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CONTENTS

EDITORIAL

ORIGINAL ARTICLES

CASE REPORTS

EDITORIAL

Admissibility of scientific evidence in forensic practice: The Frye test and Daubert standard

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Introduction

Our society always has a high regard for conclusions based on science. Therefore, expert testimony that is allegedly scientific and favors one side of a case or the other is often the critical factor that sways the final decision at all levels of the court system.

Consequently, there is great incentive for lawyers to find experts with scientific credentials to present favorable opinions said to be grounded in the scientific method, even when the major issues in a case are not technical (Mohr JC, 2000).

In the United States of America, the early attempt to control **the quality of expert testimony** arose out of a 1923 Supreme Court decision (Frye v United States) concerning admissibility of evidence from an early version of a lie detector machine (polygraph). The Court concluded that the results of the "blood pressure deception test" should not be admitted into evidence because the test lacked "general acceptance in the relevant scientific community."(Kassirer JP & Cecil S. 2002; Petroski H. 1999)

Polygraph screening is completely without any theoretical foundation and has absolutely no validity...the diagnostic value of this type of testing is no more than that of astrology or tea-leaf reading. There is no reason to expect that these "machines" are reliable when used on people with chronic

medical conditions (D C Richardson, 2008). As a matter of fact, the American Medical Association has taken a stand against these machines and testified before Congress in support of the 1988 Employee Polygraph Protection Act. Once again, there is no way to explain why a subject who is healthy or ill may have "failed." These "tests" are simply not a reliable way to measure truthfulness.

Federal Rules of Evidence

In the 1960s, the US Supreme Court developed rules of evidence for use in the federal courts. These rules, officially called the Rules of Evidence for United States District Courts and Magistrates and known simply as the "Federal Rules of Evidence (FDE) which were substancially revised. It was then adopted by Congress, effectively on the July 1st 1975.

With respect of the general admissibility of evidence, FRE Rule 402 provides that all relevant evidence is admissible unless otherwise proscribed. With respect to expert testimony, FRE Rule 702 provides that:

"If scientific, technical, or other specialized knowledge will assist the trier of fact to understand the evidence or to determine a fact in issue, a witness qualified as an expert by knowledge, skill, experience training, or education, may testify thereto in the form of an opinion or otherwise."

The value assigned by the legal system to scientific evidence and opinions leads directly to several difficult-to answer questions, such as "when is an opinion really based on science?" and "who should make this determination?" (Gasparino C, 2004)

Although the Frye rule that arose out of this issues provided judges with some guidance for controlling the admissibility of expert testimony, in practice juries were often presented with expert testimony that was clearly false or heavily biased. Judges who were concerned about the quality of the testimony given in their courts tended to concentrate on establishing the validity of the expert's credentials, leaving the evaluation of the validity of the content of the expert's testimony to the jurors.

The court should prevent "junk science" from being introduced and considered in any case tried by pseudo-expert through the maxim of **"ipse dixit"** (roughly translated as **"because I say so"**).

Frye standard

The ***Frye*** **standard** (wikipedia, 2008) is a legal precedent regarding the admissibility of scientific examinations or experiments in legal proceedings. This standard comes from the case *Frye vs. United States* (293 F. 1013 (DC Cir 1923)) District of Columbia Circuit Court in 1923.

To meet the *Frye* standard, scientific evidence presented to the court must be interpreted by the court as **"generally accepted" by a meaningful segment of the associated scientific community**. This applies to procedures, principles or techniques that may be presented in the proceedings of a court case.

In practical application of this standard, those who were proponents of a particular scientific issue had to provide a number of experts to speak to the validity of the science behind the issue in question.

Novel techniques, placed under the scrutiny of this standard forced courts to examine papers, books and judice precedence on the subject at hand to make determinations as to the reliability and "general acceptance."

Daubert standard

The **Daubert standard** is a legal precedent set in 1993 by the Supreme Court of the United States of America regarding the admissibility of expert witnesses' testimony during federal legal proceedings. The citation is Daubert v. Merrell Dow Pharmaceuticals, 509 U.S. 579 (1993).

The reason of the court to apply Daubert standard is to exclude the presentation of unqualified evidence to the jury. In other word, it is usually used to exclude the testimony of an expert witness who has no such expertise or used questionable methods to obtain the information.

Under the Daubert standard, the trial judges must evaluate proffered expert witnesses to determine whether their testimony is both “relevant” and “reliable”. He will use a two-pronged test of admissibility.

1. The relevancy prong:
   The relevancy of a testimony refers to whether or not the expert’s evidence “fits” the facts of the case. For example, you may invite a psychiatrist to tell the jury if a patient had a panic attack on the night

of a crime. However, the psychiatrist would not be allowed to testify if the fact that the severe panic attack that occurred on the night of the crime, was not relevant to the issue at hand in the trial.

2. The reliability prong:
   The Supreme Court explained that in order for expert testimony to be considered reliable, the expert must have derived his or her conclusions from the scientific method. The Court offered "general observations" of whether proffered evidence was based on the **scientific method** and it can be checked with the following checklist:
   - Empirical testing: the theory or technique must be falsifiable, refutable, and testable.
   - Subjected to peer review and publication.
   - Known or potential error rate and the existence and maintenance of standards concerning its operation.
   - Whether the theory and technique is generally accepted by a relevant scientific community.

In most but not all jurisdictions, the Daubert standard has superseded the Frye standard.

Kumho Tire Company v Patrick Carmichael

One of the example is, in 1999, in Kumho Tire v Carmichael, the court directly addressed the problem of expert opinions that were offered in fields not traditionally considered to be science.26 (26. Kumho Tire Company v Patrick Carmichael. US Supreme Court, 526 US 137 (1999). (Kumho, 2003)

In Kumho, an impressively credentialed expert had examined a twice-repaired essentially bald tire and concluded that failure of the tire was due to a manufacturing or design defect. It was apparent that the expert opinion was not based on “Daubert science”, but it was in fact the opinion of a tire expert. In excluding this expert's opinion, the court reasoned that all expert testimony must have a valid and reliable basis, "whether it is accounting or rocket science." (Faigman DL, 2002)

For forensic practitioners or pathologists, the importance of Kumho is that it unequivocally extended the court's standards for expert scientific testimony to types of testimony that had attempted to avoid Daubert by claiming for legal purposes to be not quite science. As noted by Kassirer and Cecil (2002) in JAMA, Kumho "tethered the standard of admissibility of testimony by physicians to the professional standards of the practice of medicine."

However, sometimes judges have difficulty in evaluating technical expert testimony. For example, in January 2002, Judge Louis Pollak concluded that because fingerprint "matching" did not have a known error rate, a "match" could not be accepted in court as scientific testimony. The judge subsequently reversed himself, leaving unanswered the question of what types of expert testimony must meet strict Daubert criteria and when it is permissible to allow ipse dixit ("because I say so") expert testimony to be heard by a jury. (Kennedy D, 2003)

When assessing the admissibility of novel scientific evidence, some courts limit their review to the application of the traditional evidentiary test of relevancy. Under this test, scientific evidence is admissible if the testifying expert is duly qualified, the expert's opinion is relevant and will assist the fact finder, and the testimony is not so prejudicial as to outweigh its probative value. For example, in United States v. Baller (1975), the U.S. Court of Appeals for the Fourth Circuit applied this test of admissibility to testimony relating to the then new technique of voiceprint or spectrographic identification. Because that expert testimony was found by the court to be relevant and not overly prejudicial, it was admitted.

Forensic Practice

In pathology malpractice litigation, juries have the responsibility to decide whether a pathologist's conduct in a particular instance fell below an acceptable professional standard of care, and if so, was that conduct a proximate cause of injury to the plaintiff. As with any test that we are familiar with in medicine, for a variety of reasons, the jury test is vulnerable to both false-positive and false-negative results. The misleading expert witness testimony is an important factor that can result in either a false finding of malpractice or a false finding of no malpractice.

Unfortunately, in an adversarial system, it is unlikely that all expert testimony will ever be completely congruent with clinical practice.

However we (forensic practitioners) should not feel despair, FRE Rule 403 provides that evidence may be excluded if its:

“probative value is substantially outweighed by the danger of unfair prejudice, confusion of the issues, or misleading the jury, or by considerations of undue delay, waste of time, or needless presentation of cumulative evidence.”

This means despite all the possibility of evidences given by the so called experts, the trial judge is given the task to make decision or judgement whether to include or exclude any expert witness' evidence in the case at hand. In other word, the scientific evidence **may not be used at all** to decide on the verdict in the case tried, even though the forensic practitioner/s may think otherwise.

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ORIGINAL ARTICLE

Prospective study of victims and offenders of sexual offences

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Abstract

This prospective study was conducted at South Delhi, India over period of about two years at casualty of a tertiary hospital**.** A total number of 207 cases were examined during this period, 90 (43.47%) were victims of sexual assault and 117 (56.53%) were accused (offenders). Both were brought to the Casualty for medical examination. South Delhi is thickly populated with people ranging from lower socio-economic group to high income group of varied occupation. It also has thick population of migrant workers from rural India and neighboring countries. This study was planned to make medical examination more objective and meaningful in view of increasing crime rate. This study revealed that maximum number of victims (25.55%) were brought for medical examination 5-7 days after the incident. Maximum number of assailants (47%) brought for medical examination 5-14 days after the incidence. Majority i.e. 62(68.88%) of alleged rape victims were in the age group between 11 to 20 year. In a total 90 alleged sexual offence victims, 80 (88.88%) were female victims and 10 (11.11%) were male victims. Victim and accused were brought together for medical examination in 41(19.80%) cases. Maximum numbers (64.10%) of assailants were of the age group of 16 to 25 year. In maximum number incidence of sexual offences occurred in victims' houses (41.11%) followed by in assailant's house (28.88%). From education point of view 28.88% of victims were illiterate, 33.33% of victims were literate up to class V (primary) standard. 92.22% of victims and 88.88% of assailants were from low socio-economic group. In term of relations of assailants with their victims, 44.44% were having acquaintance and 18.80% were complete strangers. 25.55% victims were involved in forcible rape, 43.33% were involved in consensual rape. Only 11.11% were involved in unnatural sexual offences and most of the victims were all male in sodomy cases. Only 25.55% victims of alleged rape cases had some physical injuries which varied from simple to grievous injuries. In 85.55% of rape victims rupture of hymen was seen at multiple sites, but all were old ruptures. One (1.11%) victim of alleged rape case became pregnant following rape and delivered a

full term male baby in the casualty. Forensic materials were collected from victims in 81.11% cases. The results were positive in 5.55% of cases. Forensic materials were collected from assailant in 21.36% cases. The results were positive in 0.85% of cases. The single, separated, divorced, or widowed female i.e. without a mate, is five times more prone to sexual assault than the married, and those having the mate with her. Area populated by factory and industrial labour , and slum clusters had highest number of cases.

Key Words: Sexual assault victim, sexual offender, medical examination in sexual assault, natural sexual offences, unnatural sexual offences, sexual offences

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Introduction

Sexual offences are acts of sexual intercourse with a second person or an animal to obtain sexual gratification. In our society usually two types of sexual offences are seen. First variety is of sexual perversions or sexual deviations. These include acts, in which gratification is obtained without sexual intercourse e.g. sadism, masochism, voyeurism, exhibitionism, etc. Such perversions or deviations are due to aberration of the mind. Second variety includes deliberate sexual crimes by person e.g. rape, sodomy etc. Sexual offences are omnipresent crime in all countries of the world.

The law and customs of the society normally permit heterosexual intercourse between a man and his own wife as provided by the nature. Sexual intercourse by a man with a woman, other than his wife would constitute an offence particularly in absence of a valid consent. When act of sexual intercourse is done in natural manner with use of sex organs, it is called natural sexual offence, e.g. rape, incest and adultery. But when the act of sexual intercourse is done against the order of nature it is called unnatural sexual offence, e.g. sodomy, buccal coitus, tribadism and bestiality.

The worst victims of this heinous crime are the vulnerable group of our society, i.e. women and children. They mostly do not report because of the

consequent marital discord, harassment at the hand of police, relatives and humiliating cross-examination in the court by defense counsel. So, only a few cases are reported. To add to the misery, people find it difficult to talk about it and the police and legal system find it equally difficult to deal with it.

The problem becomes more complex as there seems to be numerous myths and misconceptions about rape such as who get raped, why they get raped, where and how rape occurs, etc. Such misconception frequently prevent the rape victim from receiving the necessary medical, legal and psychological support and care they require to recover from the emotional trauma of the assault. A detailed scientific study of alleged rape cases only can clear the clouds of myths and misconceptions.

Doctors (gynecologists, casualty medical officers and forensic experts) who are put in this difficult situation have a greater responsibility as not only they have to give a humanitarian support, clinical and psychological treatment to the victim but also have to exert to collect medical evidence as a substantial requirement for proper prosecution and, later on have to interpret them in the court of law. Collection of medical evidences, their documentation and interpretation become valuable particularly in the cases where there is no other witness to the incident. Present literature has equipped the physician to explore and establish the age of victims, sexual activity, traumatic intercourse, identification of the accused, presence of resistance emotional trauma, etc. In these circumstances a collaborative, scientific study of case history, medical evidence, laboratory findings, circumstantial evidence and their interpretation in the court of law needs urgent attention.

In Penal Codes of the various countries, the definition of ‘Rape’ is almost the same except slight change in the wording. Under Section 375 of Indian Penal Code, ‘Rape’ has been defined as, unlawful sexual intercourse by a man with his own wife below the age of fifteen, or any other woman below the age of sixteen, or any other woman above the age of sixteen against her will, without her consent or when it has been obtained by unlawful means, i.e. putting her in fear of death or hurt or by impersonation that is when the man knows that he is no her husband and her consent has been given because she believes that he is the man to whom she is lawfully married.

Section 377 of I.P.C. defines the unnatural sexual offences, whoever voluntarily has carnal intercourse against the order of nature with any man, woman or animal shall be punished with transportation for life, or with imprisonment of either description for a term which may extend to ten years and shall also be liable to fine.

The law further states that it is not necessary that there should be complete penetration of penis with emission of semen and ruptured hymen. Partial penetration of penis within the labia majora or vulva or pudendum with or without emission of semen or even an attempt at penetration is quite sufficient for the purpose of law.

