed by Elastica van Gieson staining, with destruction of the elastic fibers being a predominant change (A). Hematoxylin-Eosin staining of square site of Fig. 2(A) showing lymphocyte and plasma cell infiltration and bleeding around the destroyed elsatic fibers (B).

Discussion

Aortitis is pathologically defined as inflammation of the aortic wall [2]. However, there is no uniform clinical definition or classification of vasculitis, including aortitis, and various syndromes have been referred to under the heading of vasculitis, depending on the site affected, the underlying disease, etc. According to the CHCC2012 adopted at Chapel Hill in 2012 to establish a uniform definition for vasculitis, vasculitis involving the aorta includes that associated with systemic diseases such as giant cell arteritis (GCA), Takayasu's arteritis, rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and Behcet's disease [3].

In the present case, there was no history of systemic diseases such as RA, and it was possible to rule out systemic disease as the cause of the aortitis. GCA more commonly occurs in elderly people; however, it is characterized histologically by the presence of polynucleated giant calls and granulomas, differing from the histological findings in the present case. Takayasu's arteritis appears more frequently in younger persons, and differs from the present case in that it is

characterized by the involvement also of branching blood vessels other than the aorta. Thus, the vasculitis seen in the present case did not satisfy any of the definitions adopted by CHCC2012.

Recently, the new disease entity “idiopathic aortitis” has been proposed, characterized by evidence of non-infectious aortitis in the absence of underlying vascular diseases such as GCA or Takayasu’s arteritis, or indeed any multi-system diseases such as RA, SLE, or Behcet’s disease [2, 4]. Idiopathic aortitis confined to the thoracic aorta is often not associated with any clinical manifestations of inflammation and is detected accidentally based on the detection of lymphoplasmacytic infiltration of the aortic wall in histopathological specimens of the aorta obtained at the time of surgical resection for thoracic aortic aneurysm, etc.[2]. In the present case, a diagnosis of idiopathic aortitis was made on the basis of the histopathological findings in the absence of any underlying diseases and any evidence of vasculitis involving other blood vessels.

In a previous study carried out to determine the incidence of aortitis, the complication of thoracic aortic aneurysms by aortitis was reported. Of the 70638 individuals in Malmö City (Sweden), with a population of approximately 240,000, who died during the 27-year period from 1958 to 1985, autopsy was performed in 85% and the causes of death were analyzed [5]. Among these, the complication of thoracic aortic disease by aortitis was seen in 9 of the 216 patients with fatal thoracic aortic dissection, 8 of the 63 patients with ruptured thoracic aortic aneurysm, and 22 of the 205 patients with asymptomatic thoracic aortic aneurysm. Thus, evidence of aortitis was found in 39 (8.1%) of the 484 cases of thoracic aortic dissection or aneurysm.

In addition, another study of aortitis in cases operated for thoracic aortic aneurysm has been reported [6]. According to this report, aortitis was detected in 39 (4.9%) of 788 cases of thoracic aortic aneurysm; the condition was non-infectious in 38 of the 39 cases (4.8%). The underlying disease was GCA in 30 cases, inflammatory aneurysm in 3 cases, aortitis accompanied by non-specific lymphocyte/plasma cell infiltration in 2 cases, Takayasu’s arteritis in 1 case, SLE in 1 case, and Behcet’s disease in 1 case. Of these cases, cases with aortitis characterized by the histological findings of non-specific lymphoplasmacytic infiltration were considered to fall under the definition of idiopathic aortitis, seen at an estimated frequency of 0.3% of all cases with thoracic aortic aneurysm and 5.1% of all cases with aortitis.

Although aortitis is known to be associated frequently with aortic aneurysm or dissection, aortic rupture in the absence of aneurysm in such cases is considered to be rare, with only one such case of rupture associated with GCA having been reported [2, 7]. Hemopericardium due to perforation of the ascending aorta usually develops as a complication of rupture of an aortic aneurysm or aortic dissection, infection or neoplasm, other than the cases attributable to trauma; rare causes include penetrating atherosclerotic ulceration (PAU), a disease entity proposed in 1986 by Stanson et al, and spontaneous rupture. In cases of PAU, ulceration in an atheroma of the tunica intima can occur, causing perforation of the internal elastic lamina, and if it spreads to the tunica media, loading of the tunica media by the arterial pressure would eventually lead to the formation of an intramural hematoma and dissection. Occasionally, perforation of the internal elastic lamina does not spread to the tunica media, but directly spreads to the tunica externa, causing perforation [8, 9]. Some cases are reported as cases of spontaneous rupture; however, the vascular findings in such cases include signs of atherosclerosis and cystic necrosis of the tunica media, indicating the necessity of discussing their possible association with PAU [10, 11]. In the present case, macroscopic signs of atherosclerosis were noted and perforation due to PAU could not initially be ruled out; however, no ulceration was found at perforation site of aorta, and the histopathological finding of lymphocyte

and plasmocyte infiltration of the aortic wall allowed precise differential diagnosis as idiopathic aortitis. In other words, the definite cause of aortic rupture cannot be identified solely on the basis of the macroscopic findings at autopsy, without histopathological examination.

Patients developing hemopericardium due to thoracic aortic perforation are more likely to undergo autopsy from the standpoint of forensic medicine, because they often die suddenly without any precursor signs. The percentage of cases autopsied among all cases with this condition is particularly low in Japan and death from this disease is counted as sudden cardiac death. Even if hemopericardium can be confirmed by post-mortem CT scanning, there are diverse causes for this condition, including trauma, infection, aortic aneurysm, aortic dissection, etc., this condition, although rarely, can arise from aortitis, like in the present case. So, active autopsy and histopathological examination seem to be desirable to identify the cause of death in cases like this one.

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ANIL AGGRAWAL'S
Internet Journal of Forensic Medicine and Toxicology
The world's first online journal devoted entirely to forensic medicine and toxicology · Published 1 January and 1 July

VOL. 16, NO. 1 · JANUARY – JUNE 2015
Downloaded from www.anilaggrawal.com · ISSN 0972-8074 (Online) · https://www.anilaggrawal.com/ij/vol_016_no_001/papers/paper003.html

Malingering Of A Skin Eruption As A Burn Scar: Case Report.

Luv Sharma[1*], Kamal Aggarwal[2]

[1]Professor, Department of Forensic Medicine, Pt. B. D. Sharma Post-Graduate Institute of Medical Sciences, University of Health Sciences, Rohtak-124001, Haryana, India

[2]Professor, Department of Skin & V.D., Pt. B. D. Sharma Post-Graduate Institute of Medical Sciences, University of Health Sciences, Rohtak-124001, Haryana, India

Contact: Dr. Luv Sharma · drluvksharma@yahoo.com

CITE THIS ARTICLE

Sharma L, Aggarwal K. Malingering Of A Skin Eruption As A Burn Scar: Case Report.. *Anil Aggrawal's Internet Journal of Forensic Medicine and Toxicology* [serial online], 2015; Vol. 16, No. 1 (January – June 2015): [about 12 p]. Available from: https://www.anilaggrawal.com/ij/vol_016_no_001/papers/paper003.html

ABSTRACT

Scars due to thermal or mechanical injuries are commonly encountered by Forensic experts both in the living and the dead. Other conditions giving rise to scarring include vaccination and surgical scars. Scarring caused by various mechanical injuries can be recognized and differentiated from each other. Scarring is also present in certain skin diseases. Scars basically indicate the healing process of an injury and are useful in estimating time/duration of an injury. Misinterpretation of scarring is not uncommon. In the present case, a scar caused by a common skin disease was claimed to be that of torture with a red iron rod. The investigating police brought the victim, a small boy, for examination. No evidence of injury or burn was found on examination. In fact, the case was diagnosed to be of scabies. This is an uncommon situation; though the dermal ailment was common, a small boy using it to evade punishment was something new and disturbing. Protocol of medico-legal investigation of an injury patient with scars should include clinical evaluation and other relevant investigations before arriving at any conclusion on their manner and type as they may turn out to be due to common disease processes.

Keywords: Scars, dermal, misrepresentation

Introduction

"He jests at scars that never felt a wound." [1] William Shakespeare's famous quote holds true for most situations when a scar is encountered in medical practice. Scars (also called cicatrices) are areas of fibrous tissue (fibrosis) that replace normal skin (or other tissue) after injury or disease. A

scar results from the biologic process of wound repair in the skin and other tissues of the body. Thus, scarring is a natural part of the healing process.

Scars have long been used (misused) by criminals for either evading/lessening punishment or for fabricating scars to frame others. Child abuse by burning comprises 6–20% of all child abuse cases, but misdiagnosis may arise in cases of some medical conditions.[2]

The annual incidence of child abuse was estimated to be 2.8 million by the national incidence study conducted in the USA in 1993, which is a two-fold increase compared to 1986. Awareness of child abuse has been increasing since the 1960s. Although most victims of child abuse present with cutaneous lesions, many genuine skin diseases may appear as non-accidental injuries which, if not recognized, may lead to misdiagnosis of child abuse.[3]

Incorrect diagnoses during child abuse evaluations are serious. Because skin lesions are common in abuse, it is important to consider cutaneous mimics of physical abuse.[4]

Case presentation

A 12 year old boy was brought to our facility by the State Crime branch for expert opinion regarding alleged torture marks on his body. The boy had been kidnapped by a gang of criminals who used these children as rag pickers and pickpockets. The boy was caught pickpocketing on a railway station of an adjoining state, whence his parents filed a case of kidnapping and torture against the alleged kidnapper and the case was transferred to the State Crime Branch for investigation.

Clinical & local examination

The initial examination of the boy was carried out in the general hospital of his home district where the examining doctors referred the case to our institution. On local and clinical examination, itchy papular and vesicular lesions, red in colour over the infra-umbilical area, both sides of the midline were noted (Figures 1 &2). The child on inquiry kept repeating that the lesions were due to torture using a hot iron rod. He also kept on scratching the affected area due to intense pruritus. There was no evidence of thermal or mechanical injury at the sites mentioned as injuries in the Medico-legal report. The lesions were diagnosed as scabies of at least 4 weeks duration as evidenced by the popular & vesicular lesions. The skin scrape test was also positive for scabies mites. The itching is due to a delayed hypersensitivity reaction (Type IV) to the mites, their eggs or feces which develops after approximately 4 weeks of the infestation.

Discussion

Several skin lesions may simulate burns. Disruption of the skin layers or its blood supply due to any reason produces similar lesions that mimic a burn injury. Some of these diseases are Stevens-Johnson disease, Lyell's syndrome, erythema multiforme, necrotizing fasciitis, automatic skin necrosis and coagulation disturbances (purpura fulminans, coumarin-induced skin necrosis syndrome), pemphigoid, and subacute cutaneous lupus erythematosus. All of these diseases affect the function and viability of skin and underlying tissues in a way resembling the loss of skin in burns.[5]

Perera conducted a study on 100 medico-legal records of victims of torture examined at the Judicial Medical Officer's Office, Colombo, Sri Lanka between 1998 and 2001 to try to interpret scars of

torture. The objectives of this study were to produce the features of a typical scar of a few selected methods of torture and determine their characteristic distribution. Scars caused by seven selected methods of torture were taken into consideration. They were, assault with both non-pliable and pliable blunt objects, cigarette burns, burns with heated solid objects and molten plastic, ligature application with different forms of suspension and cuts with sharp objects. Total number of scars analyzed in this study was 1846, which included 740 scars of non-pliable blunt weapons, 182 of pliable blunt weapons, 102 of ligature, 59 of molten polythene, 55 of heated objects and 82 of razor blades and knives. Typical scars of each method of torture and their characteristic distribution were determined.[6]

Porzionato reports two cases of dermatological conditions mimicking as child abuse scars. The first concerns a 6-month-old girl was referred to hospital for small round ulcerations on the face and abdomen, resembling cigarette burns which were finally diagnosed as Staphyloccocal scalded skin syndrome. The second case was of a 2-month-old boy hospitalized for erythema, with bullous lesions on the abdomen; a mis-diagnosis of inflicted scalds was made which was later finally ruled out and SSSS diagnosed.[2]

Heider et al[7] have reported a case of pediatric eczema mimicking an abusive burn.

Gruson and Chang report a case of Berloque dermatitis (caused by application of Bergamot containing skin lotions) which resembled child abuse. The child had blistering rash over her face and neck. These lesions were first diagnosed to be due to child abuse but detailed examination revealed them to be lesions of Berloque dermatitis.[8]

Burning with hot objects produces markedly atrophic scars which reflect the shape of the instrument and which are sharply demarcated with narrow hypertrophic or hyper-pigmented marginal zones corresponding to an initial zone of inflammation. This may, for instance, be seen after burning with an electrically heated metal rod or a gas lighter. It is difficult to make a differential diagnosis if many scars are present. Spontaneously occurring inflammatory processes lack the characteristic marginal zone and only rarely show a pronounced loss of tissue.[9]

Conclusion

A full and thorough clinical examination must be carried out in all child abuse cases. Lesions and scars on the body may not always be consequent to torture. Many dermatological conditions may resemble scars. The United Nations has released a manual on the effective investigation and documentation of torture & other cruel, inhuman or degrading treatment or punishment called the Istanbul Protocol.[9] The protocol describes the steps to be taken while examining an alleged case of torture. In the section dealing with medical examination of such cases, stress has been laid to elicit information on physical ailments that the individual believes were associated with torture or ill treatment. Even if the after-effects of acute lesions cannot be seen months or years later, some physical findings may still remain, such as electrical current or thermal burn scars, etc. The examination should include the entire body surface in order to detect signs of generalized skin disease including signs of vitamin A, B and C deficiency, pre-torture lesions or lesions inflicted by torture, such as abrasions, contusions, lacerations, puncture wounds, burns from cigarettes or heated instruments, electrical injuries, alopecia and nail removal. Torture lesions should be described by their localization, symmetry, shape, size, colour and surface as well as their demarcation and level in relation to the surrounding skin. Photography is essential whenever

possible. Ultimately, the examiner must offer an opinion as to the origin of the lesions: inflicted or self-inflicted, accidental or the result of a disease process.

This case was simple in terms of diagnosis, i.e. a common skin disease which was easily diagnosed. The importance of proper clinical evaluation of scars brought for medico-legal examination need not be overemphasized.

Figure 1- the skin lesions later diagnosed as scabies.

Figure2- a close up of the scabies lesions

Figures:



Figure 1- Itchy papular and vesicular lesions seen on the abdomen



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ANIL AGGRAWAL'S
Internet Journal of Forensic Medicine and Toxicology
The world's first online journal devoted entirely to forensic medicine and toxicology · Published 1 January and 1 July

VOL. 16, NO. 1 · JANUARY – JUNE 2015
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Tracking Drowning Trends in Himachal Pradesh during 2006-2010.

Mukesh Kumar Thakar*, Preeti Guleria
Contact: Mukesh Kumar Thakar · mukeshk38@gmail.com

CITE THIS ARTICLE
Thakar MK and Guleria P. Tracking Drowning Trends in Himachal Pradesh during 2006-2010.. *Anil Aggrawal's Internet Journal of Forensic Medicine and Toxicology* [serial online], 2015; Vol. 16, No. 1 (January – June 2015): [about 12 p]. Available from: https://www.anilaggrawal.com/ij/vol_016_no_001/papers/paper004.html

ABSTRACT
Himachal Pradesh is the state covered with different types of water bodies and its tributaries. The state is a major hub for tourism. Every year large number of tourist visits the hill station and gets trapped. Drowning is one of the most common causes of death here particularly accidental drowning. Therefore, there is a need to investigate the following questions:

Cause of death;

Actual site where death take place;

Whether the death is anti-mortem or post-mortem? or Whether death is suicidal, accidental or homicidal?

In the present study an attempt has been made to collect data of drowning death from all the districts (12) of Himachal Pradesh and then organized in a proper sequence to trace common drowning site, mode or manner of drowning. Then further attempt has been made to link the data to a common age group (adolescent, teenager or adult) of drowned persons. The data were further analyzed districts wise to find out the minimum and maximum number of drowning cases. The present study is expected to answer the above questions and further help in generating database which can help in the reduction of the death rate pertaining to drowning cases.

Keywords: Drowning, Himachal Pradesh, accidental, suicidal and homicidal

Introduction

The state of Himachal Pradesh has a big trap of rivers, lakes and other types of water bodies, which enhances the rate and scope of drowning cases. State is dominated by mountains and their associated rivers and lakes and is demarcated from the plains of Himachal and Punjab by the

Shivaliks. Himachal has five mighty rivers namely the Chenab, Ravi, Sutlej, Beas and Yamuna flowing through it; 24 Lakes and has 3 Reservoirs. Every year heavy rainfall (the average rainfall is 152 cms or 60 inches) takes place which results in a large number of accidental drowning in the area (Ahluwalia, 1998). Therefore, Drowning is a one of the leading cause of death in the state of Himachal Pradesh (Srivastava et al., 1987). Patterns of drowning vary from one geographic area to other, depending on the local physical and social environment (Warneke et al., 1994).

Drowning, it is one of the types of asphyxial death in which the respiration is inhibited by submersion in a fluid such as water, irrespective of whether the fluid is aspirated into the lungs or not. Large number of cases related to drowning has been reported every year in Himachal Pradesh. Earlier various studies have been conducted by various scientists like Walker and Crawford,1968; Park et al.,1974; Srivastava et al., 1987; Howland et al., 1996; Joseph et al., 2003 and Lunetta et al., 1998 and 2004 have also analysed drowning to determine their relationship with age, race, alcohol, pre-existing disease and other factors. Stevenson and Rimajova, 2003 attempted to investigate the causes of child drowning and revealed that 50 children of 5- years drowned in private swimming in Australia during 12-year observational period starting from 1998 to 2000.

The present work has been undertaken to study the most vulnerable drowning areas existing in the state of Himachal Pradesh. Similar attempts has already been made earlier by Thakar et al. (2009) by collecting data of drowning cases happened in Punjab state from (2005-2006) and studied various parameters on which they choose five different water bodies for Diatomological mapping of Punjab, which were reported to be most common site of drowning. About 536 cases of drowning were reported in Punjab from 2005 to 2006 and about 80% of the drowned victims were reported to be males while remaining 20% were found to be females, whereas District Roopnagar was reported to be distressed with maximum number of drowning cases.

This study examines annual counts of deaths from Himachal Pradesh data for a period 5 years i.e. from 2006 to 2010. This study has provided potentially useful data concerning victims and circumstances of drowning in Himachal Pradesh. The present study is based on the observation taken from the score of variables that includes type of drowning site, nature of drowning, Age groups, and gender type of drowned victim. Significant seasonal drowning patterns were found in all of the age and cause of death groups. This 5- yearly study has revealed some important facts about drowning cases occurred in Himachal Pradesh. This effort is supposed to be of immense help in various medicolegal studies related with drowning cases but need for more valid information and for further studies along various lines is also emphasised.