According to the law of India, a woman of and above the age of sixteen years is capable of giving consent to an act of sexual intercourse. But married female at or above the age of fifteen years can give valid consent to her own husband. The law of England is bit different. Here a female at or above 13 years of age can give consent. But the consent must be conscious, free and voluntary and given while she is in full possession of her faculties. The consent should also have been obtained prior to the act. It is not a defense that the consent was given after sexual connection. It is also not an excuse that the woman was a prostitute. Like any other woman, she is entitled to the protection of the law and can not be forced.

The consent becomes invalid under following conditions:

a. Consent was not free, voluntary and while she was not in full possession of her senses.
b. Consent was obtained by fraud, fear, force or impersonation.
c. Consent was obtained from a retarded, idiot or imbecile woman, who cannot fully understand the nature of the act of intercourse while the man knows the female to be mentally deficient.
d. Act was done without the knowledge of woman while she is under the influence of narcotics, drugs, and anesthetics, in a state of coma as after an epileptic fit and possibly while in a hypnotic trance.
e. Consent was taken after the act.
f. Act was done when a woman is unconscious or under influence of alcohol or drugs of any kind, she also is believed to be unable to give valid consent for the act.
g. Female is below the age of consent.

Age of accused

The law of England presumes that a boy under fourteen years of age is sexually impotent and therefore, incapable of committing rape. But in India, there is no age limit under which a boy is considered physically incapable of committing rape. The court decides the question of his potency from the evidence in the case, and is guided by section 82 and 83 of the IPC in awarding punishment. Even an old man can commit rape on a young girl.

Age of victim

No age is safe for rape as children of less than one year and old women of 85 years have been raped. Children are more frequently raped than the

adults as they cannot offer much resistance and also due to a false belief in parts of India that venereal diseases are cured by sexual intercourse with a virgin.

Aims and Objectives

1. To study the incidence of sexual offences in South Delhi.
2. To study the demographic variables of sexual offences with special references of cruelty to women and children.
3. To study and analyze the patterns of alleged rape cases in a metropolitan city and its relation to age, sex, religion, marital status, occupation, socio-economic status etc.
4. To study the relation between the victim and assailant and also places of occurrence.
5. To study the cytopathological examination of samples which are collected from the genital organs, clothes and other articles of victim / accused in relation to the sexual offences.
6. To study the clinical finding and collection of medical evidences and their interpretation.

Materials and Method

This study was carried out on 207 cases of alleged rape including both victim and accused brought by police and others for medico legal examination at the All India Institute of Medical Sciences, New Delhi between January 2001 to September 2002.

In this study, we have taken detailed history of the incidence and about the circumstances from accused, victim and police by predesigned structured proforma. The medical examination including physical examination was carried out in detail and medical evidences were collected. The findings were noted in the format given.

For examination of victim

1. Consent of the victim.
2. Detailed and complete history of incidence and other relevant fact.
3. Detailed examination of the clothing's for sign of struggle, various stains and forensic materials.
4. General physical examination for injuries.

General injuries by

1. Naked eye gynecological examination.
2. Full pelvic examination.
3. Documentation by use of toluidine blue.

Proof of sexual offence by

1. Microscopic examination of vaginal smear for motile or non motile sperms
2. Three vaginal swabs from -
    a. Vulva external to hymen
    b. Low vaginal
    c. High vaginal
3. Microscopic examination of smear for sperm or seminal component by biochemical tests.
4. Ultraviolet (fluorescence) light test for detection of seminal stains on clothing, vulva, thigh and other areas.
5. Detection of age of the victim by general physical examination, dental examination and radiological examination.
6. Oral swabs from the border of the gums where there is history of oral coitus.
7. Examination of urine for the suspected pregnancy cases.

For examination of Accused

1. Detailed and complete history of the event and other relevant fact.
2. Detailed examination of clothing for signs of struggle and stains of forensic materials.
3. General physical examination
4. Presence of smegma inside the prepuce
5. Pubic hair combings.
6. Lugol's iodine test for presence of vaginal and oral epithelial cells on glans penis
7. Blood for estimation of alcohol and abusive drugs with history of their abuse.
8. Rape victims were examined by the gynaecologist in Casualty Department and accused were examined by the Forensic expert in the Department of Forensic Medicine and Toxicology or in the Casualty.
9. Materials needed for special examination was collected, duly sealed and handed over to the investigating police officer for sending it to the Central Forensic Science Laboratory for investigation and one part of the collected samples were kept for examination in the Forensic Laboratory at hospital.

Investigations

For victim

The specimens to be collected from the victim for laboratory examination are:

1. Any stain, torn cloth, foreign materials are found on the clothes.
2. Scraping of dried bloodstains for grouping and DNA characteristics.
3. Scraping of dried seminal stains for grouping, sperm, P30 glycoprotein and DNA characteristics.
4. Hair, matted pubic hair, combed foreign hair and plucked hair.
5. Broken nails and debris from under the nails.
6. Blood for grouping, alcohol, drug, VDRL and T-Cells.
7. Saliva for secretor status.
8. Swabs from: any soiled areas of the skin, from bite marks for saliva, mouth, pharynx, vagina, cervix, anus for spermatozoa and microorganisms.
9. Other specimens (Head hair, body hair and urine.)

For accused

1. Specimens were collected are as follows:
2. Any stain on the cloth, torn cloth, missing buttons and foreign materials.
3. Scraping of blood and seminal stains for grouping and DNA characteristics.
4. Hair – matted pubic hair, combed foreign hair.
5. Debris under the nails.
6. Blood for grouping, alcohol, drugs, VDRL, HIV-anti bodies.
7. Saliva for secretion status.
8. Swabs from coronal sulcus, prepuce and urethra for evidence of sexually transmitted disease.
9. Other specimens (Head hair, body hair, and urine for drugs etc.)

Preparation of sample

Nature of sample

1. Swabs: From upper and lower vagina, vulva, posterior vaginal fornix and perianal area.
2. Stained materials: Clothes, underwear, bed sheets and pubic hair.

Swabs and stained materials

A small portion of stained cloth (about 1x1cm) or swab are taken into a porcelain tile / dish and macerated with a few drops of diluted HCl and wait for:

a. ½ hour to 1 hour for fresh stain
b. 2 hours to 4 hours for old stain

Glass slides are cleaned and smear prepared by rubbing with macerated material, and then smears are dried at room temperature, but not to be heated and fixed with gentle flame by spirit lamp or with alcohol by dipping method. Then proceed for staining method.

Fixation of slides

a. Heat fixation – by spirit lamp
b. Chemical fixation by ethyl alcohol

Methodology of examination of forensic materials

A. Microscopic examination of spermatozoa

1. Hanging drop preparation:

Done for motile sperm in fresh cases, within a few hours of incidence. A drop of suspected semen specimen is put on a glass slide. Then a few drops of distilled water are added on it for dilution and a cover slip is put on it. Then kept the slide upside down for a few minutes and examined under high power microscope.

Observation

If the sample of semen is fresh (2-3 hours), motile spermatozoa are seen.

2. Microscopic examination of spermatozoa from stain materials

A small portion of stained cloth material (about 1x1cm) taken into a porcelain tile/dish and macerated with dilute HCl or normal saline and wait for ½ hour to:

a. 1 hour for fresh stain.
b. 2 hours to 4 hours for old stain.

Glass slides are prepared by rubbing with macerated materials. Then smeared slides are dried at room temperature, but not to be heated and fixed the slides either with gentle flame by spirit lamp or chemical fixation with ethyl alcohol by dipping method.

Staining the slides

Slides are stained either with methylene blue or hematoxylin and wait for 15 to 30 minutes. Wash the slides in running water.

Then eosin is used for counter staining and wait for 2 to 5 minutes. Then wash the slides in running water.

After allowing the slides to drying at room temperature, they are examined under high power microscope.

Observation
Posterior ½ to $^{1}/_{3}^{rd}$ of the head is stained with deep red or pink colour and anterior ½ to $^{2}/_{3}^{rd}$ remain unstained.

Alternative staining
Staining with haemalum reagent and wait for 2 to 5 minutes and counter stain with eosin and wait for 2 to 5 minutes.

Observation
Tail is stained in pink colour.

B. Examination of enzymes from forensic materials

1. Barberio's Test

For determination spermine
Extracted seminal materials are taken on glass slides. Then 25%
Trichloracetic acid and saturated aqua-solution of Picric acid is added.

Observation
Yellow elliptical colour crystals are formed, if the spermine is present in the sample.

2. Florence's Test

For the detection of choline.
Macerated stained materials are taken on a glass slides by squeezing. Then one or two drops of Florence reagent (8% W/V of I_2 + 5% W/V of KI in water), put a cover slip on it and the reagent should be run freely under the cover slip.

Observation
Dark brown rhombic or needle shaped crystals are seen under the microscope and crystals are the choline peroxide.
False negative reaction is common and it depends on the present of choline in the seminal fluid.

3. Acid Phosphatase Test

For detection of acid phosphatase which is present in high concentration in seminal fluid of human being.
A small portion of stain cloth is macerated with 1% dilute HCL and fluid is taken into a porcelain tile/dish. Then substrate reagent is added i.e. 2 drops

of citric acid buffer and 2 drops of 1% W/V disodium phenyl phosphates and wait for 10 minutes for enzymatic reaction.
Then liberated phenol is detected by adding 2 drops of phenol reagent and 2 drops of 20% W/V aqua-solution of sodium carbonate.

Observation
Blue colour is developed within 10 minutes and acid phosphatase is positive.

3. Inhibition Test

2 drops of 3% W/V tartaric acid is added before the substrate reagent.

Observation
There will be inhibition of the reaction.

C. Lugol's iodine Test

For detection of vaginal or anal epithelial cells.
Macerated stained materials are taken on a glass slides and smeared it. Then put a drop of 5% lugol's iodine solution and a few drops of distilled water and wait for 2 to 3 minutes. Then examine under microscope.

Observation
Chocolate – brown color is developed due to presence of vaginal epithelial cells, which contain large amount of glycogen contents. Glycogen contents and Iodine react together to form the color.

D. Acid Fast Bacilli Test

For detection of smegma bacilli from smegma.

a. Flood the slides with carbol fuchsin solution and heated until stream rises.
b. Then stain for about 20 to 30 minutes. Do not allow to evaporate.
c. Wash with water
d. Differentiate with 20% sulfuric acid until the film is yellow and then was in water.
e. If it becomes pink, repeat the process until it remains yellow when washed.
f. Wash well in water.
g. Treat with 95% alcohol for 2 minutes.

h. Wash again in water.
i. Counter stain with methylene blue (Loeffler's) solution for 30 seconds.
j. Rinse and dry the slides.

Observation
Acid (alcohol) fast organisms are red and other organisms are blue in colour.
Smegma bacilli are almost the same as acid-fast bacilli, but they are thicker than the Tubercular bacilli.
(Smegma bacilli are rod shaped Acid fast bacilli, but thicker than Tubercular bacilli and present in smegma, in consistent for sexual inter course and takes 24 hours for collection.)

E. Ultraviolet (fluorescence) light test for detection of seminal stains from forensic materials

For detection and identification of seminal stain from the stained cloths/materials ultraviolet (fluorescence) light is used. They show a fluorescence of a bluish-white color, which is not specific as other albuminous materials and detergents also fluoresce. Fluorescence depends on choline of semen.

F. Blood for estimation of alcohol and abusive drugs with history of their abuse. Cavett Test

1 ml of blood / urine with 1 ml of saturated solution of potassium carbonate is kept in the base of the Cavett flask. In the hanging cup is put 0.5ml of 0.1N potassium dichromate in 60% V/V sulphuric acid solution. Seal the flask with lubricant and place in an oven at about 50^0C to 70^0C for ½ hour.

Observation
Colour changes from orange to green indicating presence of alcohol either ethanol or methanol.
Confirmation is done by GLC.

Results (from Observation)

This study was conducted in the time period between January 2001 to September 2002 on a total of 207 cases (both victims and accused) who were brought to the Casualty as well as in the Department of Forensic Medicine of the All India Institute of Medical Sciences by the police or others for medical examination in alleged sexual offence cases. A total 90 victims and 117 assailants were examined. Victims were examined by the gynaecologist and accused were examined by forensic expert on call.

Out of 207 sexual offences cases 90(43.47%) were victims and 117(56.53%) were accused . They were brought for medical examination in All India Institute of Medical Sciences by police in 198(95.65%) cases, by Social Worker and husband in 2(0.96%) cases of each, by relative in 1(0.48%) case and cases by parents in 4(1.93%). Out of 207 alleged sexual offence cases including victim and accused only 41 (19.80%) victims and accused brought together for medical examination.

Table 1: Distribution of alleged sexual offence cases brought for medical examination

Brought by	**Total No**	**Percentage (%)**
Police	198	95.65
Parents	4	1.93
Husband	2	0.96
Relatives	1	0.48
Social workers	2	0.96
Total	**207**	**100**

In total 90 victims, 9(10%) victims were examined on the day of the incidence, 13(14.44%) were examined on the 2nd day, 8(8.88%) on 3rd day, 5(5.55%) on the 4th day, 23(25.55%) on 5th to 7th days, 15(16.66%) on the 1st to 2nd week. Between 2nd to 3rd and 3rd to 4th weeks 4(4.44%) victims for each time gap were examined. 9 (10%) victims were examined after four weeks of incidence.

Table 2: Time gap between Incidence and medical examination of assailants

Days / weeks	Total No	Percentage (%)
Same day	9	10
Second day	13	14.44
Third day	8	8.88
Fourth day	5	5.55
5^{th} day – 7^{th} day	23	25.55
1^{st} week–2^{nd} week	15	16.66
2^{nd} week – 3^{rd} week	4	4.44
3^{rd} week – 4^{th} week	4	4.44
> 4^{th} week	9	10
Total	**90**	**100**

Out of 117 cases of assailants, 5(4.27%) were examined on the day of incident in the alleged rape cases while 15(12.82%) were examined on the 3^{rd} and 4^{th} day, 28(23.93%) were on the 5^{th} to 7^{th} day, 27(23.07%) cases on 1^{st} to 2^{nd} week, 12(10.25%) on the 2^{nd} to 3^{rd} week, 8(6.83%) cases on the 3^{rd} to 4^{th} week were examined and 12(10.25%) cases of assailants were examined after 4^{th} week.