Methodology

Collection of Data

The data regarding drowning cases were collected from the various district offices of Senior Superintendent of Police through RTI and the crude information was framed into fruitful information, which can further help in sorting drowning issues. The study has been based on the interpretation of the data related to drowning cases recorded by the district police offices during five years i.e. 2006-2010.

Himachal Pradesh has the following twelve (as shown in a map of Himachal Pradesh) districts:

- Bilaspur

- Chamba
- Hamirpur
- Kangra
- Kinnaur
- Kullu
- Lahaul Spiti
- Mandi
- Shimla
- Sirmaur
- Solan
- Una

Interpretation

After the collection of data from the various districts of Himachal Pradesh through RTI, it was subjected to interpretation in terms of the following five selected variables to generate the baseline data. District wise data has been prepared separately (individually for every year) and collectively for the five years i. e. from 2006 to 2010.

Variables Selected to generate baseline database are as follows:-

1. **Total no. of drowning cases happened in Himachal Pradesh in the five years (2006-2010) as shown in table no.- 1**

- Individually for every year
- Collectively for five years

1. **District wise percentage of Drowning (**showing maximum or the minimum number of drowning cases took place as depicted in Histogram no. 1)

- Districts with highest percentage i.e. the district in which maximum drowning took place in the years 2006-2010 (both individually for every year and collectively for the five years).
- District with lowest percentage i.e. the district in which minimum drowning took place in the years 2006-2010 (both individually for every year and collectively for the five years).

1. **Gender ratio** (the ratio involved or suffered drowning among males and females) is shown in table no. 2

- Among males and females (Percentage of maximum or minimum number of males or females involved in every year (Individually) and collectively for all the five years) has been shown in Pie chart 1.

4. Mode of drowning (Individually for each year & collectively for all the five years) as shown in table no. - 3

- Whether the mode of drowning was Suicidal;
- or it was Accidental or;
- It was a case of Homicidal drowning;
- Or it was Unknown (where it was not able to establish the actual mode of drowning)

5. **Manner of Drowning** (whether it was Accidental, Suicidal, Homicidal or it was Unknown in all the districts individually for each year and collectively for every year both among the males and females) as shown in table no.4 and Pie chart 2.

6. Season more prone to Drowning (the season in which maximum drowning cases took place and the season where minimum drowning cases took place, both individually for every year and collectively for all the years) has been shown in table no. 6 and Peak chart-1.

- Winter
- Monsoon
- Summer

Age group of drowned victims

The data was separated on the basis of age (as shown in table no. 5) and divided in to three categories, which is as follows:

- Adolescent (Age 1-14 years) as shown in table no. 5 (I) -- Category: A
- Teenage (Age 15-25 years) as shown in table no. 5 (II) -- Category: B
- Adult (age more than 25 years) as shown in table no. 5 (III) -- Category: C

Type of Water Bodies Drowning site (the site that was more prone to drowning)

- Rivers
- Canals
- Wells
- Ponds

Tables

Table 1: Total Number of Drowning Cases in Himachal Pradesh (Individually and Collectively)

S.No.	District	2006	2007	2008	2009	2010	Total 2006-2010
1.	**Bilaspur**	10	13	8	15	8	54
2.	**Chamba**	8	8	4	9	7	36
3.	**Hamirpur**	10	3	8	7	13	41
4.	**Kangra**	28	24	23	---	---	75
5.	**Kinnaur**	3	5	3	2	4	17
6.	**Kullu**	8	8	6	15	15	52
7.	**Lahaul spiti**	1	1	7	3	3	15
8.	**Mandi**	4	8	15	27	24	78
9.	**Sirmaur**	8	13	12	11	11	55
10.	**Shimla**	1	NIL	1	1	7	10
11.	**Solan**	4	6	7	8	12	37
12.	**Una**	5	9	13	9	8	44
TOTAL		**90**	**98**	**107**	**107**	**112**	**514**
Percentage		**17.5**	**19.0**	**20.8**	**20.8**	**21.7**	

(---): Data not provided.



Map of Himachal Pradesh showing various Districts

Table-2 Showing Gender ratio (Among males and females)

Year	Males	Percentage	Females	Percentage	Total
2006	67	76.13 %	21	23.86 %	88
2007	67	68.36 %	31	31.63 %	98
2008	81	77.14 %	24	23.85 %	105
2009	79	75.23 %	26	24.76 %	105
2010	83	74.10 %	29	25.89 %	112
2006-2010	**377**	**74.21 %**	**131**	**25.78 %**	**508**

Table 3: Showing Mode of Drowning in the State of Himachal Pradesh Collectively in the year 2006 to 2010

M	F	M	F	M	F	M	F
Accidental		**Suicidal**		**Homicidal**		**Unknown**	
379		84		1		50	
298	82	48	35	-	1	37	13
73.9		16.1		0.1		9.7	

*Symbols-(M-Male, F- Female)

Table 4: Showing number and manner of drowning among males and females collectively (2006-2010)

	Males	Females	Males	Females	Males	Females	Males	Females
District	**Accidental 2006-2010**		**Suicidal 2006-2010**		**Homicidal 2006-2010**		**Unknown 2006-2010**	
Bilaspur	34	5	4	4	-	-	5	2
Chamba	22	5	4	4	-	-	-	1
Hamirpur	25	8	4	3	-	-	1	-
Kangra	31	5	9	4	-	-	19	7
Kinnaur	7	4	-	-	-	-	5	1
Kullu	31	8	6	7	-	-	-	-
Lahaul spiti	8	3	-	4	-	-	-	-
Mandi	48	12	7	4	-	-	5	2
Sirmaur	41	11	1	-	-	-	2	-
Shimla	7	3	-	-	-	-	-	-
Solan	27	7	3	0	-	-	-	-
Una	17	11	10	5	-	1	-	-
Total	**298**	**82**	**48**	**35**	**-**	**1**	**37**	**13**

(-): no case reported

Table-5 -: Showing Age group of drowned victims in the state of Himachal Pradesh (Individually & collectively)

Category- A

(Age 0-14 years)

Age Group	0-14 years					
District	**2006**	**2007**	**2008**	**2009**	**2010**	**2006-2010**
Bilaspur	-	2	-	1	-	3
Chamba	-	-	1	-	-	1
Hamirpur	-	-	-	-	1	1
Kangra	4	3	2	-	-	9
Kinnaur	-	-	-	-	-	-
Kullu	-	-	-	-	2	2
Lahaul spiti	-	-	3	-	-	3
Mandi	-	1	1	4	1	7
Sirmaur	-	4	-	-	1	5
Shimla	-	-	-	-	2	2
Solan	1	-	1	2	3	7
Una	-	1	3	2	2	8
Total	**5**	**11**	**11**	**9**	**12**	**48**

Category- B

Age 15-25 years

Age Group	15-25 years					
District	**2006**	**2007**	**2008**	**2009**	**2010**	**2006-2010**
Bilaspur	1	3	5	3	4	16
Chamba	3	4	1	3	-	11
Hamirpur	4	1	2	2	6	15
Kangra	8	3	7	-	-	18
Kinnaur	1	1	1	1	1	5
Kullu	3	3	1	5	3	15
Lahaul spiti	-	-	2	-	-	2
Mandi	1	7	-	9	7	24
Sirmaur	5	5	1	3	1	15
Shimla	1	-	-	-	-	1
Solan	-	1	1	-	5	7
Una	1	3	4	5	2	15
Total	**28**	**31**	**25**	**31**	**29**	**144**

III) Category- C

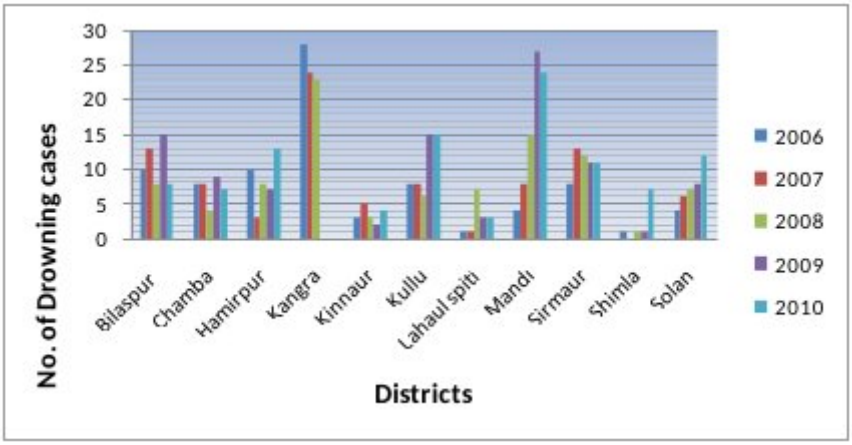
(Age more than 25 years)

Age Group	More than 25 years					
District	**2006**	**2007**	**2008**	**2009**	**2010**	**2006-2010**
Bilaspur	4	6	1	8	4	23
Chamba	5	4	2	5	7	23
Hamirpur	5	2	6	4	6	23
Kangra	14	17	12	-	-	43
Kinnaur	2	4	2	1	3	12
Kullu	4	4	5	9	10	32
Lahaul spiti	1	1	2	3	3	10
Mandi	3	7	7	14	16	47
Sirmaur	2	3	7	7	8	27
Shimla	-	-	-	1	3	4
Solan	-	1	2	4	3	10
Una	4	5	6	2	3	20
Total	**44**	**54**	**52**	**58**	**66**	**274**

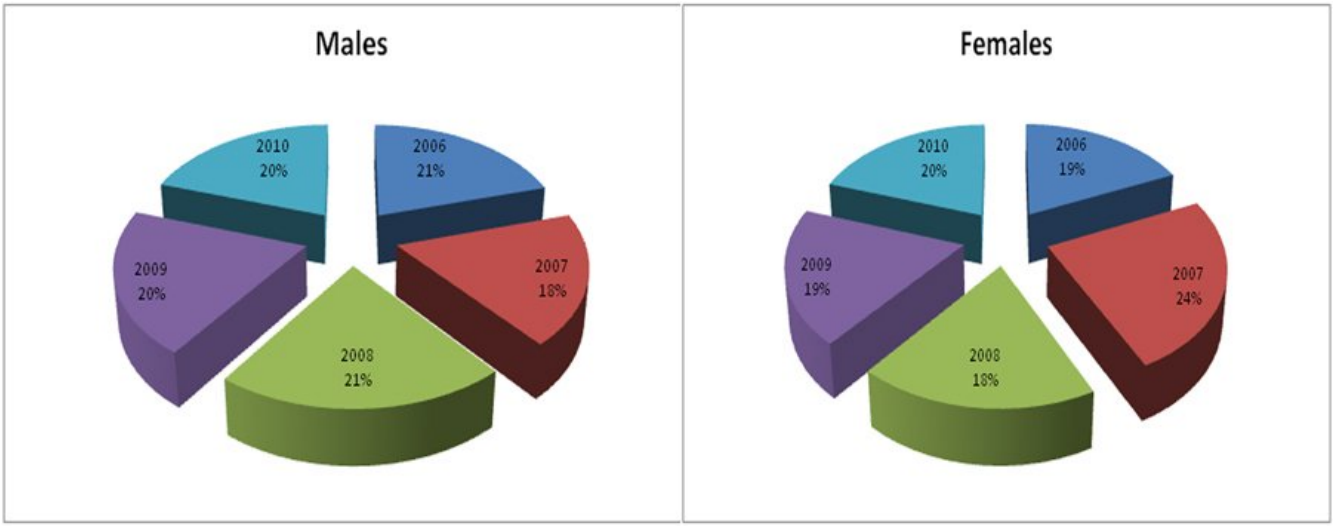
Table- 6 Showing Season Most Prone to Drowning

Year/ season	Winter	Monsoon	Summer
2006	22	27	37
2007	31	34	27
2008	28	45	26
2009	33	32	38
2010	33	24	44
2006-2010	**147**	**162**	**172**

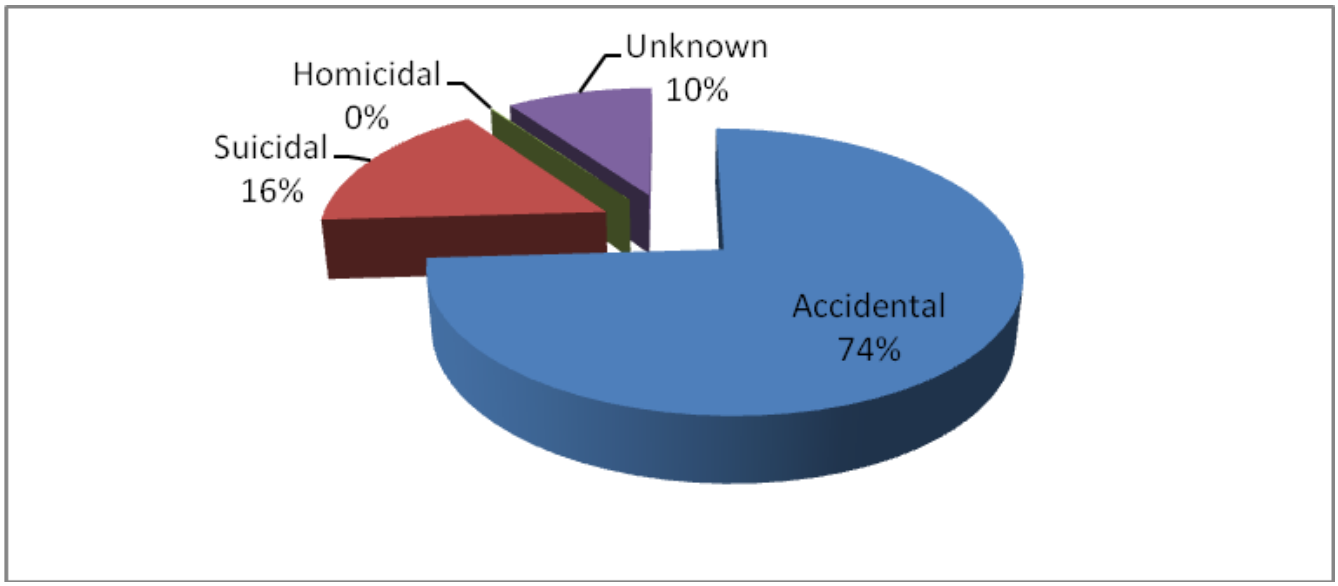
Histogram-1 Showing No. of drowning cases in various districts of Himachal Pradesh



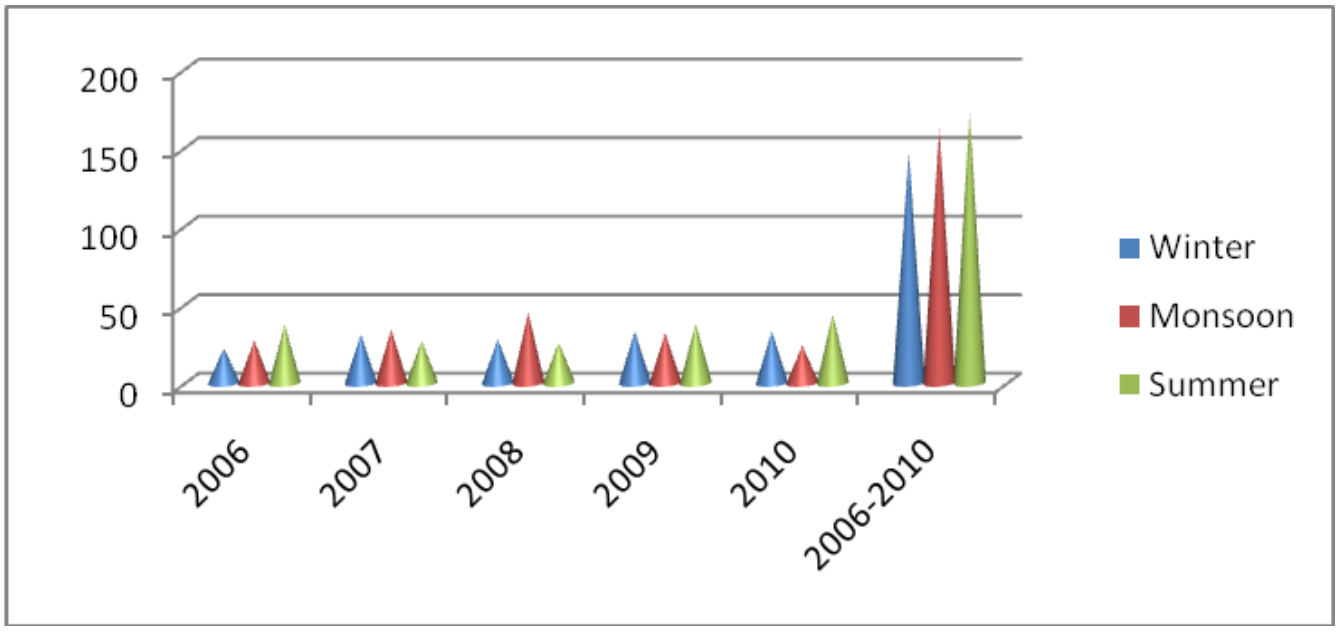
Pie chart-1 showing Percentage Male and Female Drowned in Himachal Pradesh



Piechart-2 Showing Percentage of Manner of Drowning collectively in Himachal Pradesh



Peak chart 1 -: Showing seasons prone to drowning in the state of Himachal Pradesh both (individually and collectively)



Results and Discussions

In the present study an attempt has been made to study the emerging pattern of deaths due to drowning in the state of Himachal Pradesh for the period of 2006-2010. During these five years total 514 cases of drowning have been registered in the state of Himachal Pradesh (table-1). It is evident from table-1 that there is a constant increase in the rate of drowning cases during these periods i.e. 2006 (90), 2007(98), 2008(107), 2009(107) and 2010(112) respectively (Histogram - 1). But the data of Kangra district for the year 2009-2010 was not reported by the police officials due to lack of information (table-1). The rising figures of drowned victims in the succeeding years brought a thought to take preventive measures so that drowning figures could be controlled. The data

obtained in the present study was then organized in a proper sequence to study various variables (as mentioned above in method and material) such as age, sex, cause of drowning and seasons prone to drowning of victims etc. to generate useful information for the forensic scientists working in the field. It is evident also from Histogram-1 and table-1 that maximum number of drowning cases was registered in Mandi district (78) followed by Kangra (75) district and least number of cases have been registered in the Himachal Capital Shimla (10). Table -2 and Pie chart-1represent the differences in the percentage ratio between males and females both year wise individually and collectively from 2006 to 2010, it was found that 74.2 % of the drowned deceased were males while 25.7% were females in the five years. Most of the drowning occurred in rivers and its tributaries while a few cases have reported to occurred in the wells and ponds as well.