Table 3: Age and sex incidence of sexual offences

Age in year	Victims		Accused	Total no.	Percentage (%)
	Female	Male (Sodomy)			
0-5	1	--	--	1	0.48
6-10	5	5	--	10	4.83
11-15	27	3	4	34	16.42
16-20	31	1	29	61	29.46
21-25	1	--	46	47	22.70
26-30	7	--	23	30	14.49
31-35	--	--	3	3	1.49
36-40	2	1	2	5	2.41
41-45	3	--	5	8	3.86
46-50	1	--	--	1	0.48
>50	2	--	5	7	3.38
Total	**80 (38.64%)**	**10 (4.83%)**	**117 (56.52%)**	**207**	**100**

Total 207 cases of sexual offences were seen including victims and accused . Out of 207 cases 117 (56.52%) were assailants and 90 were victims. Among victims 80(38.64%) were female victims and 10(4.83%) were male victims. Maximum number of female victims were of 11-20

year age group while male victims were in 6-15 year age group. Maximum number of assailants were involved in 20-25 year age group.

Table 4: Age distribution of victims of sexual offences

Age in year	Female victim	Male victims (Sodomy)	Total Number	Percentage (%)
0-5	1	--	1	1.11
6-10	5	5	10	11.11
11-15	27	3	30	33.33
16-20	31	1	32	35.55
21-25	1	--	1	1.11
26-30	7	--	7	7.77
31-35	--	--	--	0
36-40	2	1	3	3.33
41-45	3	--	3	3.33
46-50	1	--	1	1.11
>50	2	--	2	2.22
Total	**80 (88.88%)**	**10 (11.11%)**	**90**	**100**

In this study the age of victim was ranging between four years old child to sixty years old woman. In 90 victims, 80 (88.88%) were female victims, 10(11.11%) were male victims. All the male victims were of sodomy. Out of 90 victims, 1(1.11%) was of age group 0-5 year, 10(11.11%) cases were of 6-10 year age group (5 cases of female and male victims), 30(33.33%) cases were of 11-15 year age group, 32 (35.55%) cases were of 16-20 year age group. 15(16.66%) cases examined were of 21-50 year age group while 2 (2.22%) cases were of age group more than 50 years. The maximum (68.88%) of rape victims were of age ranging between 11 to 20 years. Incidentally 31-35 year of age group, no victim was found.

Table 4: Age distribution of the accused for sexual offences

Age in year	Total No	Percentage (%)
0-10	--	0
11-15	4	3.41
16-20	29	24.78
21-25	46	3 9.31
26-30	23	19.56
31-35	3	2.56
36-40	2	1.70
41-45	5	4.27
46-50	--	0
>50	5	4.27
Total	**117**	**100**

The age of accused ranging from 14 years (youngest) to 73 years (oldest). Out of 117 assailants 75(64.10%) were of 16-25 year age group. After 30 years onwards, the assailant involved to sexual offence is reduced. Five (4.27%) were seen in the age group more than 50 year. In this study no assailant was of 46-50 year age.

Table 5: Police Station and incidence of sexual offences

Sl. No	Name of PS	Total No	Percentage (%)
1	Okla Industrial area	32	15.45
2	Kalkaji	23	11.11
3	Sangam Vihar	20	9.66
4	Mehrauli	18	8.69
5	Badarpur	17	8.21
6	New friends colony	17	8.21
7	Malviya Nagar	11	5.31
8	HN Din	11	5.31
9	KM Pur	10	4.83
10	Sri niwas Puri	10	4.83
11	Ambedker Nagar	9	4.34
12	Lajpat Nagar	8	3.86
13	Defence colony	5	2.41
14	Hauz Khas	4	1.93
15	CR Park	3	1.44
16	Greater Kailash	2	0.96
17	Sarita Vihar	2	0.96
18	Lodhi Colony	2	0.96
19	Other PS	3	1.44
Total		**207**	**100**

Other PS :- 1. Sarojini nagar – 1, 2. RK. Poram – 1, 3. New Ashok Nagar-

Out of 207 cases of alleged sexual offence victims and assailants, the highest number of cases 32(15.45%) were brought by the police of Okhla Industrial Area and followed by the Kalkaji 23(11.11%), Sangam Vihar 20(9.66%), Mehrauli 18(8.96%) Badarpur and New Friends Colony 17(8.21%) from each, Malviya Nagar and H.N. Din 11(5.31%) from each, K.M.Pur and Sriniwaspuri 10(4.83%) from each, and as such all of the eighteen police stations under jurisdiction of South District of Delhi brought the cases of sexual offences for medical examination at All India Institute of Medical Sciences.

Other three police station-Sarojini Nagar, R.K. Puram and New Ashok Nagar who are out of jurisdiction of South District of Delhi brought one case each for medical examination.

Table 5: Places of incidence of sexual offences

Place of incidence	Total No	Percentage (%)
Victim's House	37	41.11
Accused House	26	28.88
Relative's House	9	10
Road side / isolated places	7	7.77
Jungle / field	7	7.77
Guest house/ hotel	3	3.41
School / Madarasa	1	1.11
Total	**90**	**100**

In total 90 cases of alleged sexual offence victims, in 37(41.11%) cases incidence occurred in the house of victim, in 26(28.88%) cases in the house of accused, in 9(10%) cases in the house of relative, in 7(7.77%) cases on the road sides or isolated places, in 7(7.77%) cases in jungle or field, in 3(3.41%) cases incidence occurred in Guest House or Hotel and in 1 (1.11%) case incidence occurred in Madarsa.

Table 6: Religion wise distribution of cases of alleged rape victims and assialants

Religion	TotalNoVictims	Percentage (%)	Total No. Assailants	Percentage (%)
Hindu	68	75.55	95	81.19
Muslim	20	22.22	22	18.80
Christian	1	1.11	--	0
Sikh	1	1.11	--	0
Other	--	0	--	0
Total	**90**	**100**	**117**	**100**

Out of 90 cases of alleged sexual offence victims, 68(75.55%) were Hindu, 20(22.22) were Muslim, 1(1.11%) was Christian and 1 (1.11%) was Sikh.
In total number of 117 cases of alleged sexual offence cases of assailants, 95(81.19%) were Hindu, and 22(18.80%) were Muslim. No Christian, Sikh and other religions were found involved in this present study

Age wise and marital status wise distribution of victims
Out of 90 cases of alleged sexual offence victims, 73 (81.11%) victims were unmarried 15(16.66%) were married and 2(2.22%) victims were widow. Maximum unmarried victims i.e. 59(80.82%) were in the age group of 11 to 20 years. Married victims maximum were affected in the age group of 25 to 45 years.

Table 7: Age wise and marital status wise distribution of assailants

Age in year	Unmarried	Married	Total Number	Percentage (%)
0-10	--	--	--	0
11-15	4(100%)	--	4	3.41
16-20	27(92.42%)	1(3.57%)	28	23.93
21-25	47(100%)	--	47	39.31
26-30	19(82.60%)	4(17.23%)	23	19.58
31-35	2(66.66%)	1(33.33%)	3	2.56
36-40	1(50%)	1(50%)	2	4.27
41-45	1(20%)	4(80%)	5	4.27
46-50	--	--	--	0
>50	--	5(100%)	5	4.27
Total	**101 (86.32%)**	**16 (13.67%)**	**117**	**100**

Out of 117 cases of alleged sexual offence assailants, 101(86.32%) were unmarried and 16(13.67%) were married. Out of 117 cases of assailants 74(63.24%) of unmarried cases were in the age group of 16 to 25 years. Among the married assailants, maximum were involved in the age group of 26 to 45 years.

Table 7: Relationship of assailants with victims

Type of relation	Total No	Percentage (%)
Acquaintance	52	44.44
Stranger	22	18.80
Close friend	36	30.76
Neighbor	2	1.70
Between student of same school	2	1.70
Master servant	2	1.70
Teacher student	1	0.85
By lover	-	0
Total	**117**	**100**

In total numbers of 117 assailants of alleged sexual offence cases, 52(44.44%) were having acquaintance with their victims, 22(18.80%) were stranger, 36 (30.76%) were close friends, 2(1.70%) each of neighbour, student of same school and master-servant relationship and 1(0.85%) was teacher-student. But no lover was seen to be involved in the sexual offence in this study.

Table 8: Distribution of types of alleged sexual offences

Type of rape	Total No	Percentage (%)
Forcible rape	23	25.55
Invalid consensual rape	39	43.33
Statutory rape	18	20
Unnatural sexual offence	10	11.11
Attempted rape	--	0
Child molestation	-	0
Total	**90**	**100**

Among 90 cases of alleged sexual offence victims, 23(25.55%) were of forcible rape, 39(43.33%) cases were of invalid consensual, 18 (20%) cases were of statutory rape and 10(11.11%) cases were of unnatural sexual offences. All the 10 unnatural sexual offences were alleged to be of sodomy and all victims were male. There was no case of attempted rape, child molestation in this study. In maximum adult consensual rape cases, assailants had the friendship relationship with their victims and had sexual activities after absconding togather.

Injury in sexual offence victims

Out of 90 of alleged sexual offence cases, 23 (25.55%) victims had some simple to grievous injuries on their bodies. 6 (6.66%) victims had extra genital, 10(11.11%) victims had genital and 7(7.77%) victims had combined genital and extra genital injuries. They were mostly involved in forceful rape.

Inter-religion distribution of alleged sexual offences

Out of 22 Muslim assailants, five (22.72 %) were involved in inter religion sexual offences and out of 95 Hindu assailants 6(6.31%) were involved in inter–religion sexual offences.

State of hymen

Out of 90 cases of sexual offence victims, ruptured hymen was found in 77(85.55%) cases and in maximum cases there was old rupture.

Assailants involved in gang sexual offences

(In this study, out of 207 alleged sexual offence cases 7(7.77%) victims and 18(15.38%) assailants were involved in gang rape. In one case, there were 5 assailants and in another case 3 assailants were involved. Two assailants were involved in each of the rest 5 cases.

Age wise distribution of sodomy victims

Out of 90 victims of alleged sexual assault in this study 10 (11.11%) were found to be victims of sodomy. Out of these 10 cases, 5(50%) were of age

group below 10 years, 3(30%) were between 11 to 15 year of age group and 2(10%) were found above 15 years of age who were sexually assaulted by two young men in the form of gang abuse. The maximum sodomy victims were found in the age group of 6 to 15 year.

Murder victims of alleged sexual offences

During this study 4 (4.44%) victims were found with alleged sexual intercourse and followed by murder. Out of them, the youngest rape murder victim was 6 years old. All these victims were brought in our mortuary for postmortem examination. All the forensic materials were collected, sealed, handed over to police for CFSL examination and one part kept for our laboratory.

Table 9: Literacy status of sexual offence victims

Literacy status	Total No.	Percentage (%)
Illiterate	26	28.88
Class I-Class V (Primary)	30	33.33
Class VI – Class IX (Middle)	22	24.44
Class X-class XII(Secondary)	5	5.55
College standard	7	7.77
Total	**90**	**100**

Out of 90 victims of alleged sexual offences, 26 (28.88%) were totally illiterate, 30(33.33%) victims were literate from class I to class V standard, 22(24.44%) victims from class VI to class IX standard, 5(5.55%) victims from class X to class XI standard, and 7(7.77%) victims were of college standard.

Table 10: Literacy status of assailants

Literacy	Total No.	Percentage (%)
Illiterate	20	17.09
Class I-Class V	29	24.78
Class VI- Class IX	52	44.44
Class X – Class XII	8	6.83
College standard	8	6.83
Total	**117**	**100**

Among the assailants, 20(17.09%) were illiterate, 29(24.78%) were literate from class I to Class V standard, 52(44.44%) from class VI to Class IX standard, 8(6.83%) each from class X to Class XI standard and college standard.

Table 10: Socio-economic status of sexual offence victims

Socioeconomic status	Total No	Percentage (%)
Lower	83	92.22
Middle	6	6.66
Higher	1	1.11
Total	**90**	**100**

Majority of sexual offence victims were in low socio-economic status where monthly income of the victims were below Rs.5000 and in total of 83(92.22%) alleged victims were found in this group. Six (6.66%) victims were in middle socio-economic status where monthly income was Rs.5000 to 15000. 1(1.11%) victim was in high socio-economic status.

Table 11: Socio-economic status of alleged assailants

Socioeconomic status	Total No	Percentage (%)
Low	104	88.88
Middle	11	9.40
High	2	1.70
Total	**117**	**100**

In total 117 assailants, 104(88.88%) were of low socio-economic group in which their monthly income was less than Rs.5000, 11(9.40%) were of middle socio-economic status where monthly income was ranging form Rs.5000 to Rs.15000 per month and 2(1.70%) were of high socio-economic group where monthly income was more than Rs.15000 per month. Both the assailants in high socio-economic group were government employees.

Laboratory Investigations of sexual offence cases

For this study, total 207 alleged sexual offence cases including victims and accused were examined. Out of 207 cases, the forensic materials were collected in 73(81.11%) cases form victims and in 25(21.36%) cases from accused. In rest of the cases materials were not collected, because they were brought by police in casualty after one week or more of the incidence. Collected materials were examined in Laboratory and in 5(5.55%) cases positive results were seen in the material (vagina, undergarment and direct smear were taken on clean glass slides from the swabs) collected from victims and only in 1(0.85%) case the results was found positive in the material (penile swabs) collected from assailant. Penile swabs taken from 25 assailant, positive results for vaginal cells were seen in only in one (0.85%) case. Among the samples of pubic hairs preserved, seminal stain or spermatozoa was detected in none.

Table 12: Results of laboratory tests performed on materials collected from victims of alleged sexual offences

Tests performed	Total no	Positive	Percentage (%)
Microscopic examination of spermatozoa	73	5	5.55
Acid phosphatase test	73	3	3.33
Florence test	73	2	2.22
Barberior's test	73	--	0
Hanging drop preparation	10	-	0

Out of total 90 victims of alleged sexual offence cases, that were examined for this study, forensic materials were collected in 73 (81.11%) cases and tests were performed. On all of the collected samples, microscopic examination for spermatozoa, Acid phosphatase tests, Florence test, Barberio's test were done. The results for spermatozoa were positive in 5(5.55%) cases. The results for Acid phosphatase was found to be positive in 3(3.33%) cases. The results for Florence test were found positive 2(2.22%) cases., Barberio`s test was negative in all the cases. Hanging drop test for motility of sperm was done in 10 cases but results was found to be negative in all the cases.

Table 13: Laboratory tests of assailants

Tests performed	Total no.	Positive	Percentage (%)
Microscopic examination of spermatozoa	25	1	0.85
Acid phosphatase test	25	-	-
Florence test	25	-	-
Barberio's test	25	--	-
Lugol's iodine test	25	1	0.85

Out of 117 alleged assailants cases, forensic materials were collected in 25(21.36%) cases and laboratory tests were performed in Laboratory. Microscopic examination for spermatozoa was done in 25 cases and found positive in one (0.85%) case, Lugol's iodine test was done in 25 cases, found positive only in one (0.85%) case, test for Acid Fast Bacilli was performed in 6 cases and found all negative.

Discussion

Sexual offence is one of the most heinous crimes against women. On the initiative of various social organizations and women welfare organizations,

both legal and administrative steps have been taken by the Government to give protection to women against such sexual crimes. In this study, a total of 207 alleged rape cases of South Delhi including both victims and assailants that were brought to All India Institute of Medical Sciences, New Delhi for medical examination from January 2001 to September 2002. Out of 207 sexual offence cases, 90 (43.47%) were victims and 117 (56.52%) were assailants. Among the victims, 23 (25.55%) cases were of forcible rape, 39 (43.33%) cases were of adult consensual rape, 18 (20%) cases were of statutory rape and 10 (11.11%) cases were of unnatural sexual offences. All the unnatural sexual offences were alleged for sodomy and all were male victims. For medical examination of the rape cases, 198 (95.65%) cases were brought by police (victims and accused).

Religion of victim and assailant

In this study, out of 90 sexual offence victims, 68 (75.55%) were Hindu, 20 (22.22%) were Muslim and each was Christian and Sikh 1. In the case of 117 assailants, 95 (81.19%) were Hindu and 22 (18.80%) were Muslim. Out of 22 Muslim assailants, 5(22.72%) were involved in inter religion sexual offence cases, 6(6.31%) cases of 95 Hindu assailants involved in inter-religion sexual offence cases.

Schiff (1973) found that in his study 52 white and 48 black victims of the 100 women reporting the sexual attacks. However Woodling (1975) reported nearly ninety (90%) reported rapes, as inter-racial rather than intra-racial.

In this study, 7(7.77%) of 90 victims and 18(15.38%) of 117 assailants were involved in group rape cases. Out of 18 assailants of group rape cases, five cases (5.55%) had 2 assailants. One case three assailants and one case five assailants involved in sexual offence.

Wright and West (1981) from Cambridge University, Institute of Criminology, in their evaluation of a comparison of group offences and lone assaults (Rape) and concluded that of the 97 non-white suspects, 40.6% were involved in group attacks, while only 6.9% of 189 white suspects were involved in group attacks, a significant difference.

In the study of Royice B. Evertt and Gordon K. Jimerson review of 117 women case records examined at Oklahoma. The usual victim was single and under the age of 25 years and attacked by a single assailant.

Evrard (1977) revealed the number of assailants in 125 cases. In 105 (83.3%) there was single assailant as compared to 84.61% in this study. In

his study, there were 2 assailants as compared to five (5.55%) in this study. In other cases, there were 3 assailants while in this study there was only one case. In two cases, 4 assailants participated in the sexual offences. There were 5 assailants, who raped a women in one case and also same in our study.

Fimate L (1998) reported in his study that 68.6% Meitei Hindu, 14.0% Muslim, 11.6% and 5.8% non Manipuri were sexual assaulted.

Age of victims

In this study, the age of the victims ranged from between four year old child to sixty years old women. Out of 90 victim of alleged sexual offence cases 80 (88.88%) were female victims and 10(11.11%) were male victims. All the male victims were of sodomy cases. Among the 90 victims, 1(1.11%) was 4 ½ years old female child, 10(11.11%) were of age group of 6-10 years, 30(33.33%) were of age group of 11-15 years, 32 (35.55%) were of age group 16-30 year, 15 (16.66%) were of age group 21-50 years. The maximum numbers (68.88%) of rape victim were found in the age group of 11-20 years age group.

According to Schiff (1973), the youngest victim was 10 year old and oldest a white women of 77 years , raped in her home by a 18 years old Negro boy. The largest age group was from 10-20 years of age and of 44 numbers of victims , 9 women were over 50 years of age, 8 were of 31-40 years age group.

Woodiling (1975) confirmed the median age of victim ranging from 15-24 years. While Evrad (1977) revealed the age of victims, ranged from 2-61 years. A model age was 15 years seems quite striking. Hayman and Lanza (1972) revealed age of victims ranging between fifteen (15) months to 82 years of age.

S. K. Teoh (1979) found all victims were young and below 30 years of age. Twenty (20%) percent of them were less than 10 years of age .

J D Mont et al (2000) reported that majority of victims of sexual assault were of 15-20 years age group.

Fimate L(1998) found in his study that the majority (40.7%) victims of sexual assault cases were in the age group of 13 - 20 year and child victims below 12 year of age 19.8% were affected and no victim above the 50 years.

Bhardaj et al(1995) reported that majority (62%) of sexual victims were in the age group of 11-20 year and children under o-10 year age group were 20%. Sagar et al(1992) reported in his study that the commonest group was 11-20 ie.68.4%.

Age of assailants

For this study, the age of the assailants ranged from 14 years old boy to 73 years old man. The maximum numbers of assailants were seen in the age group of 21 to 25 years i.e. 46(39.31%) in numbers followed by 29(24.78%) numbers in the age group of 16-20 years and 23(19.56%) cases in the age group of 26-35 years. This study shows that the maximum numbers of assailants i.e. 75(64.10%) cases were involved for sexual offences in the age group of 16-25 years. It also show that the youngest assailant was 14 years old boy who was involved in unnatural sexual offence i.e. sodomy with a boy of room mate of the hostel and the oldest assailant was of 73 years old who was involved in sexual intercourse with his maid-servant at his own residence.

Schiff (1973) in his review, found that the maximum numbers of assailants were involved for sexual intercourse in the age group of 17-25 years. The oldest assailant being 43 years old white man, the youngest, the aforementioned 10 years old Negro boy who participated with his two brothers in a sexual assault on a 10 years old Negro .

Evrard (1977) revealed the number of assailants in 125 cases. In 105 (83.3%) there was single assailant as compared to 84.61% in this study.
Richard (1981) concluded that, 65.3% suspects were under 21 year of age, compared with 27.3% of the suspects in individual assaults.

Heyman and Lanza (1972) found in their studies that, the aggressors showed the expected age distribution with few children, 12 years of age, 16% adolescents, 44% young adult, 39% were 25 years of age of older. The sex section states that practically all aggressors apprehended were under 30 years of age.

Sagar MS et al (1992) reported that in his study that 64% assailants were in the age group of 16-25 year and no accused was seen after 40 year of age .

Marital status

In this study, 73 (81.11%) victims were unmarried, while 15(16.66%) victims were married who became victimized for alleged rape cases. In 2(2.22%) cases victims were widow. Maximum i.e. 59 (80.82%) unmarried

alleged victims were in the age group of 11-20 years. But in the married women, the 26-45 years of age group were affected much.

Schiff (1973), found that 48 victims were single, 19 married, 10 widowed, and the remaining 33 either divorced or separated and 19 women were married at the time of attack. The author had a similar conclusion that, the single, separated, divorced, or widowed female i.e.without a mate, is five times more prone to sexual assault than the married and having the mate with her.

In contrast with the findings of the author, 49 victims, including 10 unmarried women had given birth to one or more children . No unmarried girl, who delivered child following rape was noticed .

Woodling (1975) stated that 80% of all sexually assaulted victims were single. S. K. Toeh (1979) reported 7 married victims out of 45, who specifically pointed out that 17(44.6%) cases out of 38 unmarried victims have had intact hymen at the time of examination.

In this study, out of 117 assailants, 101(86.32%) assailants were unmarried, while only 16(13.67%) assailants were married who were involved in sexual offences. Among the unmarried assailants, maximum i.e. 74(63.24%) assailants were in the age group of 16-25 years.
D J Mont (2000) in his study reported that majority (65.2%) of victims of sexual assaults were unmarried. Fimate L (1998) found in his study that 57.0% victims were single22.0 married and 1.2%, divorce.

Time elapsed between incident and medical examination

In this study, only 9 (10%) victims were examined on the same day of the incident, while 13 (14.44%) victims were seen on second day, 8 (8.88%) victims were examined on third day and maximum victims of alleged rape cases were examined after one week and onwards or even after month. But in case of 117 assailants, 5(4.27%) were examined on the same day of incidence 15 (12.82%) were on the second day and 5 (4.27%) were examined on the 3rd and 4th days. But maximum numbers were examined after one week and onwards or even after months. Some victims and assailants were also examined even after one year.

Evrard (1977) stated that 96 victims (73%) were examined within 24 hours of sexual assault, while 16 (12.7%) were not seen until at least 3 days had been passed. 11victims were presented by themselves for medical examination more than 7 days after the attack.

D J Mont (2000) in his study reported that 40.1% of victims of sexual assault were presented to hospital 2-6 hours after the incident.

Place of incidence

In this study, 90 victims of alleged sexual offence cases were examined, among which 37 (41.11%) were raped at the house of victims, 26 (28.88%) were raped at house of accused, 9(10%) were at assailants relatives house, 7(7.77%) cases were raped at road sides/isolation places and the same number of cases were at jungle/field, 3(3.41%) were at guest house or hotel and 1(1.11%) case was assaulted at madarasa by his teacher who had the unnatural sexual offence through anus.

The findings of our study, in contrast with the findings of A. F. Schiff (1973) who specifically pointed out that, the automobile, probably because of its availability, mobility and service as a private, fairly comfortable space, continues to be favorite scene of the crime, figuring in 27 cases. In the past report, the car was used in 37% of the attacks. The second most popular site was victims own dwelling (26%). 9% accused used the assailant's home or apartment. The remaining 38% transpired in empty school rooms, abandoned houses, hotel rooms and open spaces such as beaches at night, isolated vacant lots, behind buildings, in parks, in a dark alley, and one instance, behind a church.

Evrard (1977), stated that 39% of sexual assault occurred in victims or assailants house. In the young adult group 22% of assault occurred in the victims home and 7.17% in home of male friend or acquaintances.

Teoh (1979), in his review of 45 cases revealed that twelve incidents, occurred in the home, while 7 incidents in neighbouring house, 4 occurred in a party and 7 incidents happened at out door.

Victim-assailant relationship

In this study, the maximum number of assailants i.e. 52 (44.44%) were having acquaintance with their victims followed by 36 (30.76%) assailants had the relation with their victims as close friend, 22 (18.80%) assailants were completely strangers to their victims, 2 (1.70%) assailants neighbors, 2 (1.70%) were students of same school, 2 (1.70%) were having master-servant relation with their victims. One (0.85%) case was teacher-student relation with the victim. It is interestingly that no lover was involved in sexual offences in present study

According to Teoh (1979), the majority of assailants were known to the victim i.e. out of 45 were related, 8 were neighbours and other 8 were friends. Another 5 of the accused were boy friends , ten were strangers . In another 5 cases , four of accused had been alleged to have raped 2 victims each .

Evrard (1977), reported that majority of assaults in the pre-pubertal groups were by relatives (50%) or friends (33%). D J Mont (2000) reported that in 49.2% of cases the assailant was stranger or known for less than 24 hours. Fimate L(1998) found that 69.7% were aquaintaince, 25.6% stranger and 4.7% just known by face.

Weapon used

Schiff (1973) stated that in 62% of cases assailant depended upon threats. From 70-100% of all victims were threatened with death or great bodily harm, as noticed by Woodling(1977). In their studies 50-63% fought their assailant and offered significant physical resistance.

Evrard (1977), revealed that co-ercine force was used by 38 aggressors (30%), 23 threatened the victim with sadly harm or death and 7 used knife, 6 a gun and another weapons to intimidate the victim.

Wright and West (!981) reported that more victims in individual attacks were verbally threatened, while in group attacks, more were punched or kicked.

However, the author found facts as follows: 6 (21.4%) victims surrendered to their assailants as a result of threat to kill and violence. While 3 (10.7%) victims each surrendered due to genuine fear of death, grave hunt or by bare threats only. In one (3.5%) case knife was used with oral threat to life.

The findings in this study are in contrast with the above authors, the assailants of forcible rape has not used such weapons during committing offences, but they applied reasonable oral threats and some extents of physical force. But in suspected rape-murder cases i.e. 4 (4.44%) victims, in 1 (1.11%) case produced multiple stab-wound with sharp knife, 1 (1.11%) case was manual strangulated and in another 1 (1.11%) case ligature strangulation was done.

Alcoholism and alleged rape cases

In this present study, none of the assailants and victims of alleged rape cases had given the history of consumption of alcohol or drug addiction during committing of sexual offences.

In the observation of Schiff (1973), alcohol consumption occurred frequently among the victims of sexual assault and their assailants.

Evrard (1977) , historically revealed drug usage by victims, within 24 hours prior to assault, in 36 patients. Thirty patients admitted using alcohol, while two had used marijuana, two among them used combinations of drugs and two heroin shortly before the event.

Wright and West(1981) in their comparison study of group and lone assaults observed that the victims in group offences were more likely to have been drinking before hand and more likely to sustain minor injuries.
D J Mont (2000) in his study reported that 41.7% of victims have consumed alcohol.

Pregnancy of alleged sexual offence victims

In our study, except 1 (1.11%) case there was no reported case for pregnancy following rape of alleged rape victims. The victim, who was pregnant following rape , was brought by her parents to AIIMS Casualty with complains of pain in abdomen. After careful per abdomen examination, she was found pregnant and started labour pain. She then delivered a full term male baby.

Schiff (1973) had given an alarming and contrast results to this study. In his study 49 victims, including 10 unmarried women had given birth to one or more children (up to 8).

Evrard (1977) reported good medical outcome since no pregnancy resulted in his cases.