Table -3 illustrates the percentage of the *pattern of drowning* in Himachal Pradesh i.e. *accidental, suicidal or homicidal*. From the data analysis it can be safely observed that most of the cases (73.9%) reported due to drowning are of Accidental while suicidal and Homicidal are 16.1% and 0.1% respectively (Table-3 and Pie chart-2), Among accidental cases reported Males were 298 as compared to Females (82). Similar trend has been observed in suicidal (16.1%) mode of drowning, in which maximum numbers was males (48) as compared to females (35). But only one case of Homicidal (0.1%) drowning (1female) was reported which is almost negligible when compared to other mode of death due to drowning.

The details of the *manner of drowning* among males and females in various districts of Himachal Pradesh state has been give in Table-4. It has also been observed from the data analysis that maximum accidental drowning is reported in Mandi district which includes 48 males 12 female, whereas minimum accidental drowning cases was reported in Shimla district, which includes 7 male and 3 females from 2006 to 2010. Maximum suicidal cases of drowning have been reported in Una district (includes 10males and 5females) whereas minimum suicidal drowning cases was reported in Solan district (3males and surprisingly no female). No case of suicidal drowning was reported in Kinnaur and Shimla district during 2006-2010. Only one case of female homicidal drowning was reported from Una district.

In total 37 cases of male drowning and 13 of female, exact mode of drowning is unknown which is 9.7% of total drowning cases (Table-3). Maximum cases (26) of Unknown mode of drowning were reported from Kangra district which includes males (19) and females (7) followed by Hamirpur (01) and Chamba district (01).

Table- 5 shows details about the various *age groups* which was grouped into three following Categories:

- Adolescent (1-15 years) -- Category - A
- Teenage (15-25years) -- Category - B and
- Adult (25 and above) -- Category - C

It was also observed that Maximum number of drowning cases reported belongs to adult age group (274) out of total 514 cases (table-5, Category-C). It is evident from (table-5, Category-C) that maximum drowning cases reported from Mandi district (47) followed by Kangra district (43).

In teenage group, total number of cases reported was 144 (table-5, Category-B) with maximum number of drowning cases reported in Mandi district (24) followed by Kangra district (18).

In case of adolescent age (table-5, Category-A), total number of 48 cases of drowning were reported with maximum number of cases reported from Kangra district (9) followed by Una district (8).

Results of *Seasons most prone* to drowning have been given in table- 6. It shows that maximum numbers of drowning cases reported in Summer Season (172) followed by Monsoon (162) and then 147 in winter season (Peak chart-1 and table- 6).

To summarise the trend in the state of Himachal Pradesh, the Mandi district is seems to be more prone to drowning deaths whereas the Shimla district is comparatively safer. Accidental drowning cases reported have been found to be more as compare to other mode of drowning; it might be attributed to non-regularisation and protection of the sites around the rivers and their tributaries. People try to be adventurous because of which very recently 24 young students from Hyderabad College drowned in Beas River near Thalaut in Mandi district after large quantity of water being released from Larji reservoir without any prior notice.

Conclusion

The interpretation of this elaborate data of the drowning cases reported in the state of Himachal Pradesh from year 2006 to year 2010 have provided us with very relevant information related to different variables selected for the present study and showed variations in the drowning frequencies related to sex, age, and season of drowning. The baseline data has been generated for five years(2006-2010) and very fruitful information has been drawn; such as the maximum number of drowning cases occurred in summer season and the manner of drowning was reported to be accidental which included mainly male; and about the pattern of drowning. The study was carried as a preliminary work based on the information given by the police officials through RTI. It was a five year data only; the study can further be extended by adding data of more years into it.

The conclusions drawn out of this elaborate data expected to be helpful to the various Law Enforcement agencies in general and to the Forensic community in particular.

Acknowledgements

We are thankful to Himachal Pradesh Police authorities for providing us with this valuable information to generate the baseline data and which will be further helpful in conducting research works.

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ANIL AGGRAWAL'S
Internet Journal of Forensic Medicine and Toxicology
The world's first online journal devoted entirely to forensic medicine and toxicology · Published 1 January and 1 July

VOL. 16, NO. 1 · JANUARY – JUNE 2015
Downloaded from www.anilaggrawal.com · ISSN 0972-8074 (Online) · https://www.anilaggrawal.com/ij/vol_016_no_001/papers/paper005.html

The damaging in the granular cells and expression patterns of CIRBP, RBM3, HSP70, HIF-1, AIF1, SIRT1,Ngb, cFos, p53 and CCC9 in the postmortem human cerebellums obtained from individuals who died due to hanging, strangulation, drowning, or asphyxia by anaphylaxis or food aspiration may be closely linking with agonal duration.

S Furukawa[1], K Nishi[1], S Morita[1], M Hitosugi[1], L Wingenfeld[2]

[1]Department of Legal Medicine, Shiga University of Medical Science, Setatsukinowa-cho, Otsu, Shiga, Japan
[2]Institute of Legal Medicine, Munich University, Munich, Germany

Contact: Satoshi Furukawa · 31041220@belle.shiga-med.ac.jp

CITE THIS ARTICLE

Furukawa S, Nishi K, Morita S, Hitosugi M, Wingenfeld L. The damaging in the granular cells and expression patterns of CIRBP, RBM3, HSP70, HIF-1, AIF1, SIRT1,Ngb, cFos, p53 and CCC9 in the postmortem human cerebellums obtained from individuals who died due to hanging, strangulation, drowning, or asphyxia by anaphylaxis or food aspiration may be closely linking with agonal duration.. *Anil Aggrawal's Internet Journal of Forensic Medicine and Toxicology* [serial online], 2015; Vol. 16, No. 1 (January – June 2015): [about 18 p]. Available from: https://www.anilaggrawal.com/ij/vol_016_no_001/papers/paper005.html

ABSTRACT

We encountered several cases concerning to ischemic and hypoxic deaths at routine autopsy cases. These victims were individuals who died due to suicidal hanging, autoerotic accidental hanging, self-ligature strangulation, manual strangulation, strangulation by a rope, accidental and suicidal drowning, anaphylaxis shock, and asphyxia by food aspiration. Theiragonal duration and postmortem interval were different, that is, agonal duration distributed from direct death to 4days and postmortem interval was from

12hours to 40 days, respectively. We examined the damaging degree of the granular cell layer in the each cerebellum using an application of Photshop andexpression pattern of antigens in the cerebellums by immunohistochemstry using anti CIRBP, RBM3, HIF-1, AIF1, SIRT1, Ngb, HSP 70, cFos, p53 and CCC9 antibodies. The results indicate the co-relationship between damaging of the granular cells and agonal duration, andshowed that expression pattern of antigens may be relate

with agonal duration, although it was not significant. The antigens expressed during agonal duration might be kept even in postmortem interval. In a special case in which an individual was killed and buried for 40 days under ground after being strangulated, the antigens related to stress and ischemic signaling pathway such as HSP70, CCC9, CIRBP, RBM3 and SIRT 1 were detectable in the molecular layer and dentate nucleus of the cerebellum. The co-relationship between the damaging of granular cells and agonal duration may be more reliable compared with those of Purkinje cells and agonal duration. The results obtained in this study indicate that the damaging of granular cells is useful tool to estimate agonal duration, and postmortem humanbrain tissue may be utilizable for diagnosis and examination of brain diseases. The results obtained in this study indicated that CIRBP and RBM3 were hypoxic or ischemic related antigens and not the cold inducible antigens.

Keywords: Cerebellum, Purkinje cells, Granular cell layer, Dentate

nucleus, Hypoxia, Ischemia, CIRBP, RBM3, SIRT1, Ngb, CCC9 and HSP 70

Introduction

The cells could bring their action to protect harmful effects assigned by the environmental items. Although cerebral ischemia occurs in the context either of focal reduction in blood flowing to the brain regions and/or cerebral infarction, a global deficit status occurs only in animal study. However the global deficit actually occurs in forensic practical cases such as hanging, strangulation, food aspiration, drowning death and/or anaphylactic reaction.

The brain is vulnerability to ischemic and hypoxic status. It is widely accepted that in hypoxia and ischemic cases an increase in the transcription level of specific genes, including those encoding for hypoxia-inducing factor

1(HIF1)(1,2), apoptotic inducing factors (AIF-1) (3), and stress related factors containingCold inducing RNA binding protein (CIRBP)(4)and RNA binding motif 3 (RBM3)(5) which are recognized as a cold stress inducing protein and were intensively expressed in the postmortem human heart muscle obtained from accidental hypothermic death cases (6). Neuroglobin (Ngb)(7), Complement component C 9(8,9), c-Fos(10), Protein 53(p53)(11), Sirtuin 1 (SIRT1)(12) andHeat shock protein 70(HSP70)(13) were also expect to express in the hypoxia or ischemic cerebellum.

The morphological and histological changes recognized in the cerebellums obtained from brain death cases have been investigated around 1990 (14), howeverimmunohistochemical studies using antibodies against apoptotic-, stress-, necrotic- and/or hypoxic-related antigens are rare until recent. We have been performing the forensic autopsy as a routine working and encountered several suicidal hanging and self-ligature strangulation cases in which there are directly, acute, sub-acute and prolong death individuals (15), and other deaths due to different types of asphyxia and with different durations.

Although various studies have been examined the consequences of hypoxia on cerebellar neurons, a controversy still exists concerning the mode of toxicity expressed in Purkinje cells following hypoxic insult (16). In the present study we examined the degree of damaging of granular cellsand

Purkinje cells during agonal time in the first step and the differences among the expression of stress, hypoxic and apoptotic related antigens described above were studied in the next step.

Concerning hypoxic changes in Purkinje cell, Hausmann et al(17) studied using 52 individuals with a history of acute or prolonged cerebellar hypoxia/ischemia before death. Various and different kind of cause of death were contained in their study.In this study we examined the relationship between degree of damaging granular cells and expression pattern of hypoxia, apoptotic and/or necrotic antigens in the cerebellum obtained from individuals who died mainly due to compression of cervical arteries.

Concerning to the using of postmortem human brain to investigate the brain disorder, Sheedy et al (18) proposed that histological situation of cerebellar granule cell layer is a useful marker of tissue integrity. We also like to describe some comments on the autolysis of the brain tissue during the postmortem interval.

Materials and Methods

In this study we selected and examined the cerebellum obtained from suicidal hanging cases containing direct, sub-acute and delayed death cases， self strangulation cases, manual strangulation cases, drowning cases, anaphylaxis case, and asphyxia case due to food aspiration to the larynx and/or the trachea. We did not use the cerebellum obtained from patients who died due to illness such as cardiac infarction, pulmonary diseases, and or cerebral infarction, since it is difficult to determine the onset of ischemia or hypoxic state to the cerebellum in these illness. For example, we have one autopsy case where the granular cells were obviously damaged in 54 year-old-man who died due to cardiac infarction with coronary artery thrombosis during right in driving his car. The autopsy revealed that he had been have several times heart attach. Characterization of antibodies used or examined in this study was summarized in Table 1.

Table 1. Characteristics of the antibodies used in this study

Antibody	Maker	Clone	Species	AG-retrieval	incubation	AB-dilution
CIRBP	Protein Tech	10209-2-AP	R	autoclave	overnight	1:400
RBM3	Protein Tech	14363-1-AP	R	autoclave	overnight	1:200
SIRT1	Novus	E104:	R	autoclave	overnight	1:400
HSP70	santa cruz	polyclonal:	G	autoclave	overnight	1:400
HIF-1α	NOVUS	NB100-479	R	autoclave	overnight	1:400
P53	santa cruz	FL-393 :	G	autoclave	overnight	1:400
C-Fos	BioLegend	Poly6414	R	autoclave	overnight	1:400
Ngb	Sigma-Aldrich	Poly-clone	R	autoclave	overnight	1:400
AIF1	Lifespan	Poly-clone	R	autoclave	overnight	1:400
CCC9	Leica	10A6	M	autoclave	overnight	1:400

[1] Tissue preparation and analysis of Granular cell Damaging

The tissue sections with about 0.5cm thickness were obtained from the cerebellum at autopsy, Department of Legal Medicine, Shiga University of Medical Science. Tissues containing the dentate nucleus, and cerebellar cortex were fixed in 10% of formaldehyde for 24 hours and embedded in paraffin. Threeμm of tissue sections were prepared for conventional and immunihistochemical staining using antibodies described in Table 1. The staining procedure for immunohistochemistry was followed our previous report (4).

Damage of granular cell layer was evaluated by an observer blinded to the group assignment of the section according to the report by our previous report(19). In shortly the evaluation of alteration of the granular cells was performed using a Photoshop application. After taking each photograph at microscopy level the area with blue color in the granular cell layer, that is, nucleuses of granular cells stained by hematoxylin, was measured by an application and with same magnification, that is, blue color area indicates existence of live nuclear of the granular cells. The frequency of remaining granular cells was indicated as percentage of the total number of pixel and was grouped. The numerical value was the mean value of five different areas.

The damage and decreasing of the Purkinje cells was also evaluated by image analytic technique. The images of cerebellar sections stained by HE were captured using an objective lens with a magnification of 4. The total length of the Purkinje cell layer from 8 images from each individual was calculated using BZ-II Image Analysis Application. And then the number of the Purkinje cells

with nucleus was counted. The existence of the Purkinje cells was showed a number per 1mm of the Purkinje cell layer.

[2] Cases examined.

Case 1. (Autopsy No.1222)

A 39 year-old woman with depression died due to hanging using a vinyl rope.

Small number of petechea was detected in her conjunctiva, and single ligature mark was clearly observed. In this case re-perfusion of blood to the brain was not presumed.

Case 2. (Autopsy No. 1225)

A 38-year-old woman died due to hanging by a rope after she killed her own

son. At autopsy clear ligature mark was found around her neck and ligature mark indicated typical hanging manner. In this case re-perfusion of blood to the brain was not presumed, however her postmortem interval was presumed around two days.

Case 3. (Autopsy No. 58749)

A 74 year-old housewife was found to be hanging by her husband. When the husband immediately cut the ligature, her body full down and she got a bruise on the posterior of head. The autopsy revealed the no facial congestion, multiple costal fractures, bleeding in the fatty tissue in the heart and left temporal of the head and mild cerebral contusion on the lower part of both side in frontal lobes. In this case, occlusion of cervical arteries occurred in a short time, and re-perfusion of blood to the brain might be performed in a few minutes.

Case 4. (Autopsy No. 1257)

A 45 year-old woman was found her death due to hanging. The body without clothing except a scanty was standing by a stairs in her house. Her face showed severe congestion and number of petechea in the conjunctivae was detected. Her death seemed to be accident due to autoerotic performance.

The autopsy revealed a small amount of bleeding in the right sternocleidomastoid muscle and no fracture of hyoid bone and thyroid cartilage. The brain showed congestion and no bleeding. In this case intermittent occlusion may be occurred during her autoerotic performance.

Case 5. (Autopsy No. 1320)

A 43 year-old man was found in his working car. The body lied on the back seat of a low displacement car and covered with a blanket. There was a ligature mark on his neck but no ligature in the car. The result of autopsy suspected that he attempted suicidal hanging but he could not die because of snapping a ligature. His death was presumed relayed hanging death, and agonal duration was presumed two or three hours. Re-perfusion of blood to the brain obviously was presumed.

Case 6. (Autopsy No. 1255)

A 67-year-old woman performed hanging with sitting position. Although she carried to a hospital by an emergency car, she died 20 hours after finding of her hanging. Re-perfusion of blood to the brain obviously was presumed.

Case 7. (Autopsy No. 1117)

A 51 year-old man was found death due to self-strangulation with a cotton rope in his room. The severe congestion over the ligature mark, and small blisters were observed between two ligature marks. A large number of petechiea in the conjunctiva and mouth mucosa was also recognized.Agonal duration presumed to be longer than hanging death.

Case 8. (Autopsy No. 1239)

A 31 year-old man was found death in a prison room. He wound a sleeve of his T-shirt around his neck, and severe congestion and petechia on his face, conjunctiva and mouth mucosa. Agonal duration presumed to be longer than hanging death.

Case 9. (Autopsy No.1254)

A 26 year-old man drowned in a public poor during his diving training and he was rescued and transformed to a hospital, however he died after 20 hours.

Case 10. (Autopsy No.59458)

A 71 year-old woman japed into a river. After being transformed to a hospital for rescue and medical treatment, she died two days later.

Case 11. (Autopsy No.1142)

A 54-year-old woman was killed by her acquaintance with manual strangulation. In one hour after hear death the body was found by her daughter in her living room. The agonal duration was presumed to be a bit longer than self-strangulation cases, since it may be occur the defense to murderer.

Case 12. (Autopsy No.1272)

A 47-yaer-old woman was found under the ground in front of her house.

Police research revealed that her husband killed her with manual strangulation and after keeping her body in a forest for several days, she had been burying for about 40 days. At discovering her body, the body was rapped with thin plastic film and covered with plastic seat.The agonal duration was presumed to be a bit longer than self-strangulation cases, however the postmortem interval was longest in this examination.

Case 13. (Autopsy No.1242)

A-51-year old woman fell into anaphylaxis shock by intake of barium salt for stomach examination, and she died after 4 days in an emergency hospital.

Case 14. (Autopsy No.1359)

A 53-year old man was found death. Autopsy revealed that food mass entered to larynx and he died due to accidental ingestion. His agonal duration was presumed to be several minutes.

The postmortem interval of each case was within4 days except one individual from case 12.

Results

The damaging in the granular cells and Purkinje cells of each individual was described in Table 2, and results obtained by immunohistchemistry were listed in Tables3-1 and 3-2.

Table 2. Remaining of the granular cells and Purkinje cells in the cerebellum.