Evertt and Jimerson (!977) in his review of 117 women case records, found five patients became pregnant at the time of assault. No patient was known to have become pregnant as a result of rape.

Voigt(1972) from Denmark reported that out of 387 cases, 8 women were pregnant at the time of examination, six (6) of them presumably as a result of the sexual offence. Teoh(1979) found 5 patients having evidence of pregnancy at the time of examination.

Medical examination of hymen of victims

In this study 77 (85.55%) alleged sexual offence victims out of 90 cases, including married and unmarried were found having rupture hymen at multiple sites and all were having old tears, while in13 (14.44%) victims hymen remained intact and all were unmarried. No fresh hymen rupture was seen in this study.

Voigt (1972) from Denmark reported that, in 39% out of 387 cases, hymen were annular with a regular shape (lobulate or fimbriate) were seen. A circumference of 9 to 10 cm was regarded as the minimum to permit coitus was revealed where there was no evidence of fresh or absolute proof of previous hymen rupture. From above findings, it can be concluded that, sexual intercourse is not necessarily accompanied by defloration.

Injuries on victims

For our study, 6 (6.66%) victims of alleged sexual offence case had extra genital injuries, 10 (11.11%) victims had genital injuries. In 7 (7.77%) victims genital and extra genital injuries were found and in 77 (85.55%) victims had old rupture of hymen. But remaining 13 (14.44%) victims had no such genital or extra genital or even hymen rupture.

Schiff (1973) reported sixty persons having no injuries, with 30% having simple extra genital injuries, 1% only had genital as well as extra genital injuries.

Evarard (1977)reported in his study that, 15 women sustained mild physical injuries, 46 moderate and four had severe injuries.

Heyman and Lanza (1971) reported in their study that 106 had major physical injuries, or emotional trauma. 3 children and 12 adult required hospitalization. Ten (10) children along with 48 adults required emergency room treatment. There were 20 severe vaginal and vagino perineal lacerations and 199 were having minor genital injuries.

But in this study no victims required hospitalization for the genital or extra genital injuries. Interestingly in many cases, there were medical evidence of physical injury consistent with the allegations. This observation is supported by Hyman and Lanza in several hundred cases.

Evertt and Jimerson (1977) reported serious physical injury in 15 patients out of 117 women case records while 60 received minor injuries, 6 children required hospitalization for reparative surgery. Only one patient required

hospitalization for severe emotional stress. According to Voigt from Denmark severe injury was seen only in one case i.e. in 0.5% of all female over the age of 15 years. Attempted strangulation was reported in 53 cases, marks of grip were found on the neck in 38 cases, 12 showed the petechial haemorrhages in the conjunctivae.

However in this present study strangulation was done in two cases. One case by manual strangulation and in other case it was by ligature strangulation.
Teoh (1979) reported that out of 45 victims who were examined, less than half (20) had incidence of physical injuries, 14 sustained some trauma in vagina, the most common were introital bruises and congestion was found in seven cases.

D J Mont (2000) reported that in 49.2% of cases physical resistance was offered by the victim. Signs of use of physical force was seen in 52.9% of cases. Injuries were seen on the body of victim in 64.2% of cases. 3.2% of victims were hospitalized.

Laboratory reports

In this study, forensic materials were collected from 73 (81.11%) cases of victims, 25 (21.36%) cases of assailants. Out of 73 victims, 5 (5.55%) cases were found to be positive for spermatozoa. In 3 (3.33%) cases acid phosphatase test was positive and in 2 (2.22%) cases Florence test was found to be positive. But in assailants i.e. 117 cases only 1 (0.85%) case was found positive for spermatozoa and vaginal epithelial cells positive from the penile swabs.

Schiff (1973) reported that identification of spermatozoa in the vagina was made in 72% of the cases, while acid phosphatase was positive in 19% of the cases. He also stated that the discovery of spermatozoa anywhere in the female genital tract is not prima facie evidence that a crime had been committed and on the other hand, the absence of spermatozoa is not proof positive that a crime has not been committed. Spermatozoa could be absent because of other causes such as childhood diseases, excessive sexual intercourse, assailant having vasectomy operation and also lapse between – incident and examination of the victim or non ejaculation by male during offence.

Evrard (1977) reported that he carried out sperm and acid phsophatase tests in 97 (77%) cases, while sperm was found in 40 (31.7%) and acid phosphatase was positive in 35 (27.8%) cases. It is difficult to explain, except through laboratory error, why 5 patients were sperm positive and acid phosphatase negative. He also performed VDRL test of 89 (70.6%)

cases, all came negative, pregnancy test was performed at initial examination and was negative on 54 women and found positive in one patient only .

Voigt(1973) in his study reported that motile sperms were observed up to 3-4 hours and immotile sperms up to 66 hours after coitus.

In the study of Teoh (1979) 5 specimens showed presence of spermatozoa.
Study of Willott and Allard (1982) showed the longest time after intercourse that spermatozoa have been found on a total of 2410 case work swabs are as follows :

Internal vaginal swabs	120 hours
External vaginal swabs	120 hours
Rectal swabs	65 hours
Anal swabs	4 hours
Oral swabs	6 hours (9 hours on lips)

Spermatozoa were found on a cervical swabs taken after 179 hours (7½ days) of sexual intercourse.

Davies and Wilson (1974) studies showed that the persistence of spermatozoa, seminal acid phosphatase, choline and seminal blood group antigens in the human vagina after sexual intercourse had been studied and following results were obtained.

Spermatozoa were usually found up to 3 days after intercourse. Smears without spermatozoa were obtained from swabs taken as early as 28 hours, but remained rare often until 2 days after intercourse.

Seminal acid phosphatase sometimes remained detectable up to 3 days after sexual intercourse. The test was most useful on swabs taken within one day and rarely useful after 2 days.

Choline was usually only detectable on swabs taken within 1 day of sexual intercourse and even within this time many negative results were obtained. The probability of positive results declined swiftly after 14 hours.

Seminal blood group antigens were only detected on swabs taken within 48 hours of intercourse. The changes of obtaining a positive result decreased swiftly within an increasing time interval after intercourse. It has been noticed that there is an alarming rise in the incidence of sexual offences in Delhi. Many cases go unreported, because of the social stigma attached to them

Conclusions

1. A total of 207 cases (both victims and accused) were examined between January 2001 to September 2002,who were brought by the police to the AIIMS Casualty for medical examination, allegedly involved in various types of sexual offences.
2. Out of these 90 (43.47%) were victims and 117(56.53%) were accused.
3. Out of 18 police stations falling in the Jurisdiction of South Delhi maximum incidence were seen in Okhla Industrial Area.
4. Majority of alleged Sexual Offence cases 198 (95.65%) were brought by the police for medical examination.
5. Victim and accused were brought together for medical examination in 41(19.80%) cases.
6. Maximum number of victims (25.55%) brought for medical examination 5-7 days after the incidence followed by 7-14 days (6.66%).
7. Maximum number of assailants (47%) brought for medical examination 5-14 days after the incidence.
8. Maximum numbers i.e. 62(68.88%) of alleged rape victims were in the age group between 11 to 20 year.
9. In total 90 alleged sexual offence victims, 80 (88.88%) were female victims and 10 (11.11%) were male victims.
10. Maximum number (64.10%)of assailants were of the age group of 16 to 25 year.
11. Maximum incidence of sexual offences occurred in victim's houses (41.11%) followed by in assailant's houses(28.88%).
12. Maximum victims were Hindu (75.55%). Others were Muslim(22.22%) Christian(11.11%) and Sikh(11.11%).
13. Among the assailants involved in alleged sexual offence cases 81.19% were Hindu, 18.80% were Muslim.
14. 22.72%of Muslim assailants and 6.31%of Hindu assailants were involved in the inter- religion sexual offences.
15. 28.88% of victims were illiterate, 33.33% of victims were literate up to class V standard.
16. 92.22% of victims and 88.88% of assailants were from low socio-economic group.
17. In relation of assailants with their victims, 44.44% were having acquaintance and 18.80% were complete strangers.
18. 25.55% victims were involved in forcible rape, 43.33% were involved in consensual rape and 11.11% were involved in unnatural sexual offences and unnatural sexual victims were all male victims of sodomy cases.
19. Only 25.55% victims of alleged rape cases had some simple to grievous injuries.

20. In 85.55% of rape victims rupture of hymen was seen at multiple sites, but all were old ruptures.
21. Alcohol consumption was not reported in the present study.
22. One (1.11%) victim of alleged rape case became pregnant following rape and delivered a full term male baby in the AIIMS casualty.
23. Forensic materials were collected from victims in 81.11% cases. The results were positive in 5.55% of cases. Forensic materials were collected from assailant in 21.36% cases. The results were positive in 0.85% of cases.
24. In Forensic materials collected from victims, spermatozoa were found positive in 5.55% cases, acid phosphatase test was positive in 3.33% cases, and Florence's test was found positive in 2.22% cases.
25. In Forensic materials collected from assailants, spermatozoa was found positive in 10.85% cases and in one case vaginal epithelial cells were found positive.
26. The single, separated, divorced, or widowed female i.e. without a mate, is five times more prone to sexual assault than the married and having the mate with her.

Declaration

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ORIGINAL ARTICLE

An observation of histology changes with various age of skin injury

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Abstract

262 skin samples were obtained from 95 forensic cases of various injuries (abrasion, laceration and contusion). The cases comprised of 87 males and 8 female corpses which showed no sign of putrefaction. The post infliction injury time ranges from less than 1 hour to more than 72 hours. Our overall observation revealed the earliest appearance of various inflammatory cells and fibroblasts is in the following order: neutrophils, macrophages, fibroblasts, and lymphocytes at 35 minutes, 1 hour 10 minutes, 6 hours 45 minutes and 21 hours 55 minutes respectively. The authors feel this finding can be used as a general guide for forensic cases in estimating the minimum age of skin injury (human) from histopathology examination.

Keywords: Skin injury, antemortem wound, fibroblast, macrophage, neutrophil

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Introduction

In forensic pathology, it is very crucial to determine either the injuries sustained by a victim are inflicted during life or after death. This is important in helping the forensic pathologist to come to an opinion regarding the cause and manner of death (Ohshima 2000).

Wounds on the skin involve the body surface. The estimation of time interval between the infliction of an injury and death is based on the evolution of human skin lesions over a period of time base on normal wound healing process.

Good observation and interpretation of the injuries found on a victim using both macroscopic and microscopic examinations by the forensic pathologist will help the court to carry out justice for both the victim and the accused.

Injuries sustained by an individual during life will initiate an inflammatory response which is proportional to the magnitude of tissue offense in order to restore the normal body function (Hernandez-Cueto et. al. 2000). The series of events in response to the initial injury generally follow the definite order. Histological estimation of the age of injuries is based on the morphology of the various stages of wound healing.

Each phase of healing process is characterized by chronological events that tend to overlap but are sufficiently distinct and can be demonstrated by either conventional histology, enzyme histochemistry or immunohistology techniques.

The accuracy of morphological wound age estimation depends on the number of evaluable parameters providing reliable information on the post infliction interval base on their earliest, regular or latest appearances during the wound healing process (Betz 1995). If a parameter occurs regularly and consistently within a certain period of time, failure to demonstrate it points to a wound age of less or more than the corresponding time interval already established in published series.

Robertson and Hodge did a study on skin abrasions in 71 cases of death due to accidental injury in 1971 which showed that polymorphonuclear cells were observed in the wounds between 2 – 8 hours post infliction. In an animal study using male albino rats done by Ali et. al. in 1988, white blood cells were observed in the post mortem wounds taken after 4 hours of chemotactic agent injected into the abdominal skin. In another animal study using pig by Ordman and Gillman in 1966, polymorphonuclear cells were

observed in the wounds 48 hours post infliction. Thereafter, mononuclear cells will predominate.

The cell infiltrates are observed in the background of normal skin histology. In normal skin, cells of epidermis are keratinocytes, melanocytes and Langerhans cells, whereby cells of the dermis are fibroblasts and mast cells (Walter and Gundula 1990).

In injury after somatic death, margination and a limited emigration of leucocytes may occur. Marked cellular exudation and reactive changes in the tissue cells are seen in ante mortem wounds only.

However, the absence of tissue reaction doesn't necessary indicate post mortem origin of wounds due to several factors as described below:

- victim dies soon after infliction of injury,
- in small wounds the degree of cellular injury may not be sufficient to cause emigration of leucocytes, or there may be resolution of the reaction,
- the circulatory failure occurring in severe injuries may interfere with the normal reaction.

The intensity of local reaction to an injury depends on:

- severity of injury,
- vascularity of the injured tissue,
- presence or absence of infection or foreign body due to which the age of the ante mortem wound cant be determined within narrow limits.

In this study, type of wound examined will be focused only on wounds sustained by blunt force trauma which are bruise, abrasion and laceration. In addition, understanding of normal human skin anatomy and histology is important in assisting the interpretation of result.

Materials and methods

This is a cross sectional descriptive study on all the medico-legal post mortem cases with trauma, attended at the Hospital Kuala Lumpur from the 1st January 2006 until 30th November 2006.

This study was done to determine the age of skin injuries in post mortem cases by using histological parameters i.e. the inflammatory cells and fibroblasts. The differences in cellular inflammatory response between

bruise, laceration and abrasion is compared using both conventional and immunostaining methods.

The cases with abrasions, lacerations and bruises were sampled. The skin injuries of interest were photographed. Skin biopsies were taken from lacerations, abrasions and bruises with normal control of unaffected skin. Fusiform shape excisional or incisional biopsy with 3:1 length to width ratio which is 1.5 x 0.5 cm and 30^0 angles at the ends were taken. The depth of the skin biopsy includes the subcutaneous fat tissue (Bland KI 2000). These biopsies were preserved with 10% formalin overnight before sending to histopathology laboratory for routine H&E staining (Robertson and Hodge 1972). All samples undergo a panel of special staining and immunostains. Special stains used were Prussian–Blue and Pearl's stains to detect erythrophages, siderophages and haemosiderin laden macrophages, Giemsa stain for eosinophil granulocytes (Betz 1994) and Masson Trichrome for collagen. Immunostain which were antibody CD 3 to detect T cells (Betz 1994) and CD 20 to detect B cells were used in selected cases.

Exclusion criteria are malnutrition, cases on cytotoxic/cytostatic drugs, cases on warfarin medication, cases with malignancy or severe diabetes, bleeding disorder and decomposed cases (Betz 1994).

Results

262 skin samples were obtained from 95 cases. The cases comprised of 87 males and 8 female corpses without sign of putrefaction. The post mortem interval was less than 2 days. The duration of post infliction injury ranges from less than 1 hour to more than 72 hours.

The corpses were of wide ranges which include Malay, Chinese, Indian, Indonesian, Bangladeshi, Myanmar and Sabahan. The cause of death ranges from motor vehicle fatalities, industrial accident, transportation injury due to train, fall from height, homicide of sharp and blunt weapons as in assault/fighting cases and blunt injury to the head. In addition, another two cases were of suicide in nature with one hanging case. The majority of cases were due to motor vehicle fatalities and fall from height.

The age of decedents varies from 2 years old to 57 years of age. All the decedents were apparently well and healthy. The history was retrieved from relatives and friends.

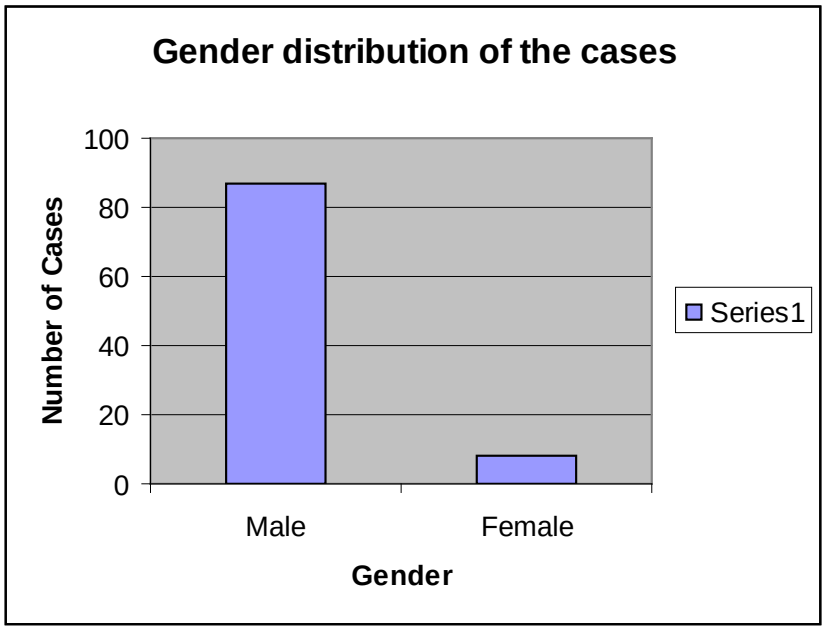

Fig. 1 : Gender distribution of the cases (male 87; female 8)

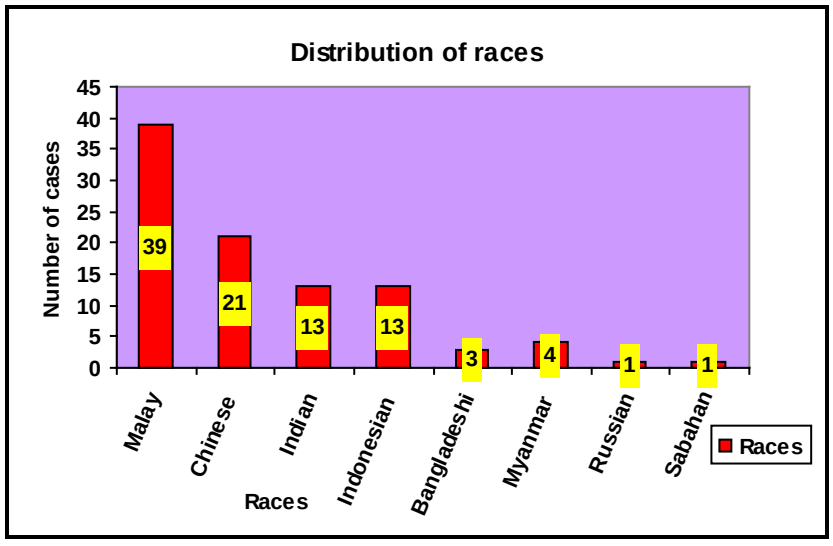

Fig. 2 : Distribution of different races in the study.

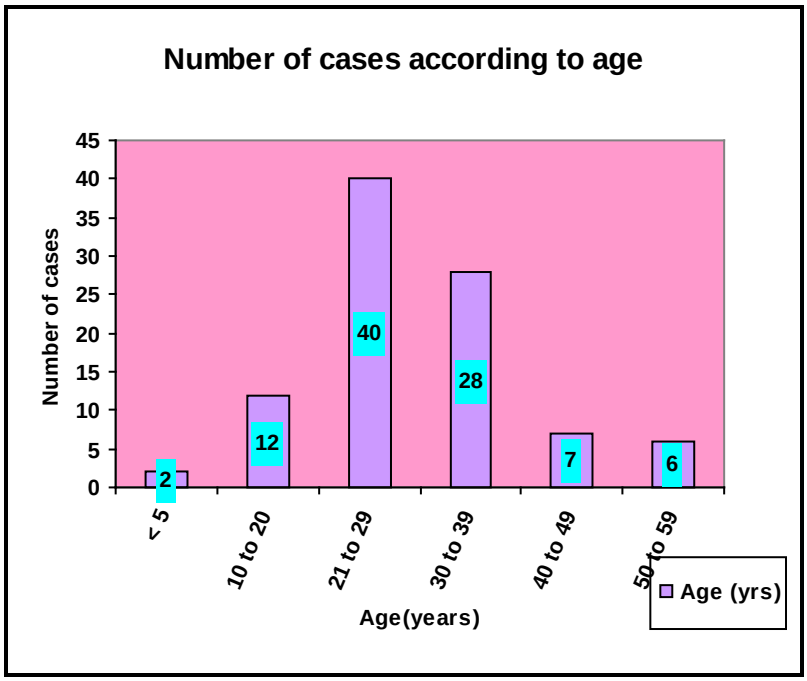

Fig. 3: The number of cases in relation to the age.

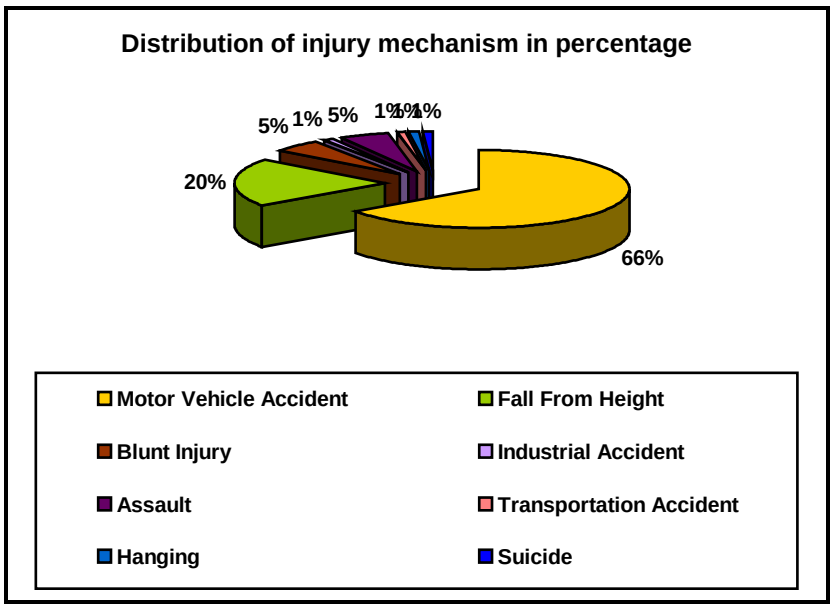

Fig. 4: The distribution of injury mechanism in percentage.

Table 1.0
Overall results on all the 95 cases for the lacerations, bruises and abrasions including controls using histology parameters

CASE	Lymphocyte	Eosinophils	Fibroblast	Erythrophage	Siderophage	Lipophage	Macrophage	Neutrophils
1A	-	-	-	-	-	-	++	++
1B	-	-	-	-	-	-	++	++
1C	-	-	-	-	-	-	+	+
2A	-	-	-	-	-	-	-	-
2B	-	-	-	-	-	-	-	-
2C	-	-	-	-	-	-	-	-
3A	-	-	-	-	-	-	-	-
3B	-	-	-	-	-	-	-	-
3C	-	-	-	-	-	-	-	-
4A	-	-	-	-	-	-	-	-
4C	-	-	-	-	-	-	-	-
5B	-	-	+	-	-	-	++	+++
6A	-	-	-	-	-	-	-	-
6B	-	-	-	-	-	-	-	-
6C	-	-	-	-	-	-	-	-
7A	-	-	-	-	-	-	-	-
7B	-	-	-	-	-	-	-	-
7C	-	-	-	-	-	-	-	-
8A	-	-	-	-	-	-	-	-
8B	-	-	-	-	-	-	-	-
8C	-	-	-	-	-	-	-	-
9A	-	-	-	-	-	-	-	-
9B	-	-	-	-	-	-	-	-
9C	-	-	-	-	-	-	-	-
10A	-	-	-	-	-	-	-	-
10B	-	-	-	-	-	-	-	-
10C	-	-	-	-	-	-	-	-
11A	-	-	-	-	-	-	-	-
11A1	-	-	-	-	-	-	-	-
11B	-	-	-	-	-	-	-	-
11C	-	-	-	-	-	-	-	-
12B1	-	-	-	-	-	-	-	-

CASE	Lymphocyte	Eosinophils	Fibroblast	Erythrophage	Siderophage	Lipophage	Macrophage	Neutrophils
12B2	-	-	-	-	-	-	-	-
13B	-	-	-	-	-	-	-	-
13C	-	-	-	-	-	-	-	-
14A	-	-	-	-	-	-	-	-
15A	-	-	-	-	-	-	-	-
15B	-	-	-	-	-	-	-	-
15C	-	-	-	-	-	-	-	-
16A	-	-	-	-	-	-	-	-
16B	-	-	-	-	-	-	-	-
16C	-	-	-	-	-	-	-	-
17A	-	-	-	-	-	-	-	-
17B	-	-	-	-	-	-	-	-
17C	-	-	-	-	-	-	-	-
18A	-	-	-	-	-	-	-	-
18B	-	-	-	-	-	-	-	-
18C	-	-	-	-	-	-	-	-
19A	-	-	-	-	-	-	-	-
19B	-	-	-	-	-	-	-	-
19C	-	-	-	-	-	-	-	-
20B	-	-	-	-	-	-	-	-
20C	-	-	-	-	-	-	-	-
21A	-	-	-	-	-	-	-	-
21C	-	-	-	-	-	-	-	-
22A	-	-	-	-	-	-	-	-
22B	-	-	-	-	-	-	-	-
22C	-	-	-	-	-	-	-	-
23A	-	-	-	-	-	-	-	+
23B	-	-	-	-	-	-	-	-
23C	-	-	-	-	-	-	-	-
24B	-	-	-	-	-	-	-	-
24C	-	-	-	-	-	-	-	-
25B	-	-	-	-	-	-	-	+++
25C	-	-	-	-	-	-	-	+++
26A	-	-	-	-	-	-	++	+++
26B	-	-	-	-	-	-	++	+++
26C	-	-	-	-	-	-	-	-

CASE	Lymphocyte	Eosinophils	Fibroblast	Erythrophage	Siderophage	Lipophage	Macrophage	Neutrophils
27A	-	-	-	-	-	-	-	-
27B	-	-	-	-	-	-	-	-
27C	-	-	-	-	-	-	-	-
28A	-	-	-	-	-	-	-	-
28B	-	-	-	-	-	-	-	-
28C	-	-	-	-	-	-	-	-
29A	-	-	-	-	-	-	+	++
29B	-	-	-	-	-	-	+	++
30B	+	-	+	-	-	-	+++	++
30C	+	-	+	-	-	-	+++	++
31A	-	-	-	-	-	-	-	-
31B	-	-	-	-	-	-	-	-
31C	-	-	-	-	-	-	-	-
30B	+	-	+	-	-	-	+++	++
30C	+	-	+	-	-	-	+++	++
31A	-	-	-	-	-	-	-	-
31B	-	-	-	-	-	-	-	-
31C	-	-	-	-	-	-	-	-
32A	+	-	++	-	-	-	++	+
32B	+	-	+	-		-	++	+
32C	+	-	+	-	-	-	++	+
33A	-	-	-	-	-	-	-	-
33A1	-	-	-				-	-
33B	-	-	-	-		-	-	-
33C	-	-	-	-	-	-	-	-
34A	-	-	-	-	-	-	-	-
34B	-	-	-	-		-	-	-
34C	-	-	-	-	-	-	-	-
35A	-	-	-	-	-	-	-	-
35B	-	-	-	-	-	-	-	-
35C	-	-	-	-	-	-	-	-
36A	-	-	-	-	-	-	-	-
36B	-	-	-	-	-	-	-	-
36C	-	-	-	-	-	-	-	-
37A	-	-	-	-	-	-	-	-
37B	-	-	-	-	-	-	-	-

CASE	Lymphocyte	Eosinophils	Fibroblast	Erythrophage	Siderophage	Lipophage	Macrophage	Neutrophils
37B1	-	-	-	-	-	-	-	-
37C	-	-	-	-	-	-	-	-
38C	-	-	-	-	-	-	-	-
39A	-	-	-	-	-	-	-	-
39B	-	-	-	-	-	-	-	-
39C	-	-	-	-	-	-	-	-
40A	-	-	-	-	-	-	-	-
40B	-	-	-	-	-	-	-	-
40C	-	-	-	-	-	-	-	-
41A	-	-	-	-	-	-	-	+++
41B	-	-	-	-	-	-	-	+++
41C	-	-	-	-	-	-	-	+++
42A	-	-	-	-	-	-	+	+++
42B	-	-	-	-	-	-	+	+++
42C	-	-	-	-	-	-	+	+++
43B	-	-	-	-	-	-	-	-
43B1	-	-	-	-	-	-	-	-
43C	-	-	-	-	-	-	-	-
44A	-	-	-	-	-	-	-	-
44B	-	-	-	-	-	-	-	-
44C	-	-	-	-	-	-	-	-
45A	-	-	-	-	-	-	-	-
45B	-	-	-	-	-	-	-	-
45C	-	-	-	-	-	-	-	-
46B	+	-	-	-	+	+	++	+++
46C	-	-	-	-	-	-	-	-
47A	-	-	-	-	-	-	-	+++
47B	-	-	-	-	-	-	-	+++
47C	-	-	-	-	-	-	-	+++
48A	-	-	-	-	-	-	-	-
48B	+	-	+	+	-	-	+++	++
48C	-	-	-	-	-	-	-	-
49A	-	-	-	-	-	-	-	-
49B	-	-	-	-	-	-	-	-
49C	-	-	-	-	-	-	-	-
50B	-	-	-	-	-	-	++	+++