Case	NO.	Sex	Age	Remain GC	Remain PC	Esti-PI	Esti-AG	Cause of Death
1	1222	F	39	52.4%	6.1cells	22h	3m	Hanging
2	1225	F	38	47.1%	5.8cells	36h	3m	Hanging
3	58749	F	74	52.5%	6.5cells	24h	3m	Hanging with brain contusion
4	1257	F	45	38.3%	3.7cells	50h	3m*	Accidental hanging
5	1320	M	49	19.1%	3.6cells	4d	3h	Delayed hanging death
6	1255	F	62	8.4%	3.4cells	25h	20h	Brain death due to hanging
7	1117	M	51	46.1%	4.1cells	8h	15m	Self-ligature strangulation
8	1239	M	31	46.8%	3.1cells	36h	15m	Self-ligature strangulation
9	1254	M	26	25.4%	3.5cells	30h	20h	Drowning
10	59458	F	71	18.5%	0.8cells	25h	2d	Drowning
11	1142	F	54	36.5%	2.8cells	12h	20m	Manual strangulation
12	1272	F	36	20.3%	3.1cells	43d	20m	Manual strangulation
13	1242	F	51	12.2%	1.7cells	24h	4d	Anaphylaxis
14	1359	M	53	36.2%	1.5cells	34h	10m	Aspiration

Remain GC; Frequency of remaining granular cells, Remain PC; Remaining Purkinje cells, Esti-PI; Estimated postmortem interval (hours or days), Esti-AG; Estimated agonal durati (minutes or days), : *; autoerotic case, repeated performance was estimated. Theevaluation procedures for the granular cells and Purkinje cells were described in the text.

Table 3-1. Results obtained from immunohistochemistry

Case No.	Cells	Antibodies									
		CI	RB	HS	SIR	HIF	Ngb	c-Fos	p-53	AIF	CCC9
1.1222	Gc*	+	+	+	-	-	-	-	-	-	-
	PcN	+	+	-	-	-	-	-	-	-	-
	PcC	+	+	-	-	-	+	+	-	-	-
	Mc*	+	+	+	-	-	+	+	-	-	-
	DN	+	+	-	-	-	-	-	-	-	+
	DC	+	+	+	+	+	+	+	+	+	+
	W*	+	+	+	-	-	+	+	-	+	-
2.1225	Gc*	+	+	-	-	-	-	-	-	-	+
	PcN	+	+	-	-	-	-	-	-	-	-
	PcC	+	+	-	-	-	+	+	+	+	+
	Mc*	+	+	+	-	-	-	-	-	+	+
	DN	+	+	-	-	-	-	-	+	+	-
	DC	+	+	+	-	-	+	+	+	+	+
	W*	+	+	+	-	+	+	-	+	+	+
3.58749	Gc*	+	+	+	-	-	-	+	+	+	+
	PcN	+	+	-	-	-	-	-	-	-	-
	PcC	+	-	-	-	+	+	+	+	+	-
	Mc*	+	+	+	-	+	+	+	+	+	+
	DN	+	+	-	-	-	-	-	-	-	+
	DC	+	+	+	-	+	+	+	+	+	+
	W*	+	+	+	-	-	+	+	-	+	-
4.1257	Gc*	+	+	+	-	-	+	+	+	+	+
	PcN	+	+	-	-	-	-	-	-	-	-
	PcC	+	-	-	-	-	-	+	+	+	+
	Mc*	+	+	+	-	-	-	-	-	+	+
	DN	+	+	-	-	-	-	-	-	-	+
	DC	+	+	+	-	-	-	-	-	-	-
	W*	+	+	+	-	+	-	-	-	-	+
5.132	Gc*	+	+	-	-	+	-	+	-	+	+
	PcN	+	+	-	-	-	-	-	-	-	+
	PcC	+	+	-	-	-	+	+	+	+	+
	Mc*	+	+	+	-	+	-	-	-	+	+
	DN	+	+	-	-	-	-	-	-	+	-
	DC	+	+	+	+	+	+	+	+	-	+
	W*	+	+	+	+	+	-	+	+	+	+
6.1255	Gc*	-	-	+	-	-	+	+	+	-	-
	PcN	+	+	-	-	-	-	-	-	-	-
	PcC	-	+	-	-	-	+	+	+	-	-
	Mc*	+	+	+	-	+	-	+	+	-	-
	DN	-	-	-	-	-	-	-	-	-	-
	DC	+	+	+	-	-	-	-	-	-	+
	W*	+	+	+	+	-	+	+	-	+	-
7.1117	Gc*	+	+	+	-	-	-	-	-	-	+
	PcN	+	+	-	-	-	-	-	-	-	-
	PcC	+	+	-	-	-	+	+	+	+	+
	Mc*	+	+	+	-	-	-	-	-	-	+
	DN	+	+	-	-	-	-	-	-	-	+
	DC	+	+	+	-	-	-	+	+	-	+
	W*	+	+	-	-	-	+	+	+	+	+

CI;CIRBP, RB;RBM3, HS;HSP-70, SIR;SIRT 1, HIF;HIF-1, Nbg; Neuro-globin, GC; Granular cells, PcN; Purkinje cell nucleus, PcC; Purkinje cell cytoplasm, Mc; Cells in molecular cell layer , Dn; Dentate cell nucleus, Dc;Dentate cell cytoplasm, Reactivity; +; positive, -;negative, *; result of reactivity detected in cytoplasm or nucleus was described.

Table 3-2. Results obtained from immunohistochemistry

Case No.	Cells	Antibodies									
		CI	RB	HS	SIR	HIF	Ngb	c-Fos	p-53	AIF	CCC9
8.1239	Gc *	+	+	+	-	-	-	-	-	-	+
	PcN	+	+	-	-	-	-	-	-	-	-
	PcC	+	+	-	-	-	+	+	+	+	+
	Mc*	+	+	+	-	-	-	-	-	-	+
	DN	+	+	-	-	-	-	-	-	-	-
	DC	+	+	+	-	-	-	+	+	+	+
	W*	+	+	-	-	-	+	+	+	+	+
9.1254	Gc*	+	+	+	-	-	-	-	-	-	-
	PcN	-	-	-	-	-	-	-	-	-	-
	PcC	-	-	-	-	-	-	+	-	-	-
	Mc*	+	+	-	-	+	-	-	-	-	+
	DN	-	-	-	-	-	-	-	-	-	+
	DC	-	-	-	-	-	-	-	-	-	-
	W*	+	+	+	-	-	-	+	-	-	+
10.59458	Gc*	+	+	-	-	+	-	+	-	+	+
	PcN	+	+	-	-	-	-	-	-	-	+
	PcC	+	+	-	-	-	+	+	+	+	+
	Mc*	+	+	+	-	+	-	-	-	+	+
	DN	+	+	-	-	-	-	-	-	+	-
	DC	+	+	+	+	+	+	+	+	-	+
	W*	+	+	+	+	+	-	+	+	+	+
11.1142	Gc*	+	+	+	-	-	+	+	+	+	+
	PcN	+	+	-	-	-	-	-	-	-	-
	PcC	+	-	-	-	-	-	-	-	-	-
	Mc*	+	+	+	-	-	-	-	-	-	-
	DN	+	+	-	-	-	-	-	-	-	-
	DC	+	+	+	-	+	-	-	-	-	-
	W*	+	+	+	-	-	-	-	-	-	-
12.1272	Gc*	-	-	-	-	-	-	-	-	-	-
	PcN	-	-	-	-	-	-	-	-	-	-
	PcC	+	-	-	-	-	-	-	-	-	-
	Mc*	+	+	+	+	-	-	-	-	-	+
	DN	+	+	+	-	-	-	-	-	+	-
	DC	+	+	-	-	-	-	-	-	-	+
	W*	+	+	-	+	-	-	-	+	-	-
13.1242	Gc*	-	-	-	-	-	-	-	-	-	-
	PcN	-	-	-	-	-	-	-	-	-	-
	PcC	-	-	-	-	-	-	-	-	-	-
	Mc*	-	-	-	-	-	-	-	-	-	-
	DN	-	-	-	-	-	-	-	-	-	-
	DC	+	+	+	+	+	+	+	+	+	+
	W*	-	-	-	-	-	+	+	-	-	-
14.1359	Gc*	+	+	+	-	-	+	+	+	+	+
	PcN	+	+	-	-	-	-	-	-	-	-
	PcC	+	-	-	-	-	-	-	-	-	-
	Mc*	+	+	+	-	-	-	-	-	-	-
	DN	+	+	-	-	-	-	-	-	-	-
	DC	+	+	+	-	+	-	-	-	-	-
	W*	+	+	+	-	-	-	-	-	-	-

CI;CIRBP, RB;RBM3, HS;HSP-70, SIR;SIRT 1, HIF;HIF-1, Nbg; Neuro-globin, GC; Granular cells, PcN; Purkinje cell nucleus, PcC; Purkinje cell cytoplasm, Mc; Cells in molecular cell layer , Dn; Dentate cell nucleus, Dc;Dentate cell cytoplasm, Reactivity; +; positive, -;negative, *; result of reactivity detected in cytoplasm or nucleus was described.

1. Granular cells

[1]Hematoxylin-Eosin staining

In the granular cell layer the morphology was different, that is, complete disappear of cells with cell debris or fibrous material in some individuals, remarkable decreasing of cells in other individuals and numerous remaining cells in another individuals. For example, the granular cell layer from severe level degeneration individuals showed a diffuse decrease in number the nuclei stained by hematoxylin and diffuse pallor of the background without glial reaction and with cell debris as shown in Figure 1, and the changes in high remaining individuals were not obvious and showed no remarkable difference compared with those from brain injure deaths due to traffic

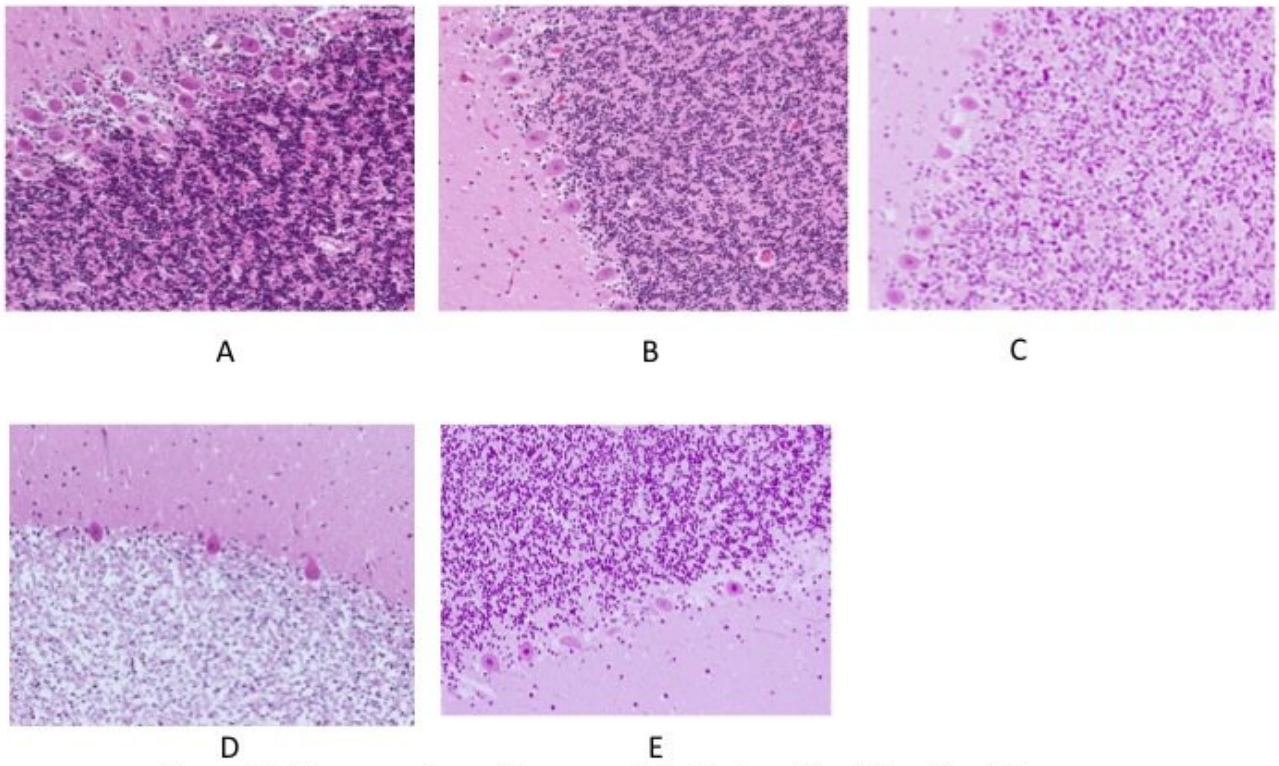


Figure 1. The granular cell layer and Purkinje cells stained by HE
A; High remaining level from Case 3. B; middle remaining level from case 4.
C; low remaining level from case 9, D; severe level from case 6. E;
low remaining from case 12. The granular cells were damaged with agonal
duration but Purkinje cells kept the morphology.

accidents (date not shown).

The results obtained from evaluation of the damaging of the granular cell layer using an application of Photo-shop were listed in Table 2. The frequency of remaining granular cells was indicated as percentage of the total number of pixel and classed into four degrees, that is, high remaining level; over or around 50% of cell was stained by hematoxylin, middle remaining level; from 30 to 40% of the cells were stained, low remaining level; around 20% of cells were stained, and severe degeneration level; around 10% of cells were remained with large amount of cell debris (synaptic glomeruli). Case 1,2,3,7,and 8 were grouped into high remaining level, case 4,11 and 14 were in moderate group, case 5, 9, 10 and 12 were in low group and case 6 and 13 were grouped into severe level. As an interesting case, the granular cell layer of the cerebellum obtained from case

12 individual whose postmortem interval was presumed over 40 days, decreased the stain-ability, however the morphology of the granular cells have been well keeping compared with the longer agonal duration cases that was classed into severe level.

Changing of granular cell layer was remarkable compared with that of Purkinje cells in lower and severe level groups where the morphology of Purkinje cells was still remained.

[2] Immunohistochemical staining

In the granular cell layer at high level remaining, a large amount of granular cell nucleus showed good and intensive reactivity with anti CIRBP and RBM3 antibodies. HSP70 showed weak or no

reactivity with the nucleus of the granular cells, however it showed good reactivity with cytoplasm and synaptic glomeruli of some cells, presumed Golgi cells and Lugar cells in the granular cell layer and the Purkinje cells layer, respectively. Antibodies againstcFos, AIF1, p53, Ngb and CCC9 showed weak reactivity with cytoplasm of the granular cells and in the synaptic glomeruli of the granular cell layer. The synaptic glomeruli showed just like cell debris.

The granular cells at middle remaining level showed moderate reactivity with anti RBM3 antibody and weak reactivity with CIRBP antibody in the nucleus, and anti HIF1, SIRT1 and Ngb showed no reactivity with the granular cells at middle remaining level. There is slight reactivity between cell debris, synaptic glomeruli, with anti CIRBP, RBM3, HIF1, SIRT1 and Ngb in the granular cell layer at low level remaining.

2. Purkinje cells

[1]Hematoxylin-Eosin staining

The Purkinje cells showed various changes such as disappear, shrunken eosionophilic cytoplasm with smudgy dark nuclei, or vacuolated, homogenous, eosinophilic or pale cytoplasm with pale or piknotic nuclei.

The Purkinje cells exhibited homogeneity in color and shape at high remaining level. With decreasing of the cells in the granular cell layer, the degeneration of Purkinje cells was also progressed. Purkinje cells from case

12 remained the morphology with normal nucleus in many cells, although staining ability was decreased.

[2]Immunohistochemical staining

Purkinje cells in the cerebellum at high remaining level showed clear reactivity with anti CIRBP both in the nucleus and cytoplasm, and intensive reactivity with RBM3 only in the nucleus (Figure 2).

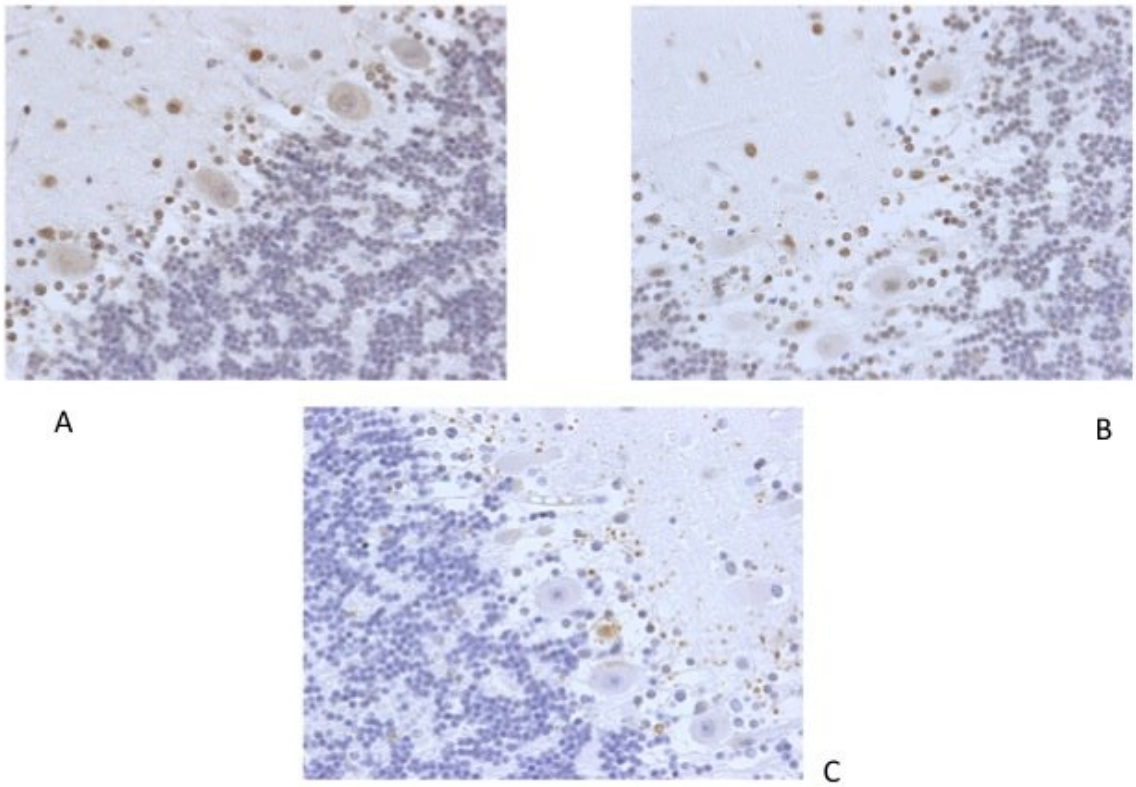


Figure 2. Results of immunohistochemical staining of Purkinje cells from case 3. A; Anti CIRBP showed good reactivity with cytoplasm of the cells, B; Anti RBM3 stained the nucleuses of Purkinje cells, C; Anti HSP70 showed clear reactivity with cytoplasm in cells around Purkinje cells but no with Purkinje cells. (×400)

Anti cFos, Ngb and

CCC9 showed weak reactivity with cytoplasm and no with nucleus. Anti

HSP70 showed good reactivity cytoplasm in the cells around Purkinje cells (Figure 2), however, reactivity of the antibody with cytoplasm in the Purkinje cells decreased with progression of damaging of granular cells.