CASE	Lymphocyte	Eosinophils	Fibroblast	Erythrophage	Siderophage	Lipophage	Macrophage	Neutrophils
52C	-	-	-	-	-	-	-	-
53A	-	-	-	-	-	-	-	+++
53B1	-	-	-	-	-	-	-	++
53C	-	-	-	-	-	-	-	++
54A	-	-	-	-	-	-	-	-
54B	-	-	-	-	-	-	-	-
54C	-	-	-	-	-	-	-	-
55A	-	-	-	-	-	-	-	+++
55B	-	-	-	-	-	-	-	+++
55C	-	-	-	-	-	-	-	+++
56A	-	-	-	-	-	-	+	+++
56B	-	-	-	-	-	-	++	+++
56C	-	-	-	-	-	-	+	+++
57A	-	-	-	-	-	-	-	-
57B	-	-	-	-	-	-	-	-
57C	-	-	-	-	-	-	-	-
58A	-	-	-	-	-	-	-	-
58B	-	-	-	-	-	-	-	-
58C	-	-	-	-	-	-	-	-
59B	-	-	-	-	-	-	-	-
59C	-	-	-	-	-	-	-	-
60B	+	-	-	-	-	-	++	+++
61A	-	-	-	-	-	-	-	-
61B	-	-	-	-	-	-	-	-
61C	-	-	-	-	-	-	-	-
62A	-	-	-	-	-	-	-	-
62B	-	-	-	-	-	-	-	-
62C	-	-	-	-	-	-	-	-
63A	-	-	-	-	-	-	-	-
63B	-	-	-	-	-	-	-	-
63C	-	-	-	-	-	-	-	-
64A	-	-	-	-	-	-	-	-
64B	-	-	-	-	-	-	-	-
64C	-	-	-	-	-	-	-	-
65A	-	-	-	-	-	-	-	-
65B	-	-	-	-	-	-	-	-

CASE	Lymphocyte	Eosinophils	Fibroblast	Erythrophage	Siderophage	Lipophage	Macrophage	Neutrophils
65C	-	-	-	-	-	-	-	-
66A	-	-	-	-	-	-	-	-
66B	-	-	-	-	-	-	-	-
66C	-	-	-	-	-	-	-	-
67A	-	-	-	-	-	-	-	-
67B	-	-	-	-	-	-	-	-
67C	-	-	-	-	-	-	-	-
68A	-	-	-	-	-	-	-	-
68B	-	-	-	-	-	-	-	-
68C	-	-	-	-	-	-	-	-
69A	-	-	-	-	-	-	-	-
69B	-	-	-	-	-	-	-	-
69C	-	-	-	-	-	-	-	-
70A	-	-	-	-	-	-	-	-
70B	-	-	-	-	-	-	-	-
70C	-	-	-	-	-	-	-	-
71B	+	-	+	-	-	-	++	+++
71C	-	-	+	-	-	-	++	+++
72A	-	-	-	-	-	-	-	-
72B	-	-	-	-	-	-	-	-
72C	-	-	-	-	-	-	-	-
73A	-	-	-	-	-	-	-	-
73B	-	-	-	-	-	-	-	-
73C	-	-	-	-	-	-	-	-
74A	-	-	-	-	-	-	-	-
74B	-	-	-	-	-	-	-	-
74C	-	-	-	-	-	-	-	-
75A	-	-	-	-	-	-	-	+++
75B	-	-	-	-	-	-	-	++
75C	-	-	-	-	-	-	-	++
76B	-	-	-	-	-	-	-	-
76C	-	-	-	-	-	-	-	-
77B	-	-	-	-	-	-	+	+++
78A	-	-	-	-	-	-	-	-
78B	-	-	-	-	-	-	-	-
78C	-	-	-	-	-	-	-	-

CASE	Lymphocyte	Eosinophils	Fibroblast	Erythrophage	Siderophage	Lipophage	Macrophage	Neutrophils
79A	-	-	-	-	-	-	-	-
79B	-	-	-	-	-	-	-	-
79C	-	-	-	-	-	-	-	-
80A	-	-	+	-	-	-	++	+++
80B	-	-	+	-	-	-	++	+++
80C	-	-	+	-	-	-	++	+++
81A	-	-	-	-	-	-	-	-
81B	-	-	-	-	-	-	-	-
81C	-	-	-	-	-	-	-	-
82A	-	-	-	-	-	-	-	-
82B	-	-	-	-	-	-	-	-
82C	-	-	-	-	-	-	-	-
83A	-	-	+	-	+	-	++	+++
83B	-	-	+	-	-	-	+	++
83C	-	-	+	-	-	-	+	++
84B	-	-	-	-	-	-	-	+
84C	-	-	-	-	-	-	-	+
84A	-	-	-	-	-	-	-	-
84B	-	-	-	-	-	-	-	-
84C	-	-	-	-	-	-	-	-
85A	-	-	-	-	-	-	-	-
85B	-	-	-	-	-	-	-	-
85C	-	-	-	-	-	-	-	-
86A	-	-	-	-	-	-	-	-
86B	-	-	-	-	-	-	-	-
86C	-	-	-	-	-	-	-	-
87B	-	-	-	-	-	-	-	+
87C	-	-	-	-	-	-	-	+
88A	-	-	-	-	-	-	-	-
88B	-	-	-	-	-	-	-	-
88C	-	-	-	-	-	-	-	-
89A	-	-	-	-	-	-	-	-
89B	-	-	-	-	-	-	-	-
89C	-	-	-	-	-	-	-	-
90A	-	-	-	-	-	-	-	-
90B	-	-	-	-	-	-	-	-

CASE	Lymphocyte	Eosinophils	Fibroblast	Erythrophage	Siderophage	Lipophage	Macrophage	Neutrophils
90C	-	-	-	-	-	-	-	-
91A	-	-	-	-	-	-	-	-
91B	-	-	-	-	-	-	-	-
91C	-	-	-	-	-	-	-	-
92A	-	-	+	-	-	-	+	+++
92B	-	-	+	-	-	-	+	+++
92C	-	-	-	-	-	-	-	-
93A	-	-	-	-	-	-	-	-
93B	-	-	-	-	-	-	-	-
93C	-	-	-	-	-	-	-	-
4A	-	-	-	-	-	-	++	+++
94B	-	-	-	-	-	-	+	++
94C	-	-	-	-	-	-	-	++
95A	-	-	-	-	-	-		+
95B	-	-	-	-	-	-		+++
95C	-	-	-	-	-	-	-	-

Indicators: - = negative / no inflammatory cell
+ = <10 cells/10 random HPF outside the bleeding area
++ = 10 - 20 cells/10 random HPF outside the bleeding area
+++ = >20 cells/10 random HPF outside the bleeding area
A = Laceration, **B** = Bruises, **C** = Abrasion

N/B:
All the 95 cases of control showed absence of bleeding microscopically and did not produce any inflammatory reaction. The controls were taken from the the contra-lateral sites of uninjured skin.

Table 1.1: LACERATION

Duration (hours)	Cases	Lymphocyte	Eosinophils	Fibroblast	Erythrophage	Siderophage	Lipophage	Macrophage	Neutrophils
< 1	23	-	-	-	-	-	-	-	+
	25	-	-	-	-	-	-	-	+++
	26	-	-	-	-	-	-	++	+++
	53	-	-	-	-	-	-	-	+++
	73	-	-	-	-	-	-	-	+
	83	-	-	-	-	-	-	-	+++
	95	-	-	-	-	-	-	-	+
1 - 12	1	-	-	-	-	-	-	++	++
	41	-	-	-	-	-	-	-	+++
	47	-	-	-	-	-	-	-	+++
	75	-	-	-	-	-	-	-	+++
	80	-	-	-	-	-	-	-	+++
12 - 24	29	-	-	-	-	-	-	+	++
	42	-	-	-	-	-	-	+	+++
	55	-	-	-	-	-	-	-	+++
	56	-	-	-	-	-	-	-	+++
	92	-	-	-	-	-	-	-	+++
	94	-	-	-	-	-	-	-	+++
24 – 72	32	+	-	++	-	-	-	++	+

Table 1.2: BRUISE

Duration (hours)	Cases	Lymphocyte	Eosinophils	Fibroblast	Erythrophage	Siderophage	Lipophage	Macrophage	Neutrophils
< 1	5	-	-	-	-	-	-	++	+++
	25	-	-	-	-	-	-	-	+++
	26	-	-	-	-	-	-	++	+++
	46	-	-	-	-	-	-	++	+++
	53	-	-	-	-	-	-	-	++
	73	-	-	-	-	-	-	-	+
	77	-	-	-	-	-	-	+	+++
	87	-	-	-	-	-	-	-	+
	95	-	-	-	-	-	-	-	+++
1 - 12	1	-	-	-	-	-	-	++	++
	12B1	-	-	-	-	-	-	-	++
	41	-	-	-	-	-	-	-	+++
	47	-	-	-	-	-	-	-	+++
	50	-	-	-	-	-	-	++	+++
	75	-	-	-	-	-	-	-	++
	80	-	-	+	-	-	-	++	+++
	84	-	-	-	-	-	-	-	++
12 - 24	29	-	-	-	-	-	-	+	++
	42	-	-	-	-	-	-	+	+++
	48	+	-	+	-	-	-	+++	++
	55	-	-	-	-	-	-	-	+++
	56	-	-	-	-	-	-	++	+++
	92	-	-	-	-	-	-	+	+++
	94	-	-	-	-	-	-	+	++
24 - 72	32	+	-	+	-	-	-	++	+
	71	+	-	+	-	-	-	++	+++
> 72	30	+	-	+	-	-	-	+++	++

Table 1.3: ABRASION

Duration (hours)	Cases	Lymphocyte	Eosinophils	Fibroblast	Erythrophage	Siderophage	Lipophage	Macrophage	Neutrophils
< 1	25	-	-	-	-	-	-	-	+++
	53	-	-	-	-	-	-	-	++
	73	-	-	-	-	-	-	-	+
	83	-	-	-	-	-	-	+	++
	87	-	-	-	-	-	-	-	+
1 - 12	1	-	-	-	-	-	-	+	+
	41	-	-	-	-	-	-	-	+++
	47	-	-	-	-	-	-	-	+++
	75	-	-	-	-	-	-	-	++
	80	-	-	+	-	-	-	++	+++
	84	-	-	-	-	-	-	-	+
12 - 24	42	-	-	-	-	-	-	+	+++
	55	-	-	-	-	-	-	-	+++
	56	-	-	-	-	-	-	+	+++
	94	-	-	-	-	-	-	-	++
24 - 72	32	+	-	+	-	-	-	++	+
	71	-	-	+	-	-	-	++	+++
> 72	30	+	-	+	-	-	-	+++	++

Table 1.4
Type of inflammatory cells and earliest appearances in the lesions

Type of cell	Earliest appearances
Neutrophils	35 minutes
Macrophage	1 hour 10 minutes
Fibroblast	6 hours 45 minutes
Lymphocyte	21 hours 55 minutes

The descriptions for the negative results are as below:

- Case 2, 3, 4, 8, 9, 11, 13, 14, 15, 16, 17, 18, 20, 21, 22, 24, 26, 27, 28, 31, 33, 35, 36, 37, 38, 39, 40, 43, 44, 45, 49, 52, 54, 57, 58, 62, 64, 65, 66, 67, 68, 69, 70, 72, 73, 74, 76, 78, 81, 82, 85, 86, 88, 90, 91 and 96 = immediate death (died at scene post trauma)

- Case 6, 7, 10, 19, 34, 57, 61, 67, 77, 87, 93 & 95 BID (Brought In Dead) to HKL.

- Case 23 in the control sample there is focal area of chronic dermatitis (lymphocyte +++). MVA case died at scene, trapped between 2 colliding trailers.

- Case 63 died at scene but present of chronic dermatitis in the back ground noted.

- Case 59 immediate death (failed resuscitation) in A & E, Hospital Kuala Lumpur.

The descriptions for positive results are as follow:

- Case 1 died 1 hour 10 minutes post-admission
- Case 5 died 16 hours 50 minutes post-trauma
- Case 12 died 5 hour 10 minutes post-trauma

- Case 25 died 35 minutes post-trauma

- Case 29 died 19 hours 45 minutes post trauma
- Case 30 died 3 days post-assault (80 hours 39 minutes).
- Case 32 died 24 hours 30 minutes post blunt injury
- Case 42 post MVA 20 hours 15 minutes.
- Case 46 (involved in fight sustained blunt injury, died at the scene)
- Case 41 & 47 died less than 2 hours post-trauma
- Case 48 died after 21.55 hours post – trauma MVA
- Case 50 died 8 hours 45 minutes post trauma
- Case 53 died < 1 hour post trauma
- Case 55 died after 12 hours 50 minutes post MVA
- Case 56 died after 17.5 hours post trauma
- Case 60 died 26 hours and 12 minutes post fall.
- Case 71 died 38 hours 36 minutes post-trauma (industrial accident).
- Case 74 died due to stab wound to the neck; exsanguinations of blood
- Case 75 died after 11 hours post MVA
- Case 79 died within 45 minutes in A+E post trauma
- Case 80 died 6 hours 45 minutes post MVA
- Case 83 died < 1 hour post trauma
- Case 84 died 4 hours 5 minutes post MVA
- Case 89 died 1 hours 10 minutes post MVA
- Case 92 died 15 hours 10 minutes post-trauma
- Case 94 died 15 hours 25 minutes post MVA

Discussion

In this study, majority of the cases are brought in dead to the mortuary by police. This produces results which are skewed towards the immediate death or death of less than 1 hour.