Purkinje cells at middle remaining level showed slight reactivity with CIRBP in cytoplasm and clear reactivity with anti RBM3 in the nucleus.

Although no reactivity with anti HIF1, SIRT1 and CCC9 was recognized, antiNgb, cFos and HSP antibodies showed weak reactivity in the cytoplasm of some Purkinje cells.

Purkinje cells at low remaining level showed no reactivity with anti CIRBP, RBM3, HIF1, and SIRT1, however anti CCC9 (Figure 3), Ngb, cFps and HSP, showed weak reactivity with cytoplasm of some Purkinje cells which were keeping the morphology.

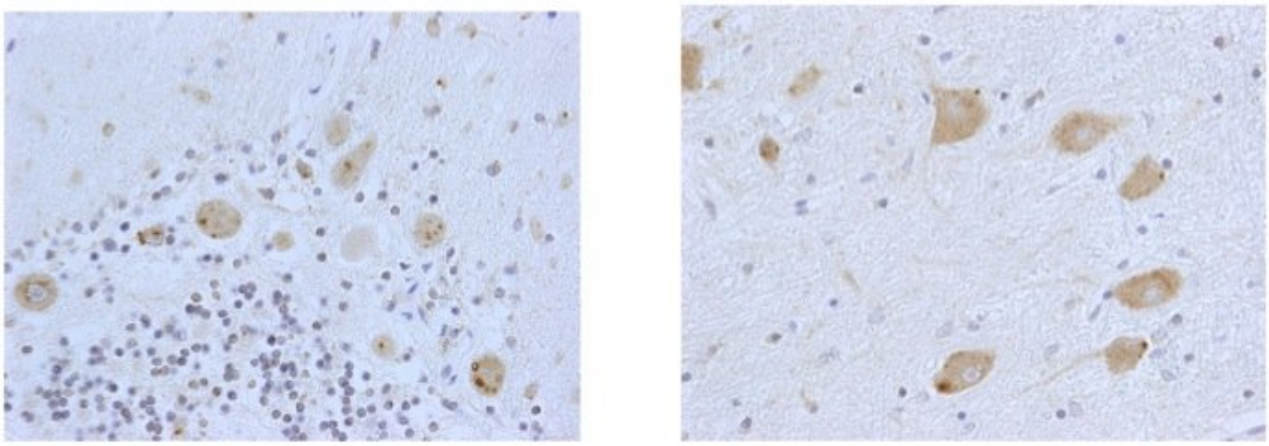


Figure 3. Anti CCC9 showed weak reactivity with Purkinje cells (A) and clear with the dantate nuclear cells (B) from Case 5.

3. Molecular layer

[1]Hematoxylin-Eosin staining

The cells in the molecular layer were detected even in individuals with severe decreasing level of granular cell layer, although the number of remaining cells was decreased, however it was difficult to identify the basket or satellite cells.

[2] Immunohistochemical staining

At high remaining level the nucleuses of many cells in the molecular layer, but not all cell, showed intensive reactivity with anti CIRBP and RBM3, and moderate reactivity with anti HSP. Although weak reactivity with cells and anti CIRBP, RBM3, HSP and cFos antibodies was recognized at low and middleremaining level, anti HIF1 and Ngb showed no reactivity with the cells. At moderate and low remaining level anti SIRT 1and CCC9 stained weakly with nucleus of some cells in the molecular layer but not all cell. At severe level weak reactivity of CIRB, RBM3, cFos and HSP with the nucleus of the some cells was detected and no or feeble with other antibodies.

4. Cells in the Dentate Nucleus

[1]Hematoxylin-Eosin staining

With the progressing of the agonal duration, the degeneration of the dentate nuclear cells increased. However cells were able to keep the morphology even in severe level cases where it was difficult to detect the cell nucleus.

[2]Immunohistochemical staining

Anti CIRBP, RBM3 and HSP showed intensive reactivity with cytoplasm of the dentate nucleus cells at high remaining level, the reactivity with these antibodies decreased with increasing of damaging of the granular cells, however weak reactivity with all antibody (Figure4), except anti HIF1, examined in this study was kept even in severe damage level of the cerebellum obtained from Case 6 whose evaluation numerical value was calculated at 8.4%.

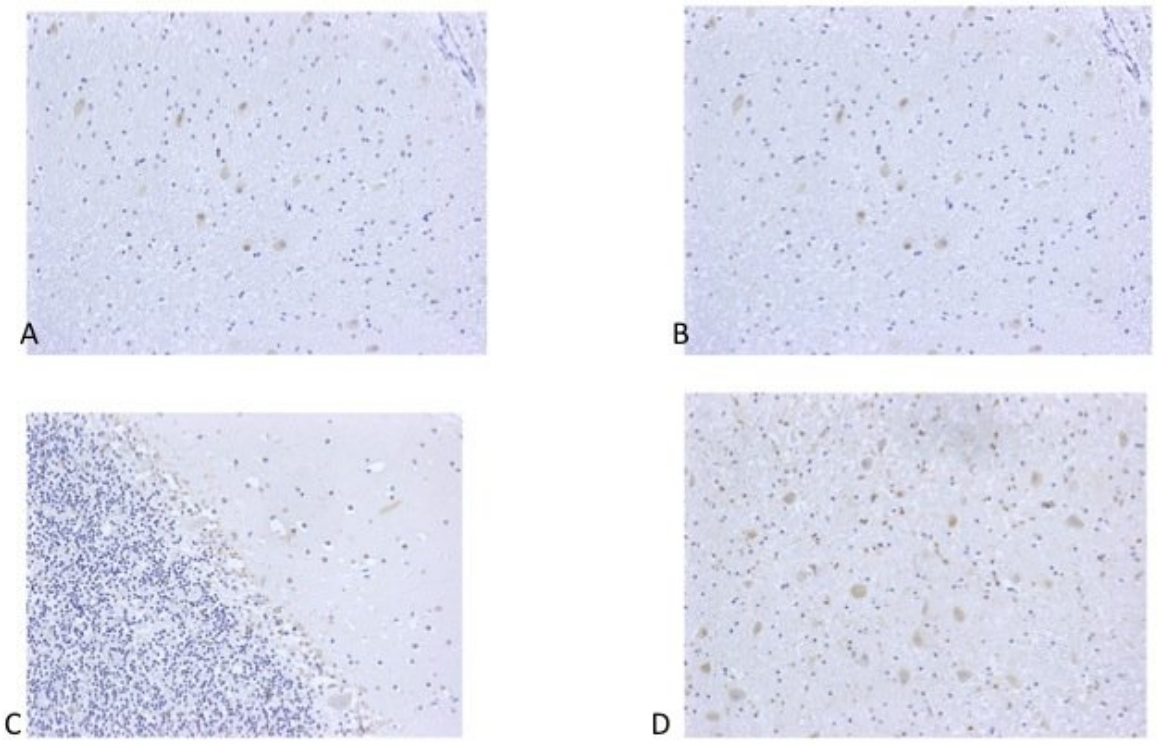


Figure 4. Cells in the molecular layer (A) and dentate nuclear cells(B) remained reactivity with anti CIRBP, and cells around Purkinje cells (C) and dentate nuclear cells(D) remained reactivity with anti HSP70. (×100) The cerebellum was obtained from case 12.

Anti SIRT 1 showed moderate reactivity with the cell nucleus around the dentate nucleus and cytoplasm of the dentate nucleus cells at moderate remaining level.

5. The cells in the white matter

[1]Hematoxylin-Eosin staining

The cells in the white matter of the cerebellum were remained even in severe level individuals, although their number was remarkably decreased.

[2]Immunohistochemical staining

Anti CIRBP, RBM3, HSP70 and cFos antibodies showed weak reactivity with nucleuses and cytoplasm of the remaining cells even in the severe level individuals. Anti AIF1, SIRT1 and Ngbalso showed weak reactivity with the cytoplasm of the remaining cells in severe damaged level individuals, however, reactivity with HIF1 was not detected.

Some cells such as cells in the dentate nucleus cells and molecular layer in the cerebellum from case 12 remained weak reactivity with anti CIRBP, HSP70 and CCC9 as shown in Figure 4.

Discussion

In this study could indicate that many antigens are expressed during agonal duration as the vital reaction or biological reaction of the bodies. In forensic field it is known as the obvious evidence that the pressure ulcer could occur during alive and not occur or not become more seriously after one's death.

Concerning to agonal duration in hanging cases, Sauvageau et al (20) reported that the death occurred at 4mintes 12 seconds± 2 minutes 29 seconds, and in our experience the death occurred at 2mints 38seconds (21).

Since in typical hanging case both side of carotid and vertebral arteries are obstructed, the death comes earlier than other death due to self-strangulation and/or manual strangulation.

The brain is critically dependent on its blood flow for a continuous supply of oxygen and glucose. Only a few minutes of severe ischemia induce the selective degeneration of certain neuronal populations, including pyramidal neurons in the CA1 region of the hippocampus, neocortical neurons in layers

3,5, and 6, and cerebellar Purkinje cells (22). As describing in a review article bySarna and Hawkes(23), it is also well known the vulnerability of the Purkinje cells to severe and transient global brain ischemia, chronic hypoxia experiments, intermittent hypoxia(24,25), toxic situation (26)and many neuronal degeneration diseases. This study indicates that the vulnerability of the granular cells may be more obvious and convenience for analysisthan that of Purkinje cells, since the changing of granular cell layer was remarkable and homogeneous compared with that of Purkinje cells in lower and severe level groups where Purkinje cells was still remained and showed various changes such as disappear, shrunken eosinophilic cytoplasm with smudgy dark nuclei, or vacuolated, homogenous, eosinophilic or pale cytoplasm with pale or pyknotic nuclei, showing difficulty in the evaluation and analysis.

There was the difference of the degree of damaging in the granular cell layer between case 11 and 12, although they were killed by manual strangulation andagonal durations of them were presumed almost the same. The differencein partly seemed to relate their postmortem intervals. There was also difference of damaging level among case 6, 10 and 13. This difference may arise from obstruction of cervical vessels in case 6 and global anoxia in cases1 10 and 13.

In our previous reports (6), antigens such as CIRBP, RBM3 and SIRT1 were expressed in the nucleus of the cardiac calls obtained from hypothermic and/or carbon monoxide intoxication death. In this study, reactivity with anti-CIRBP and RBM3 antibodies was detected in nucleuses in the cells of the cerebellum, however that of anti SIRT1 antibody was not detected in the granular cells and Purkinje cells, although the reactivity was recognized in the cytoplasm of the dentate nucleus cells of the cerebellum. The same result was observed in the cerebral cortex (15), indicating that expression of SIRT1 which plays important role to protect the brain function from many stress (27)may be most delayed one in the brain.

It is well known that CIRP and RBM3 were discovered after exposure to a moderate cold-schock and other cellular stress such as UV radiation and hypoxia(28). Although at normal temperature, CIRBP

mRNA expression was detected in the rat brain(29), Wellmann et al (30) reported that hypoxia significantly induced CIRBP and RBM3 expression in vitro. We also reported that intensive reactivity of anti CIRBP and RBM3, weak reactivity of HIF1 and HSP-70 and no reactivity with SIRT1 in the nucleus in the neuron of the frontal cortical region obtained from two male who died suicidal strangulation using a rope or T-shirt were detectable (15). These results indicate that CIRBP and RBM3 may be ischemic and/or hypoxic related antigens, although these antigens were discovered as the cold inducible antigens.

HIF-1 is a hypoxic inducing factor and detected in hypoxic tissues (1), and oxygen-regulated expression of CIRBP and RBM3 by a HIF-1-independent mechanism was reported usingthe human leukemic cell line (30), however in this study HIF1 could show weakor feeble reactivity with Purkinje cells and confirm the repot from Wellmann et al (30). Chen et al (12) reported a bidirectional link of HIF and Sirt1 signaling during hypoxia, and then weak reactivity of HIF and Sirt 1 in the celebbellum may be caused by the linkage of the genes encoded expression of HIF and Sirt 1 in the this organ.

Concerning the expression of HSP70 many studies were reported, and the expression of HSP70 in Purkinje cells reduced in patients with schizophrenia compared with individual without disease (31). In this study the expression of HSP70 was not detected in Purkinje cells, although the clear expression of the antigen in the cells was recognized in Purkinje cell layer, granular cell layer, molecular layer, dentate nucleus, and cerebellar white matter. In our previous experience (15), reactivity with HSP70 and neurons in the cerebral cortex was remarkably detected in the case 7 and 8 of this study.These results may cause by the difference of stability against the stress among the cells, indicating that the HSP70 expression system in Purkinje cell is most vulnerability, different among the brain lesions or early disappear in the cells.

Schaefer et al (32) described deposition of C5b-9 complexes can already occur at the cytoplasmic membrane surface of ischemic cells, and immnocytochemical detection of C5b-9 provides a powerful tool for detecting even single cell necrosis in the cardiac infarction cases. In this study the reactivity of CCC9 was detected mainly in the cytoplasm of the dentate nucleus cells. The cells stained by anti CCC9 may be in necrotic stage and shows the latest stage in the cell biological reaction against ischemic, because some heart cells were stained by CCC9 and other cells with normal morphology did not show the reactivity in acute cardiac infarction cases.

In this study the Purkinje cells seemed to be destroyed with increasing of disappearing of granular cells and showed clear difference in the expression pattern of CIRBP and RBM3, that is, CIRBP showed its reactivity in the cytoplasm and RBM3 was detected in the nucleus of the Purkinje cells at the level of normal stainability of granular cells. This phenomenadiffer from those in the heart muscle in which these antigens were expressed mainly in the nucleus of the cells affected in hypoxic situation (6).This result indicates that the difference in the expression of these antigens exists among the organs.

Conclusion

The results obtained in this study indicate that the intracellular distribution of each antigen confirmed the previous reports. The intercellular distribution of each antigen was different in the cerebellum. The expression of hypoxia, ischemia, apoptotic and necrotic related antigens may be expressed according to each encoded gene during agonal duration and the activity of its expression,

cascade of antigen expression pathway, may be stopped at cellular death and situation of expressed antigen and cell morphology would be kept even in postmortem interval when there was no putrefy of the body.

The cells in the dentate nucleus and white matter may be most stable to the changing during agonal duration and postmortem interval. The evaluation of granular cell layer might be useful to presume the agonal duration, confirming to the report described by Sheedy et al (18), however, we should know that the granular cells were kept their morphology for a long time in special situation such as under ground.

The present study indicates that once vessel occlusion has occurred, the concerned tissue depleted of blood supply undergoes causing neuronal injury and many signal pathways are activated leading to cell death, immune, and other responses, and also indicates that the results by cascade of signal pathways may be kept the situation that the each cascade was stopped. This phenomenonresembles to pressure ulcer (decubitus), that is, the pressure ulcer never newly becomes in postmortem interval.

Acknowledgements

Partially supported by JSPS KAKENHI Grant Number 26460876.

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ANIL AGGRAWAL'S
Internet Journal of Forensic Medicine and Toxicology
The world's first online journal devoted entirely to forensic medicine and toxicology · Published 1 January and 1 July

VOL. 16, NO. 1 · JANUARY – JUNE 2015
Downloaded from www.anilaggrawal.com · ISSN 0972-8074 (Online) · https://www.anilaggrawal.com/ij/vol_016_no_001/papers/paper006.html

Estimation Of Adult Human Stature By Anthropometric Measurements Of Inter Anterior Superior Iliac Spinal Distance – A Cross Sectional Pilot Study.

Niranjan Choudhary[1], Bhagwati Choudhary[1], Manasi Patel[2], Ajay Phatak[3], Lavlesh Kumar[4], Swapnil Sudhirkumar Agarwal[5*]

[1]MBBS Students, Pramukhswami Medical College & Shri Krishna Hospital, Karamsad, Anand, Gujarat, India
[2]Assistant Professor, Preventive and Social Medicine, Pramukhswami Medical College & Shri Krishna Hospital, Karamsad, Anand, Gujarat, India
[3]Manager, Central Research Services, Pramukhswami Medical College & Shri Krishna Hospital, Karamsad, Anand, Gujarat, India
[4]Professor & Head, Forensic Medicine & Toxicology, SBKS Medical Institute and Research Centre, Waghodia, Vadodara, Gujarat, India
[5]Professor & Head, Forensic Medicine & Toxicology, Pramukhswami Medical College & Shri Krishna Hospital, Karamsad, Anand, Gujarat, India
Contact: Swapnil Sudhirkumar Agarwal · swapnilagarwal@msn.com

CITE THIS ARTICLE
Choudhary N, Choudhary B, Patel M, Phatak A, Kumar L and Agarwal SS. Estimation Of Adult Human Stature By Anthropometric Measurements Of Inter Anterior Superior Iliac Spinal Distance – A Cross Sectional Pilot Study.. *Anil Aggrawal's Internet Journal of Forensic Medicine and Toxicology* [serial online], 2015; Vol. 16, No. 1 (January – June 2015): [about 18 p]. Available from: https://www.anilaggrawal.com/ij/vol_016_no_001/papers/paper006.html

ABSTRACT

Background

Estimation of stature forms a part of examination of an unidentified corpse, especially a dismembered one, for a forensic pathologist while aiming to establish its identity. It can be accomplished accurately by anatomical reconstruction, followed by Type I regression equations using long bones of lower limbs. Skeletal remains presented to forensic pathologists are often incomplete; one has to rely on other elements too. As no existing equations are clearly appropriate, new sample specific regression equations formulation has been attempted. **Aim:** To formulate regression equation to establish relationship between stature and inter anterior superior iliac spinal distance of living individuals. **Methodology:** Measurements were taken from 100 randomly selected medical students above 20 years of age, native of Gujarat, Indian using spreading calliper

[least count being 1 mm]. The data was analysed using SPSS Software version 11. **Results:** Three sets of regression equations were derived. It revealed that predictive value from the equations was unsatisfactory. **Conclusion:** Inter- anterior superior iliac spinal distance is not a good parameter for stature estimation. Further detailed studies are recommended to support or negate the findings.

Keywords: Inter- anterior superior iliac spinal distance, stature correlation, regression equation

Declaration

Approval from Institutional Ethics Committee was obtained prior to start of the study.

INTRODUCTION

The establishment of identity of an individual is of immense medico legal significance both in the living and dead. Forensic medicine specialists are very often faced with the problem of fixing the individuality of an unknown human body. It is easy when the remains are complete. But the real task lies when only parts of the body are recovered. Such bodies are encountered in homicides [to conceal evidence], in railway accidents, plane crashes, bomb explosions etc. Here estimation of sex, age and stature from the available skeletal remains help in identifying an individual.[1]

The most common method employed for stature estimation is use of regression equations developed from length of long bones. Such formulae, like the Karl-Pearson, Dupertius & Hadden and Trotter & Glesser, have been derived from predominantly Western populations.[2] Some studies have been conducted in India also but are few and far between, e.g., Siddiqui & Shah, Singh& Soha and Mehta & Thomas.[3]

It is necessary to have different formulae for determination of stature from the measurements of different body parts. Cases are common where only parts of the long bones are available along with an intact pelvis. There have been only few studies done to find out formulae for estimation of stature without long bones.[4] Absence of an intact long bone in such cases makes it difficult for approximate estimation of stature. As no existing equations are clearly appropriate, new sample-specific regression equations formulation is always recommended. An approach is utilized in this study to establish the relationship between statures of western Indian population and their inter-anterior superior iliac spinal distance; to develop regression equation formulae from these two variables by simple regression analysis. The formula thus obtained could be used for the determination of stature of individuals of this region.

METHODOLOGY

The study sample comprised of 100 medical students, randomly selected at a tertiary health care institute with attached medical college. The participants were aged between 20 – 22 years with mean age of 20.5 years [by this age pelvic growth is presumed to be complete]. M: F ratio of participants was 50:50. The participants constituted mixed population of Western India. Cases of stunted or enhanced bone growth or with history of skeletal injury were excluded from the study. The participants were explained about the purpose and procedure of the study and informed consent was obtained.

Data collection and anthropometry

The data collected for the study were stature and inter anterior superior iliac spinal distance. The measurements were taken with procedures and landmarks as by Krogman.[2]

Stature

Participants were asked to stand up erect against the wall with hands hanging down, feet axes parallel or slightly divergent, and head in the Frankfurt horizontal plane, without any headgear or footwear being worn; and stature recorded using an anthropometer rod set. No pressure was exerted since this was a contact measurement.

Inter anterior superior iliac spinal distance

Inter-anterior superior iliac spinal distance is the distance between two bony landmarks i.e. anterior superior iliac spine on each side. Anterior superior iliac spine is the end point of the front end of iliac crest easily palpable on both sides with subject standing in normal position with his arms hanging by the sides.

The measurement from the vertex of head to the ground was taken after bringing down the adjustable cross-bar to the head and the measurement was read from the vertical scale. Next, keeping point of one static arm of the anthropometer rod on right anterior superior iliac spine, the tip of adjustable arm of the anthropometer rod was brought to the left anterior superior iliac spine to measure the inter anterior superior iliac spinal distance in centimeters with the person in the same erect position.

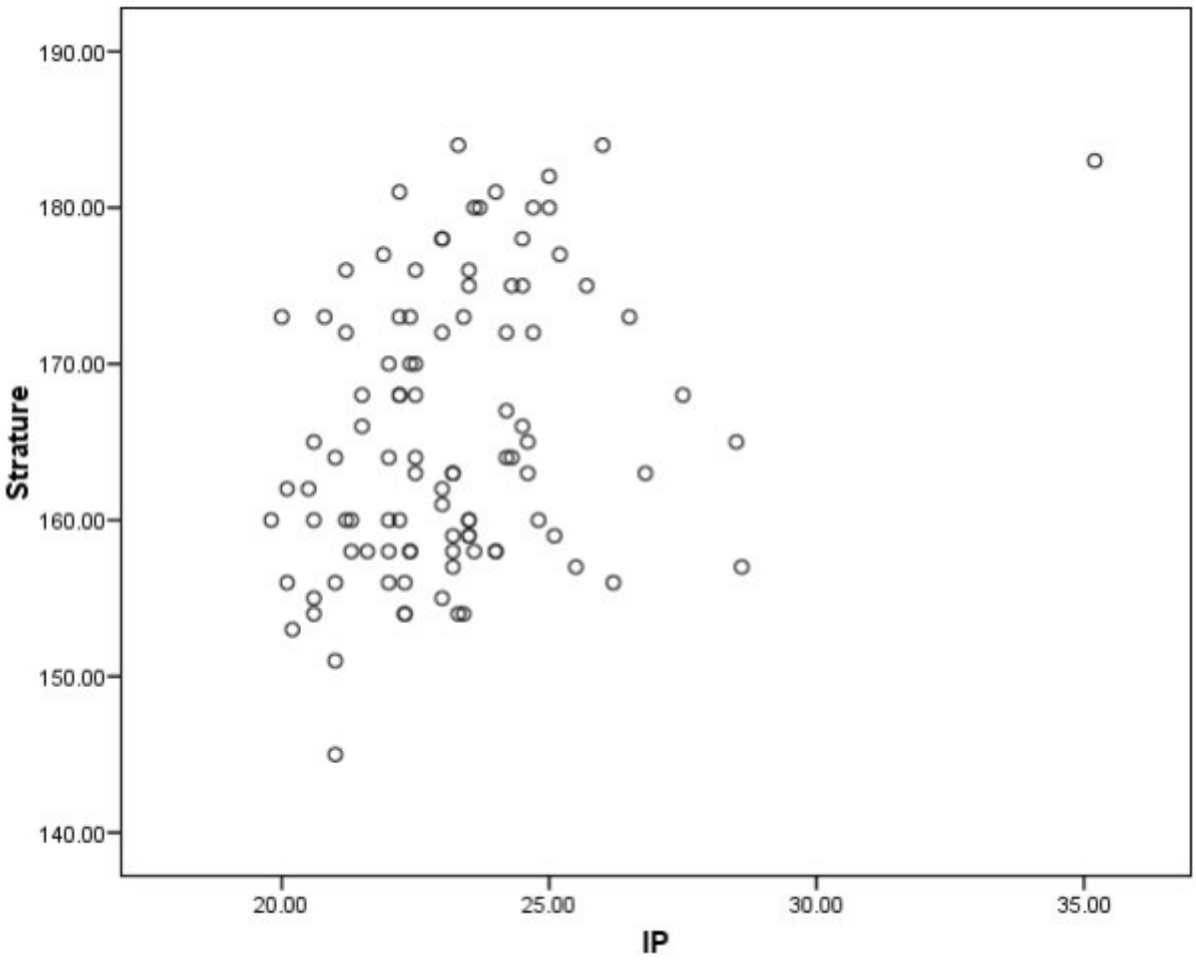
Technical/Measurement error

The data were analyzed statistically (using basic bivariate statistics and simple and multiple regression analyses) by SPSS software, version 11 to formulate regression equation. The observer bias was reduced as they underwent training for measurement of the parameters. Then 5 cases were measured in presence of the 6^{th} author and the findings were confirmed by 5^{th} author and the observer. The degree of measurement error [< + 0.2 cms] and reliability were well within accepted standards.

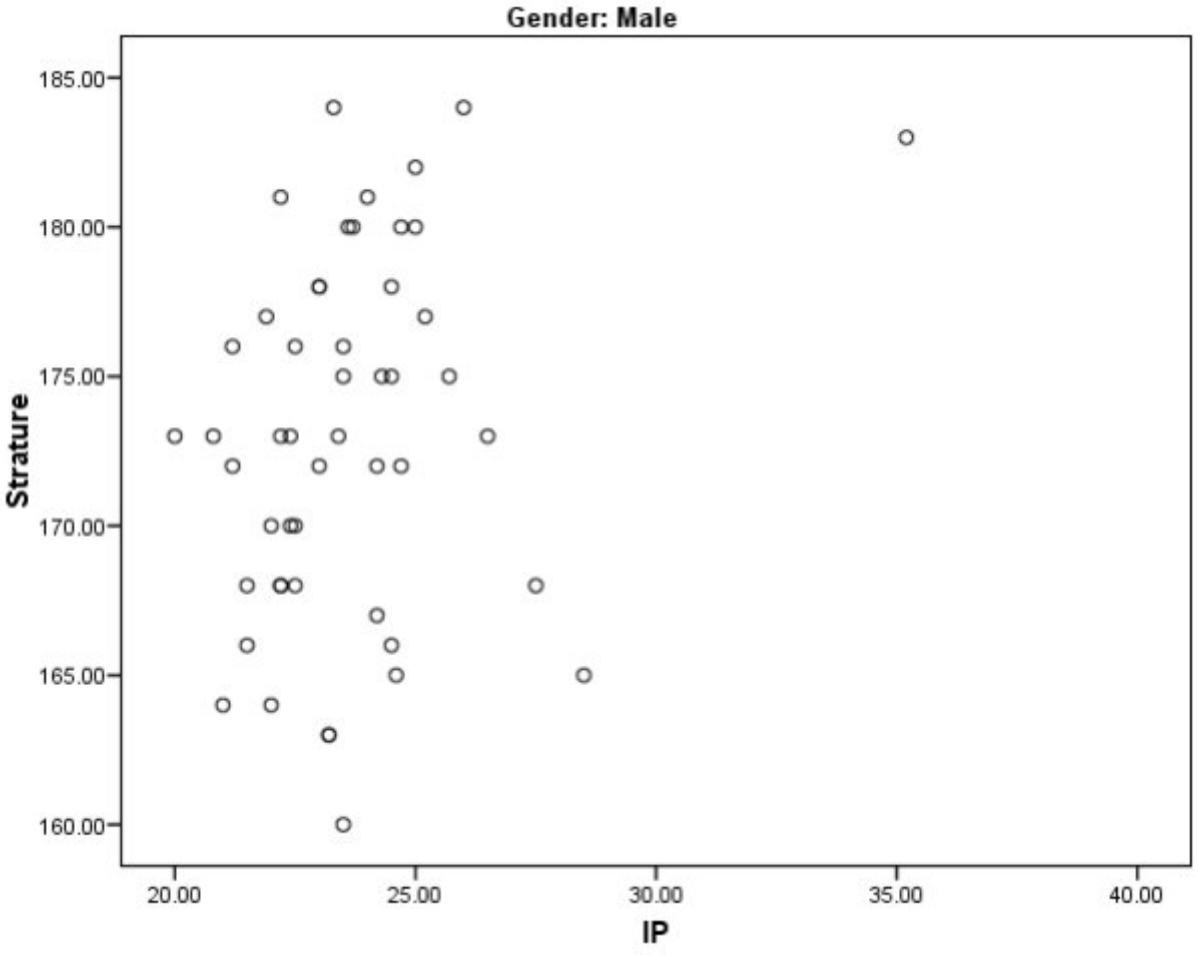
OBSERVATIONS AND ANALYSIS

Two variables were taken into consideration i.e. stature and inter anterior superior iliac spinal distance [IASIS]. Basic bivariate statistics and linear regression analyses was applied to find out correlation between the variables. Three regression equation formulae were obtained by statistical analysis from the relationship between statures inter anterior superior iliac spinal distance for females only, males only and males and females combined (Table 1). As all the subjects were of approximately same age, correlation between age and inter-acromial length was not sought. When combined data was used, no positive correlation between statures and inter anterior superior iliac spinal distance was found (Scatter plot 1). Similarly, there was insignificant correlations found between statures and inter anterior superior iliac spinal distance in either of the sexes (Scatter plots 2 & 3).

Scatter plot 1 shows insignificant correlation between stature and inter-anterior superior iliac spinal distance. Also, there was no correlation found between stature and inter-anterior superior iliac spinal distance sex wise, as shown in Scatter plots 2 & 3.

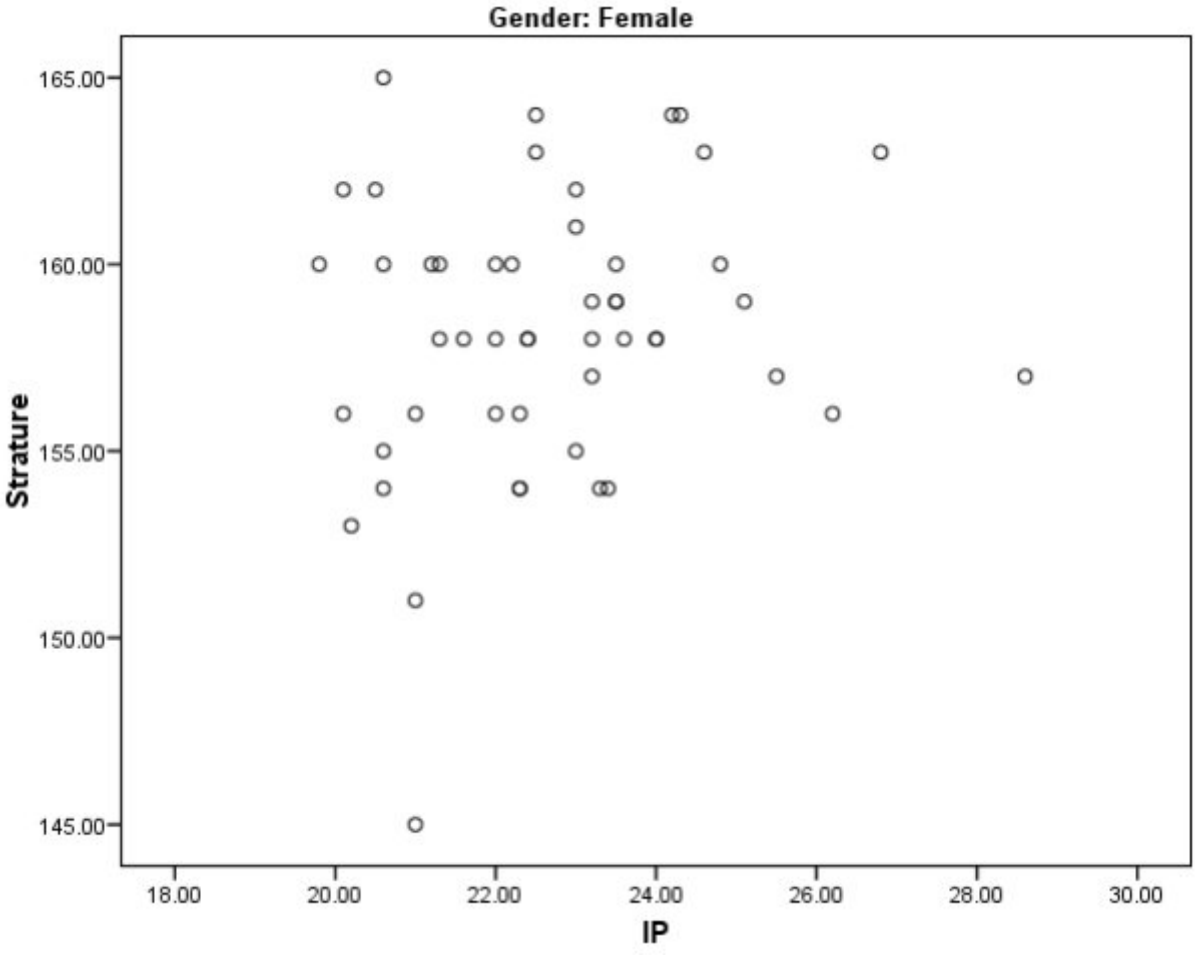


Scatter plot 1 [combined] shows that there is not much association between the two variables. Regression analysis further confirms the fact.



Scatter plot 2 [for males] shows that there is some positive association. But the p value is insignificant.

Scatter plot 3 [for females] shows that there is no/ or very little association. The regression analysis confirms the same.



In our study, all variables were quantitative; thus linear regression was applied to assess the prediction of stature from inter anterior superior iliac spinal distance.

Table 1

Variable	Regression formula for stature (y) =	Correlation coefficient	R^2
Females	150.11 + 0.357x	0.177	0.031
Males	157.28 + 0.665x	0.258	0.066
Combined	135.66 + 1.29x	0.313	0.098

Where x = IASIS distance

DISCUSSION

Population variations in anthropometric dimensions always exist and are attributed to genetic, dietary habits, environmental and other factors. This indicates that specific formulae or regression equations used in prediction of stature are only applicable to the population from which the data were collected.[4]

Various researchers with variable degree of success have attempted the estimation of stature from long bones, by using different statistical methods such as regression equations and multiplication

factors with variable degrees of success. The availability of adequate quantities of bones, choice of bones, and trained personnel has always been limitations in conducting such studies. Nevertheless, no study could be found on the use of these statistical methods to calculate stature from inter anterior superior iliac spinal distance.

The above results show that there was insignificant correlation found between stature of a person and inter-anterior superior iliac spinal distance. Though it is a fixed landmark, there might exist some errors in measurements thus giving an insignificant correlation. It can be ruled out by further similar studies and on a larger sample size. References regarding it could not be found in literature. So it might be possible that similar studies have been conducted in past but because of negative result, they haven't been reported.

CONCLUSION

The present study concluded that inter anterior superior iliac spinal distance has no forensic value in estimating stature in living individuals. This was a pilot study hence it is aspired that the results may be viewed more as indicative of the feasibility of the technique. Similar studies in other geographical areas and racial groups with larger sample size may validate or negate our observations.

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ANIL AGGRAWAL'S
Internet Journal of Forensic Medicine and Toxicology
The world's first online journal devoted entirely to forensic medicine and toxicology · Published 1 January and 1 July

VOL. 16, NO. 1 · JANUARY – JUNE 2015
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Face off and alcohol consumption – A case series.

Alok Atreya[1], Jenash Acharya[2], Tanuj Kanchan[3*]

[1]Lecturer, Department of Forensic Medicine, Manipal Teaching Hospital, Pokhara, Nepal
[2]Lecturer, Department of Forensic Medicine, Kathmandu Medical College Teaching Hospital, Kathmandu, Nepal
[3]Associate Professor, Department of Forensic Medicine, Kasturba Medical College, Mangalore (A constituent College of Manipal University), India
Contact: Tanuj Kanchan · tanujkanchan@yahoo.co.in

CITE THIS ARTICLE

Atreya A, Acharya J, Kanchan T. Face off and alcohol consumption – A case series.. *Anil Aggrawal's Internet Journal of Forensic Medicine and Toxicology* [serial online], 2015; Vol. 16, No. 1 (January – June 2015): [about 9 p]. Available from: https://www.anilaggrawal.com/ij/vol_016_no_001/papers/paper007.html

ABSTRACT

During sudden altercations, the more easily available and accessible weapons are frequently used to assault; head and face being the vulnerable parts in such fatal attacks. These incidents are mostly unplanned, and occur in a fit of rage, alcohol often being a contributory factor. The present case series highlights the unique facial injuries caused following arguments during alcohol consumption, and using easily accessible weapons in the surrounding. Gorkha Khukuri knife used as a weapon of offence in the first case is a utility knife used by Nepalese population. Fish hook used as a weapon of offence in the second case in fact is a very uncommonly used weapon in cases of assaults.

Keywords: Assault; Alcohol; Crimes; Facial injuries

Introduction

WHO estimated that alcohol contributes approximately 9% of the burden of disease and disability in developed countries, making it the third-highest risk factor for morbidity. Alcohol-related harm is a widespread problem. Two billion people worldwide are estimated to consume alcohol; 76 million of those have a diagnosable alcohol use disorder **[1]**. Alcohol, although considered a bottled poetry by many can at times be a risk factor for either being victimized or perpetrating a crime. As per the World Health Organization (WHO) estimates, approximately four and a half percent of the global burden of disease and injury is attributable to alcohol. Alcohol consumption is estimated to cause from 20% to 50% of cirrhosis of the liver, epilepsy, poisonings, road traffic accidents, inter

personal violence, sexual violence and several types of cancer **[2]**. At times when excess amount of alcohol meets young blood, matters may even get worst and the degree of violence is usually devastating. Besides the well-known effect of alcohol on cognitive and physical functions like disinhibition, where the normally suppressed behavior are expressed, alcohol also has an equal role in reducing self-control and increasing emotional liability and impulsivity **[3, 4]**.

We report a series of cases from Nepal where the victims were assaulted by their near-ones following altercations during alcohol consumption.

Case 1

A 21 years old male was brought to the emergency, who had stable vitals and Glasgow Coma Scale computed 15/15, with a complaint of multiple sharp force injuries over head and face. Alleged history suggested that he met with an argument with his fellow drinker, known to be his friend, who on sudden altercation impulsively rushed to his house nearby and hastily came back with a heavy sharp cutting weapon, allegedly a “Gorkha Khukuri Knife” and pounded on him. The weapon was glazed into the right temporal region resulting in de-gloving of the right lateral part of the face and neck slicing the pinna of right ear into two halves and exposing the underlying dura. The total length of the wound measured 22 cms **(Figure 1).** The direction of the blow was above downwards and lateral to medial which sliced the skin and soft tissue with underlying cut fracture of the temporal bone. The dura appeared intact during the primary examination but it was later learnt that while transporting the patient for Computed Tomography scan, bony specules had ruptured the dura. He was taken to Operation Theater where the dura was secured with a forceps at the point of cerebro spinal fluid leak, wound was explored, and washed and foreign body was removed under general anaesthesia. Minor craniotomy was done to blunt the bony specules. The dura and skin was sutured in layers. After a day in post-operative room, the patient was shifted to neurological intensive care unit for four days. Intravenous antibiotics coverage was given to prevent meningitis, after a pure tone audiometry was done which was found normal for right ear and the patient was discharged on 15^{th} day of hospital stay.

Case 2

An adult male fisherman, aged 32 years, had some property related issues with his brother for quite a while. It was one eventful day that they decided to solve it over few pegs of alcohol. He being single his sister-in-law insisted him to grant her a greater portion of the ancestral property, which resulted in a heated argument. On seeing his mother being verbally abused by his uncle, the elder cousin, about 18 years of age, impulsively stabbed on the cheek of the fisherman, with a “fishing hook” that was lying nearby. The victim when brought to the emergency room showed a penetrating metallic foreign body embedded in his left cheek **(Figure 2)**. He was conscious and his vitals were stable. The radiological examination did not reveal any underlying bony deformity. The foreign body was removed under local anesthesia; the wound was cleaned and sutured **(Figure 2).** The foreign body happened to be a metallic rod, alleged to be a fishing hook, with a J shaped pointed curve at one end and a hollow cylinder on the other. It was 18 cms in length and weighed about 400gms.

Discussion

Injuries linked to alcohol are categorized as intentional and unintentional injuries **[2]**. Suicide and violence after heavy drinking may be linked to intentional injuries whereas unintentional injuries include road traffic accidents, falls, drowning, poisoning etc. Almost all categories of unintentional injury are impacted by alcohol consumption. There is a strong link to the level of alcohol concentration in the blood and the resulting effects on psychomotor abilities, and higher levels of alcohol consumption create an exponential risk increase **[2]**. The stimulating effects of alcohol are mainly due to the release of norepinephrine in the brain **[5],** and also release of pituitary hormones which signal the adrenal glands to release adrenaline **[6]**. Alcohol also represses the functioning of the prefrontal cortex which is responsible for decision making **[7]**, probably the reason for people to lose their inhibitions and involve in inappropriate acts. Studies have shown that excessive risk-taking and impulsive behaviors are linked to drug addiction **[8, 9]**.

Psychomotor impairment, lengthened reaction time, impairment of judgment, emotional changes, and decreased responsiveness to social expectations are some of the features of alcohol intoxication. Men drink more often and much heavily than the females. Many adolescents and young adults characteristically drink till intoxicated. Even though there is a significant differences in drinking patterns between people of different nations, genders, age groups, and ethnicity; harm attributed by alcohol is common and falls under one of these three types viz: the toxic effect (that primarily impacts on chronic diseases), drinking in a specific event, including intoxication (primarily impacts on injuries), and dependence (due to its probable role in contributing to high volume and excessive patterns of consumption) **[10].**

Young people who drink in settings away from home, for example in groups or public spaces like bars and pubs, end in both fighting and unintentional injuries, particularly when rival peers or peer groups are involved **[11]**. A study conducted in Scotland among 82,461 patients with facial injuries revealed the involvement of alcohol in 27.2% of the cases. The incidences of facial injuries were 3.27 per 1,000 population; 4.68 per 1,000 for males; 2.00 per 1,000 for females during the study period. The incidence of alcohol-related facial injuries for the same period was 0.89 per 1,000 population; 1.54 per 1,000 for males; and 0.30 per 1,000 for females. Young age (15–19 years) and male sex were major determinants for alcohol related facial injuries. It was also shown that those residing in a socioeconomically deprived area were seven times more likely to have such injury **[12]**.

In a decade long study from New Zealand, 79% of the males had facial fractures out of a total of 26,637 cases. Inter-personal violence was attributed to be the major etiological factor as it accounted to 38% of the total cases **[13].** The present case series highlights the unique facial injuries caused following arguments during alcohol consumption using locally available household weapons/ instruments. Gorkha Khukuri knife used in the first case is an indigenous utility knife found in the majority of the Nepalese household. Fish hook used as a weapon of offence in the second case is again found in fisherman houses and in fact is a very uncommonly used weapon in cases of assaults. It is understood that during sudden altercations, the more easily available and accessible weapons are frequently used to assault, head and face being the vulnerable parts in such fatal attacks. These incidents are mostly unplanned, and occur in a fit of rage, alcohol often being a contributory factor.



Figure 1: Chop wound caused by a 'khukuri' producing a glancing injury into the right temporal region resulting in degloving of the right lateral part of the face, slicing the pinna of right ear into two halves and exposing the underlying dura. The direction of the blow was above downwards and lateral to medial which sliced the skin and soft tissue with underlying cut fracture of the temporal bone.



Figure 2: Penetrating metallic "fishing hook" embedded in the victim's left cheek that was removed under local anesthesia, the wound was cleaned and sutured

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Internet Journal of Forensic Medicine and Toxicology
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VOL. 16, NO. 1 · JANUARY – JUNE 2015
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Situs Inversus With Rupture Of Cerebral Blood Vessels - A Rare Case Report.

Yadukul S[1*], Pradeep Kumar M.P.[2]

[1]Assistant Professor, Department of Forensic Medicine and Toxicology, Chamarajanagar Institute of Medical Sciences, Chamarajanagar, India

[2]Assistant Professor, Department of Forensic Medicine and Toxicology, Bangalore Medical College and Research Institute, Bengaluru, India

Contact: Yadukul S · dr.koooool@gmail.com

CITE THIS ARTICLE

Yadukul S, Pradeep Kumar MP. Situs Inversus With Rupture Of Cerebral Blood Vessels - A Rare Case Report.. *Anil Aggrawal's Internet Journal of Forensic Medicine and Toxicology* [serial online], 2015; Vol. 16, No. 1 (January – June 2015): [about 9 p]. Available from: https://www.anilaggrawal.com/ij/vol_016_no_001/papers/paper008.html

ABSTRACT

Situs inversus (also called situs transversus) is a congenital condition in which the major visceral organs are reversed or mirrored from their normal positions. Many people with situs inversus totalis are unaware of their unusual anatomy until they seek medical attention for an unrelated condition. The most frequent cause of clinically significant non-traumatic subarachnoid hemorrhage is rupture of a *saccular (berry) aneurysm.*

Here, we present a case of a male, aged 45yrs who complained of severe head ache, collapsed and died at his work place. He was asymptomatic before the incident occurred. At autopsy, it was found that, all the visceral organs of the thorax and abdomen was transposed to the other side, including the heart. Apart from this anatomical variation no other pathological entity was evident. On opening the cranial cavity, it was found that, there was diffuse sub-dural and sub-arachnoid haemorrhage present all over. On further examination, the anterior cerebral artery was found ruptured on the right side at its junction with anterior communicating artery. A combination of situs inversus and berry aneurysm is a rare phenomenon.

Keywords: Situs inversus, Berry aneurysm, Rupture of cerebral vessel

INTRODUCTION

The term situs inversus is a short form of the latin phrase "Situs inversus Viscerum" meaning "inverted position of the internal organs". It is an Autosomal recessive pattern of inheritance.

Generally, patients with situs inversus totallis are asymptomatic and have a normal life expectancy. Prevalence of Situs inversus varies among different population but is less than 1 in 10,000[1].

The most frequent cause of clinically significant non-traumatic subarachnoid hemorrhage is rupture of a *saccular (berry) aneurysm*[2]. Autopsy and angiography studies have found that about 2% of adults harbor intracranial aneurysms, for a prevalence of 4 million persons in the United States; the aneurysm will rupture, producing Sub Arachnoid Haemorrhage, in 25,000–30,000 cases per year. Hemorrhage into the subarachnoid space may also result from vascular malformation, trauma (in which case it is usually associated with other signs of the injury), rupture of an intracerebral hemorrhage into the ventricular system, hematologic disturbances, and tumors[3].

CASE REPORT

A 45-year-old male, complained of severe head ache and collapsed while working in his factory. He was immediately taken to the nearby hospital where he was declared brought dead. The dead body was subjected to Autopsy in Department of Forensic Medicine and Toxicology, Bangalore Medical College and Research Institute, Bengaluru.

Postmortem examination revealed a body of a middle aged male, wheatish in complexion, moderately built and nourished. There were no demonstrable external injuries present over the body. Nails on both the hands showed features of clubbing (Fig 1).



Figure 1 - Clubbing in nails.

On opening the thoracic and abdominal cavity, it was noted that all the visceral organs of the thorax and abdomen was transposed to the other side, including the heart. The liver was on the left side while the heart and the spleen were present on the right side (Fig 2).



Figure 2 - Transposition of visceral organs

Apart from the transposition of the visceral organs, there was no gross pathology in any of the organs. Stomach was empty, with normal mucosa.

On removing the cranial cavity, it was observed that there was diffuse Sub-dural and sub-arachnoid hemorrhage present all over the brain (Fig 3).



Figure 3 - Diffuse Sub-dural and Sub-arachnoid hemorrhage

On further examination, it was noted that there was numerous aneurismal spots present over the cerebral vasculature. The anterior cerebral artery was found ruptured on the right side at its junction with anterior communicating artery (Fig 4).

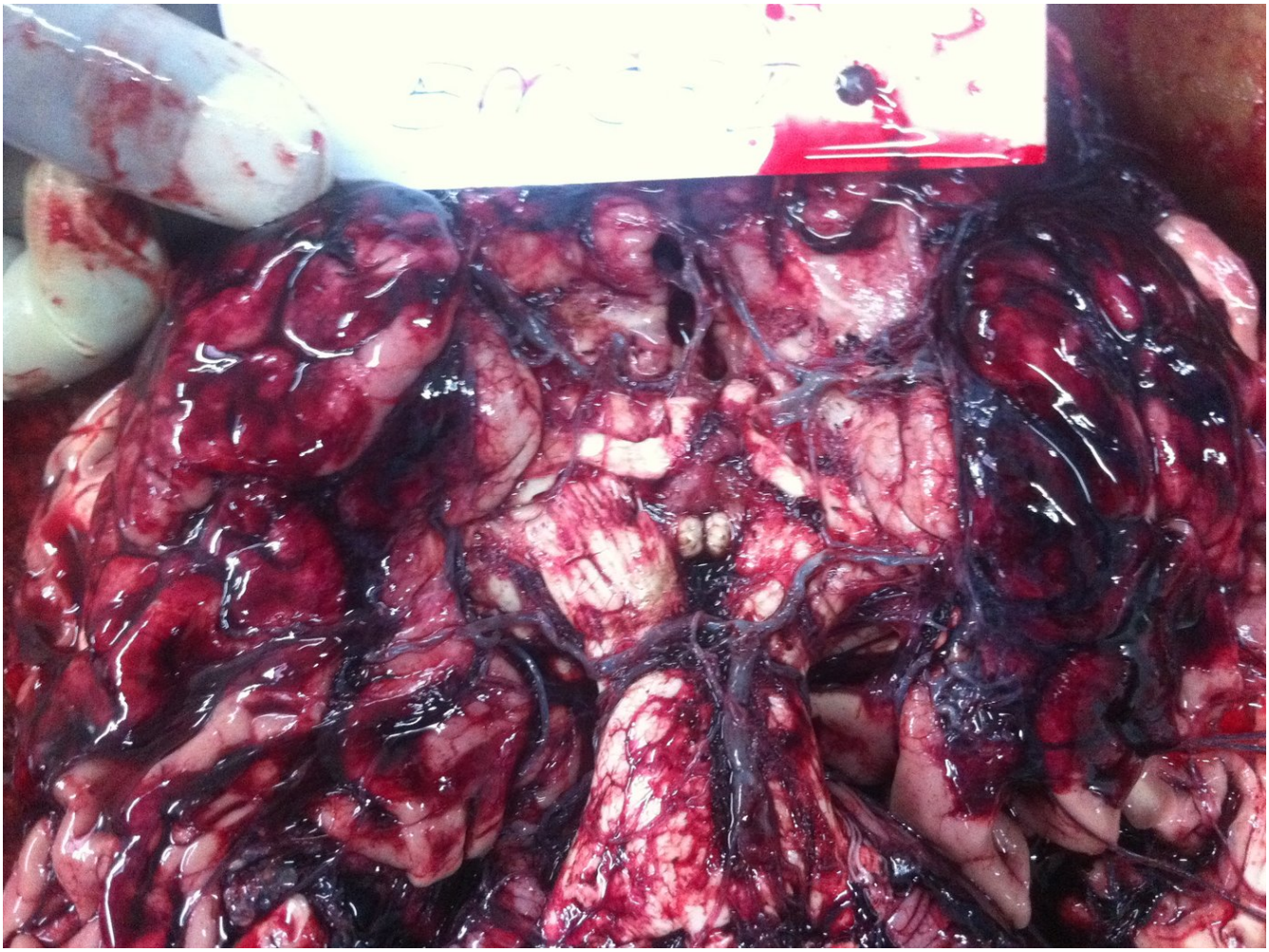


Figure 4 - Rupture of Saccular aneurysm

The cause of death was opined as a result of rupture of cerebral blood vessels.

DISCUSSION

Situs inversus (also called situs transversus or oppositus) is a congenital condition in which the major visceral organs are reversed or mirrored from their normal positions. The normal arrangement is known as situs solitus. In other rare cases, in a condition known as situs ambiguus or heterotaxy, situs cannot be determined[1]. Dextrocardia was first seen and drawn by Leonardo da Vinci in 1452–1519. However, situs inversus was first described more than a century later by Matthew Baillie. The prevalence of situs inversus varies among different populations but is less than 1 in 10,000 people. The condition affects all major structures within the thorax and abdomen. Generally, the organs are simply transposed through the sagittal plane. The heart is located on the right side of the thorax, the stomach and spleen on the right side of the abdomen and the liver and gall bladder on the left side. The left lung is trilobed and the right lung bilobed, and blood vessels, nerves, lymphatics and the intestines are also transposed[1].

The most frequent cause of clinically significant subarachnoid hemorrhage is rupture of a *saccular (berry) aneurysm.* Hemorrhage into the subarachnoid space may also result from vascular malformation, trauma (in which case it is usually associated with other signs of the injury), rupture of an intracerebral hemorrhage into the ventricular system, hematologic disturbances, and tumors. Rupture can occur at any time, but in about one-third of cases it is associated with acute increases

in intracranial pressure, such as with straining at stool or sexual orgasm[2]. Blood under arterial pressure is forced into the subarachnoid space, and individuals are stricken with sudden, excruciating headache (classically described as "the worst headache I've ever had") and rapidly lose consciousness. Between 25% and 50% of individuals die with the first rupture, although those who survive typically improve and recover consciousness in minutes[2]. Saccular aneurysms occur at the bifurcations of the large to medium-sized intracranial arteries; rupture is into the subarachnoid space in the basal cisterns and often into the parenchyma of the adjacent brain. Approximately 85% of aneurysms occur in the anterior circulation, mostly on the circle of Willis. About 20% of patients have multiple aneurysms, many at mirror sites bilaterally[3].

Situs inversus totalis with dextrocardia was detected incidentally in a 60-year-old cadaver in routine dissection kept for undergraduate medical students by D Radhika et al[4]. Congenital diaphragmatic hernia is associated with situs inversus totalis as given by CD Tesselaar et al[5]. Combination of meckel's diverticulum with situs inversus totalis has been reported by V Yazhini et al[6].

CONCLUSION

To conclude, in the present case, the person dint had any previous medical illness prior to his death except for the excruciating head ache which was experienced by him prior to his death. Situs inversus totalis usually does not cause any significant morbidity to an individual harbouring it. However, its timely diagnosis is crucial for the correct interpretation of future symptoms and results of diagnostic procedures. An antedated diagnosis of this disorder will form a baseline reference for future procedures and as such will be invaluable in preventing an unintentional mishap.

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ANIL AGGRAWAL'S
Internet Journal of Forensic Medicine and Toxicology
The world's first online journal devoted entirely to forensic medicine and toxicology · Published 1 January and 1 July

VOL. 16, NO. 1 · JANUARY – JUNE 2015
Downloaded from www.anilaggrawal.com · ISSN 0972-8074 (Online) · https://www.anilaggrawal.com/ij/vol_016_no_001/papers/paper009.html

Separation of different components of hair cosmetic (Hairsprays) using TLC and HPTLC.

Amandeep Kaur[1], Sandeep Singh Sahota[2], Rakesh Kumar Garg[3*]

[1]Research Student, Department of Forensic Science, Punjabi University, Patiala-147002, India
[2]Senior Research Scholar, Department of Forensic Science, Punjabi University, Patiala-147002, India
[3]Professor, Department of Forensic Science, Punjabi University, Patiala-147002, India

Contact: Dr. Rakesh Kumar Garg · rkgvpbi11@yahoo.com

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Kaur A, Sahota SS and Garg RK. Separation of different components of hair cosmetic (Hairsprays) using TLC and HPTLC.. *Anil Aggrawal's Internet Journal of Forensic Medicine and Toxicology* [serial online], 2015; Vol. 16, No. 1 (January – June 2015): [about 15 p]. Available from: https://www.anilaggrawal.com/ij/vol_016_no_001/papers/paper009.html

ABSTRACT

Hairspray is one of the top most selling products among cosmetics. As it is a hair product, it has a great importance from forensic point of view because hair evidence is one of the most common types of evidence encountered in criminal investigations and cosmetics (including hairspray) are used widely today. The analysis of hair with respect to its identity, source of origin and presence of any adhering material (like hair color, dyes, hair sprays or serums etc.) is very significant in different types of Forensic cases.

In the present study, an attempt has been made to study the hair spray samples (marketed) commonly used for the separation of components using thin layer chromatography and high performance thin layer chromatography based on their polarity. Eight hairspray brands were analyzed with thirty different solvent systems through TLC and by one of the best solvent system (from TLC results), through HPTLC. It is interesting to note that same solvent [Carbon tetrachloride: Cyclohexane (90: 10)], is found to be the most suitable for the separation of components of hairsprays using both the techniques. The results are clear and well separated and can be used in Forensic investigation using these techniques in order to save time and chemicals.

Keywords: Hairsprays, HPTLC, TLC, Forensic

Introduction

Chromatography is the technique for the separation of the components based on the polarity using two phase, i.e. Mobile phase (Solvent) and Stationary Phase (Silica Gel G). This technique was first reported by Russian Botanist Tswett. In 1903, he succeeded in separating leaf pigments using a solid polar stationary phase. Thin layer Chromatography and High performance thin layer chromatography are a form of planar chromatography. Thin layer chromatography is widely used in the forensic science laboraborties for the analysis of drugs[1-4], Cosmetics[5-6], inks[7-10], etc. Over the period of time thin layer chromatography has been replaced by more sensitive, accurate, fast technique i.e High performance thin layer chromatography. It is also a planar chromatography and more refined form of thin layer chromatography (TLC), it has a wide range of applications in pharmaceutical [11-15], Botany [16-18], Forensic [1-4, 7-10], Chemistry [19-21] etc. With the advancement in the field of technology, HPTLC is hypernated with other instrumental technique to know the exact structure of the compound. In the present study an attempt has been made to separate the components of different components to narrow down the search for the suspect and use the result as corroborative evidence.

Hair sprays are widely used cosmetic product in today's times. It is also known as Hair Lacquer. It contains various natural and synthetic resins in both aqueous or alcoholic solutions and a propellant. Apart from these it also contains perfumes and small amount of lanolin. [22] This composition is filled into a pressure resistant container with a spray nozzle. The spray is dispensed from a pump or aerosol spray nozzle. For propellant driven spray applications, pressurized propellant mix is added. In case of pump sprays, often creating less fine aerosols, propellants are excluded. [23] Spraying deposits a stiff layer of the polymers on the hair after the solvent evaporates. The key property that makes polymers useful as hairspray is the holding agents and it's their ability to form films upon drying. Once hair spray is applied to the hair, the liquid drops run down the hair shaft until they reach the intersection of two hair fibers. When the drops dry at this fiber intersection, they create an invisible film that bonds hairs together. [24]

Hair sprays were developed around the time of an aerosol can in the 1940s. The first hair spray used was shellac, a resinous material derived from insects to hold hair in place. It also produced a water-insoluble film that was difficult to wash out of the hair. Synthetic polymers into hair styling helped overcome this problem. The first to package it was Chase products (an aerosol manufacturer) in 1948. Historic beauty titan Helene Curtis coined the name "Hairspray" in 1950, with the release of her wildly successful product "Spray Net". By 1964, hairspray was the most successful beauty product in the country, outselling even lipstick. However the late '60s into the '70s saw a drastic decline in hairspray sales. This was a result of a few factors like the popularity of "flower child" hair-styles in that era and it was discovered that aerosol cans contained Chlorofluoro Carbons (CFCs), which were harmful to the environment—particularly the ozone layer, as well as the women using the products. Before the 1970s, hair spray contained the propellant "vinyl chloride." This is a known carcinogen linked to cancer of the liver.

Eventually, new rules and regulations came forward for the improvement of these products, and CFCs were removed from aerosol cans completely and vinyl chloride was also replaced by other propellants.

But by the 80s, Hairspray's popularity came back. Today hairsprays are formulated as flexible, medium, and maximum hold, as there is much more of a liking towards natural hair styles. [25]

The present work has been done with the aim to analyze the hairsprays present in the area or victim that can help in association of the victim or assailant in crime investigation. This is a preliminary study and not much is reported, particularly with reference to India. It is expected that it will provide significant information that will be useful in forensic investigations.

Materials and Methods

1. **Collection of samples:** Eight (commonly used) hair sprays samples of different brands were collected from different beauty parlors and salons of Punjab.
2. **Chemicals and Solvents:** The entire reagents were of Laboratory grade purchased from Merck, Mumbai. Silica Gel G was purchased from Merck, Mumbai for Thin layer chromatography. Aluminium per coated TLC plates with silica Gel 60 F254 (MERCK KGaA) of size 20×20 cm were used for HPTLC.
3. **Analytical Configuration**: HPTLC CAMAG® analytical station. It includes- (1) Applicator Linomat 5, (2) Wincat software, (3) TLC scanner 3.

Results and Discussion

In the present study, an attempt has been made to find a better solvent system for the separation of hairspray components. A total of thirty solvent systems were used to find out that with which solvent system separation is more suitable. The solvent systems used are given in Table 1. Out of these thirty solvent systems attempted eight solvents were selected as it gave maximum separation of the components of hairspray and the visualization done with the help of iodine fuming in case of thin layer chromatography and under UV (long and short) which in case of high performance thin layer chromatography. These selected eight best solvent systems are given in Table-2.

Out of the eight selected suitable solvent systems, Solvent system [Carbon tetrachloride: Cyclohexane (90: 10)] is chosen for further HPTLC analysis of the hair sprays to separate the components as it gave better separation result. Using Solvent system [carbon tetrachloride: Cyclohexane (90: 10)], Berina gave 3 spots at hRf (16,47,54), Keabeen gave 3 spots at hRf (12,17,26), Nova gave 2 spots at hRf (12,27), Nova Gold 4 spots at hRf (9,14,57,98) and Swift gave 4 spots at hRf (30,42,48,57) while Ck, Gatsby and Sora Amore gave no results.

Same Eight samples were subjected to High Performance Thin layer chromatography. Two solvent systems were selected based on the results of Thin layer chromatography i.e. Solvent system [Chloroform: Ethanol (90: 10) and Carbon tetrachloride: Cyclohexane (90: 10)]. HPTLC results obtained and visualized under UV light at 254 nm. Out of these two solvents system selected, solvent system Chloroform: Ethanol (90: 10) gave better results, separating two components of three samples out of six samples and one component of remaining five samples. Whereas, solvent system Chloroform: Ethanol (90: 10) gave no result.

Using Solvent system Carbon tetrachloride: Cyclohexane (90: 10), Berina gave only one spot with hRf at 4, CK gave two spots at 3 and 16, Gatsby gave one spot at 3, Keabeen gave one spot at 3, Nova gave two spots at 4 and 60, Nova gold gave two spots at 3 and 9, Sora amore gave two spots at 2 and 4, while Swift gave two spots at 4 and 9 (Table-3), separating all the samples of hairsprays depending upon their polarity.

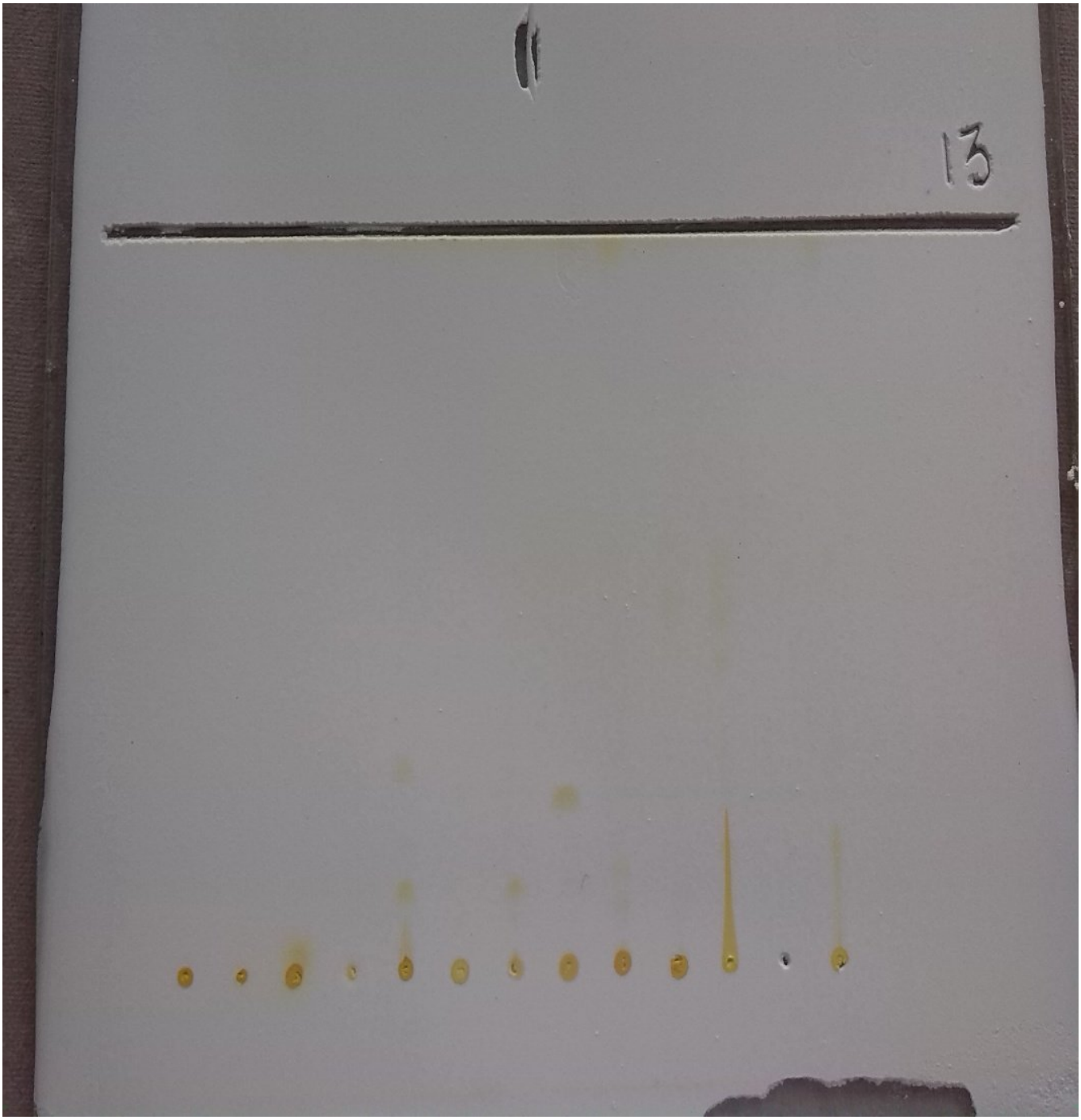


Fig. 1 -TLC result of solvent system S5

Carbon tetrachloride: Cyclohexane (90: 10)

Conclusion

Eight hairspray samples were analyzed by thirty different solvent systems through TLC and by one of the best solvent system (from TLC results), through HPTLC. Solvent system [Carbon tetrachloride: Cyclohexane (90: 10)], is found to be the most suitable for the separation of components of hairsprays using TLC and HPTLC. From the results of thin layer chromatography and High performance thin layer chromatography it is concluded that different components of hair sprays could be separated using these techniques. In this study it was found that using the same solvent system for analysis of hair sprays using these techniques, the result of the separation of the

components varies. It is interesting to note that components which could be separated by thin layer chromatography were separated using high performance thin layer chromatography. CK, Gatsby and Sora Amore gave no results using TLC, while Using HPTLC analysis gave better results. In this study a preliminary attempt has been made to separate the different components of hairsprays as the related information is not available.

1. **Conflict of Interest**: None
2. **Source of funding**: Self
3. **Acknowledgement**: We are thankful to Sophisticated Instruments Centre, Punjabi University, Patiala for providing infrastructure to conduct the study.

Table-1 Showing Solvent systems used in the present study

Solvent System Codes	Solvent System	Ratio
S1	Chloroform : Methanol	90 : 10
S2	Cyclohexane ; Chloroform	80 : 20
S3	Chloroform : Ethanol	90 : 10
S4	Chloroform : n-Hexane	75 : 25
S5	Carbon tetrachloride : Cyclohexane	90 : 10
S6	Carbon tetrachloride : n-Hexane	75 : 25
S7	Acetonitrile : Water	80 : 20
S8	Hexane : Toluene : Acetic acid	50 : 50 : 2
S9	Carbon tetrachloride : Ethanol	90 : 10
S10	Carbon tetrachloride : Methanol	90 : 10
S11	n-Hexane : Toluene : Chloroform	20 : 20 : 60
S12	Acetonitrile : Methanol	80 : 20
S13	Chloroform : Acetone	70 : 30
S14	Chloroform : Benzene : Diethyl ether	80 : 10 : 10
S15	Chloroform : Toluene : Acetonitrile	80 : 15 : 5
S16	Dichloromethane : Cyclohexane : Ethanol	65 : 25 : 10
S17	Dichloromethane : Toluene : Methanol	60 : 20 : 20
S18	Dichloromethane : Cyclohexane	80 : 20
S19	Dichloromethane : Toluene	80 : 20
S20	Chloroform : Toluene	70 : 30
S21	Dichloromethane : n-Hexane	70 : 30
S22	Chloroform : Diethylamine	90 : 10
S23	Chloroform : Methanol : Water	100 : 30 : 2
S24	Chloroform : Methanol : Water : Acetic acid	50 : 3 : 30 : 8
S25	Dichloroethane : Chloroform	60 : 40
S26	Dichloroethane : Carbon tetrachloride	60 : 40
S27	Dichloroethane : Ethanol	70 : 30
S28	Dichloroethane : Methanol	70 : 30
S29	Cyclohexane : Ethyl acetate	70 : 30
S30	Chloroform : Ethyl acetate	80 : 20

Table-2 Showing Best solvent systems

Solvent System Codes	Solvent Systems	Ratio
S2	Cyclohexane ; Chloroform	80 : 20
S3	Chloroform : Ethanol	90 : 10
S5	Carbon tetrachloride: Cyclohexane	90: 10
S7	Acetonitrile : Water	80 : 20
S8	Hexane : Toluene : Acetic acid	50 : 50 : 2
S9	Carbon tetrachloride : Ethanol	90 : 10
S10	Carbon tetrachloride : Methanol	90 : 10
S28	Dichloroethane : Methanol	70 : 30

Table-3 Showing Results of HPTLC of hairspray samples

S. No	Sample	Peaks	Rf
1	Berina	1	4
2	CK	1	3
		2	16
3	Gatsby	1	3
4	Keabeen	1	3
5	Nova	1	4
		2	60
6	Nova Gold	1	3
		2	9
7	Sora amore	1	2
		2	4
8	Swift	1	4
		2	9

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Tables

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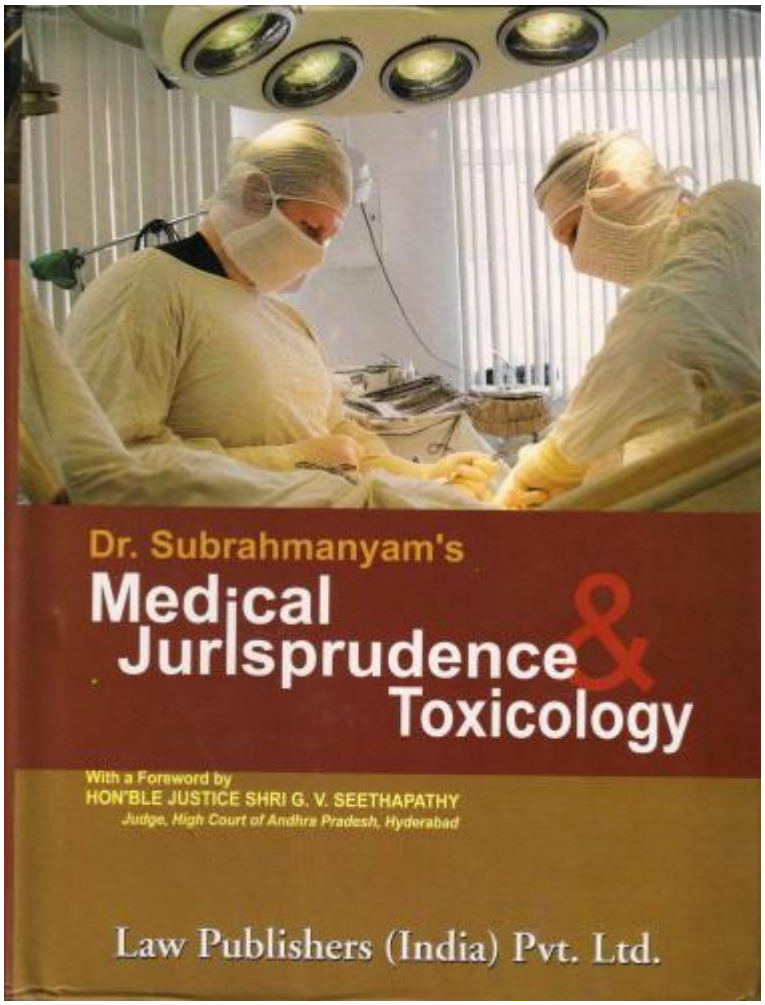
VOL. 16, NO. 1
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The book gives several photographs which further add value to the text.

The foreword by Justice GV Seethapathy rightly congratulates the author for the publication of this book.

Overall I found the book useful for postgraduates. I heartily recommend it to them. I also congratulate Dr. Subrahmanyam for taking the lead to write a book for postgraduates, which did not exist so far.

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