Most of the immediate deaths show negative results. For the case no. 74, who died because of stab wound to the neck, all of the skin samples (laceration, abrasion and bruise) showed not only absence of inflammatory cells but also absence of erythrocytes due to blood exsanguinations.

According to K. S. Narayan Reddy (2006) the severity of inflammatory reaction depends on severity of injury, vascularity and presence or absence of foreign body/infection. In this study, the case no. 12 was a 2-year old Indian girl who sustained head injury due to television set fell onto her head. Two skin bruises were sampled from her, i.e. from the left parietal scalp region and the left thigh (labeled as B1 and B2). Microscopically bruise from the left parietal scalp showed mild infiltration of neutrophils from the surrounding blood vessels. The victim died 5 hours and 10 minutes post

trauma. This case demonstrates role of injury severity and vascularity in inflammatory reaction.

In a study performed by Betz and co-worker in 1994 on 221 of human skin wounds (lacerations, surgical or stab/cut wound) from 148 male and 73 female corpses without sign of putrefaction, the earliest cells observed in human skin wound during the normal healing process were neutrophilic granulocytes within 20 – 30 minutes post infliction. The number of neutrophils decreased with increasing post infliction intervals. In a lesion aged 3 hours macrophages infiltration was observed and became a regular findings after 15 hours. Skin injury of less than 15 hours showed predominant neutrophils infiltration but at 20 hours post infliction interval macrophages predominate. Foamy macrophages are detectable at interval of 3 days post infliction and become regular findings in wound aged between 12 and 31 days. Spot-like lymphocytic infiltrations occur in a wound aged 8 days and commonly found in lesions between 20 days and 7 months. Fibroblasts are detectable in wounds with survival time of 25 hours and regularly found in the granulation tissue of lesions aged 6 days or more. Primary healing of human skin lesions occur earliest at 5 days post wound infliction manifests as complete re-epithelialization. The absence of a complete new epidermal layer indicates a survival time of less than 21 days.

In our study, the three deceased persons who died at the scene (case no. 26, no. 46 and no. 95) showed the presence of neutrophils within and outside the bleeding areas. Case no. 25 which the victim died 35 minutes post trauma showed intense neutrophilic infiltrations within and outside the bleeding area. In this case where bruise and abrasion were sampled, both lesions showed same intensity of inflammatory reactions. Therefore this study demonstrates that neutrophils infiltration of injured area started less than one hour of post-injury infliction. To be more specific, it is about 35 minutes.

In addition, four cases which are case 26, 73, 92 and 95 show absence of inflammatory reaction in abrasions which are superficial but positive for bruises and lacerations. In comparison to abrasion in case 25 which is positive, these results indicate that in impact abrasion with underlying bruising inflammatory reaction preceded by neutrophil can be expected. However as shown in this study, superficial abrasion does not elicit inflammatory reactions or it may elicit mild inflammations which undergo complete resolution.

For cases no. 5 (bruise), no. 30 (abrasion), no. 32 (bruise) and no. 77 (bruise), pigment laden macrophages are seen around and within the bleeding areas. Except for case no. 30 which is 80 hours 30 minutes post

trauma, all the other cases are less than 3 days/72 hours. Perl's staining confirms that these pigment laden macrophages do not contain haemosiderin.

This study which also cover wide age spectrum from 2 years old to 57 years old shows that age does not influence the inflammatory reaction but the severity of injury does. The neutrophils tend to predominate before 20 hours but after that macrophages increase in appearance. Macrophages are first seen to appear in this study at 1 hour and 10 minutes post trauma. The fibroblasts appear after 6 hours which can be seen as plump elongated cells coming out from blood vessels into the injured areas. However, granulation tissues are not seen in this study because the longest duration post injury infliction was 80 hours and 39 minutes. The observation to differentiate between the mature and young fibroblasts are not done in this study. Lymphocytes are first seen after 20 hours but the number is very small, less than 10 cells outside the bleeding area in random 10 high power field of light microscopic magnification.

In all the 95 cases, controls which are taken from the uninjured skin demonstrate that post mortem incised wounds show absence of bleeding microscopically and do not produce any inflammatory reactions.

In conclusion, this study shows minimum ante-mortem post injury infliction interval by positive results obtained microscopically using inflammatory cells parameters. In addition, routine staining of H&E (Hematoxylin & Eosin) is sufficient and reliable but the use of special staining and immunostains will help to produce more concrete evidence. Negative results, however, are of no practical meaning due to their rather frequent appearances (Betz 1994).

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ORIGINAL ARTICLE

Extraction and detection of atropine from viscera by new solvent system using high performance thin layer chromatography plate

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Abstract

Atropine is a basic constituent of Dhatura and is non-volatile in nature. Atropine is considered to be a cerebral deliriant.Although several instrumental methods like UV, GC and HPLC are available for separation & identification of atropine, the cost of analysis is very high. Therefore a simple, rapid & reliable HPTLC method for separation of atropine has been presented. The proposed system is well suited for day-to-day analysis. The main advantage of TLC is the low cost, simultaneous analysis of large number of samples & minimum sample preparation is required. Atropine was first extracted from viscera and then identified on the HPTLC plates by using various new solvent systems. For the detection of atropine on developed plates, Draggendroff was used as spraying reagent. For the analysis a total of 41 solvent systems were selected, in which 20 solvent systems were giving good result. R_f of sample in different 20 solvent systems was in between 0.062 to 0.887 and time taken by solvent system was in between 27 min to 146 min. In case any one of 20 solvent systems is not available, other mentioned system can also be used for the analysis of Atropine.

Key Words: Atropine, Dhatura, Solvent systems, HPTLC plate, R_f value

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Introduction

Atropine occurs as white crystals. It's molecular formula is $C_{17}H_{23}NO_3$ Fig (a) and Fig (b) and molecular mass is 289.38 g/mol. Atropine sublimes under high vacuum at 93°C to 110°C and has a melting point of 114°C to 116°C. Atropine has low solubility in water. Atropine is optically inactive and unstable in light.

Atropine is readily absorbed from mucous membranes, the skin and the intestinal tract but not from stomach[1-2]. Atropine is a constituent of Dhatura and the plant has two varieties: Dhatura Fastuosa Fig (c) & Dhatura Stramonium Fig (d). Dhatura Fastuosa grows in plain while Dhatura Stramonium grows in the range of Himalayan altitude. It is an extremely deadly plant.

Dhatura is considered as a stupefying poison,commonly used by people for robbery, kidnapping and rape. Children usually eat raw fruit Fig (e) or seeds Fig (f) mistaking them for edible fruit or capsicum seeds respectively. After the intake of Dhatura seeds, the person becomes dry as bone, red as beet, blind as a bat, hot as a hare and mad as a wet hen. 60 mg for adult and 4 mg for children are considered as a fatal dose. Death usually occurs within 24 hours.

For the treatment the stomach should be evacuated to remove the remnants of the crushed seeds by a stomach wash with either a weak solution of $KMNO_4$ or 4 to 5% tannic acid. Physostigmine in a dose of 1-4 mg or Neostigmine act as physiological antidotes.

Atropine produces many effects in the body, including relief from spasms of the gastrointestinal tract (stomach and intestines), the bladder, and the biliary tract. This is helpful in controlling conditions such as colitis, renal and irritable bowel syndrome. Atropine also reduces the secretions of many organs, thereby helping to control conditions such as excessive stomach acid production and excessive secretion from the pancreas; to reduce secretions of the nose, lungs, salivary glands, and stomach before surgery. Atropine also has effects on the heart.

It is used during surgery to maintain proper heart function, during emergencies involving the heart, and to treat certain heart disorders. Atropine is used to control laughing and crying episodes that are caused by brain tumors. Atropine also has effect on the eyes as it dilates the pupil and therefore is available in an ophthalmic (eye) formulation[10-14] Various techniques have been used for analysis of atropine such as High Performance Liquid Chromatography (HPLC), Gas Liquid Chromatography

(GLC) etc., but in present paper an attempt has been made to analyse atropine from viscera by Thin Layer Chromatography (TLC) which is very simple, cheap, and rapid and can be performed in very less time as compared to GLC and HPLC.

Materials and method

a) Material required

i) Chemical and Reagent: Methanol (Division of Glaxo India Limited, Mumbai), Acetic acid (Division of Glaxo India Limited, Mumbai), Ethylacetate (Glaxo Smithkline Pharmaceutical Limited, Mumbai), Cyclohexane (Merck Specialities Private Limited, Mumbai), Isopropanol (Glaxo Smithkline Pharmaceutical Limited, Mumbai), Nitric acid (E.Merck (India) Limited, Mumbai), Isobutanol (Glaxo Smithkline Pharmaceutical Limited, Mumbai), Ammonia (Division of Glaxo India Limited, Mumbai), Ethanol (Merck KGaA, Germany), Chloroform (Merck Limited, Mumbai), Acetonitrile (Merck Limited, Mumbai), Toluene (E.Merck (India) Limited, Mumbai), Hexane (E.Merck (India) Limited, Mumbai), Acetone (Merck Specialities Private Limited, Mumbai), Bismuthsubnitrate (Division of Glaxo India Limited, Mumbai), Potassium iodide (Division of Glaxo India Limited, Mumbai).

(ii) Glassware: Glass chromatographic chamber, beaker, conical flask, pipette and fine capillary tube of borosil make were used.

iii) Equipment: Precoated HPTLC with silica gel $60F_{254}$ (Merck kGaA, Germany).

b) Preparation of standard solution

1000-ppm solution of Atropine was prepared in methanol by dissolving 0.1 gm of Atropine in 100 ml of methanol.

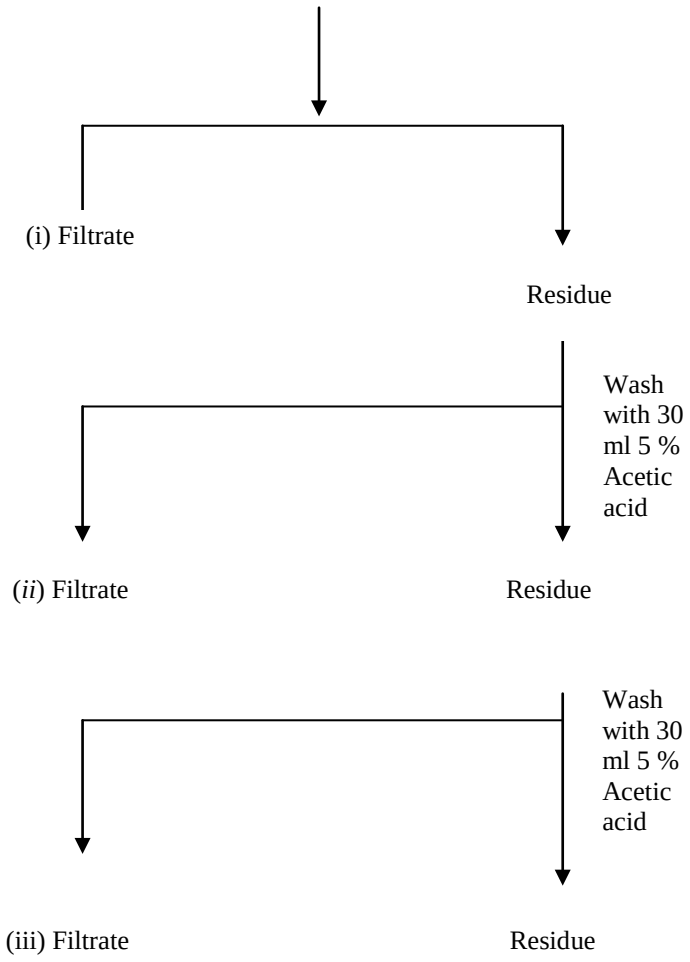
c) Preparation of Draggendroff spraying reagent

i) 2 gm of Bismuthsubnitrate is dissolved in 25 ml of glacial acetic acid and to it 100 ml of water is added.
ii) 40 gm of Potassium iodide is dissolved in 100 ml of water.
10 ml of (i) is mixed with 10 ml of (ii) and 25 ml of glacial acetic acid is added. The solution is then diluted with 100 ml of water.

d) Extraction of atropine from viscera[14]

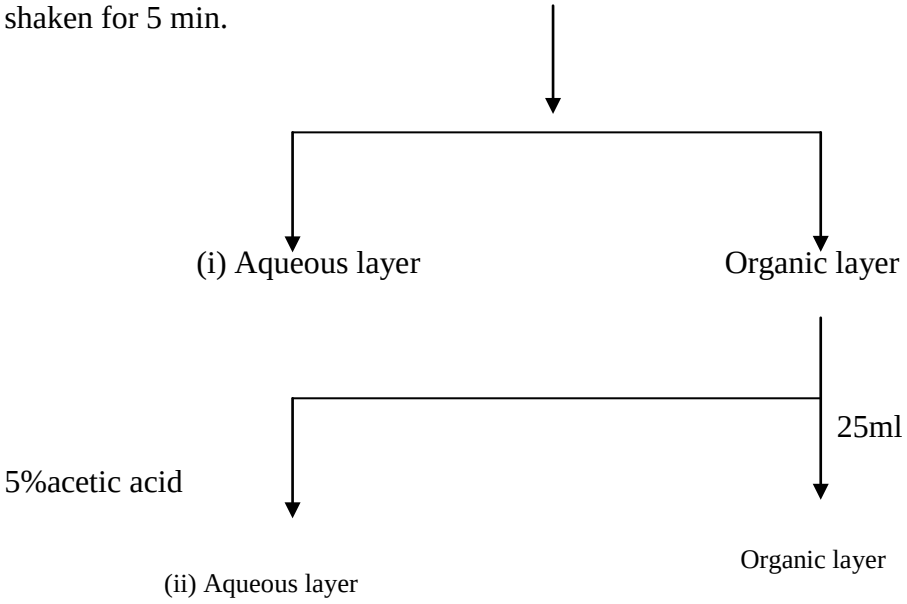
Step I

7 gm tissue (cut into fine pieces) + 100 ml of 5 % acetic acid + solid ammonium sulphate in 500 ml beaker is heated on water bath for 4 hours. The tissue proteins are coagulated. The contents are filtrated off without suction through filter paper pulp

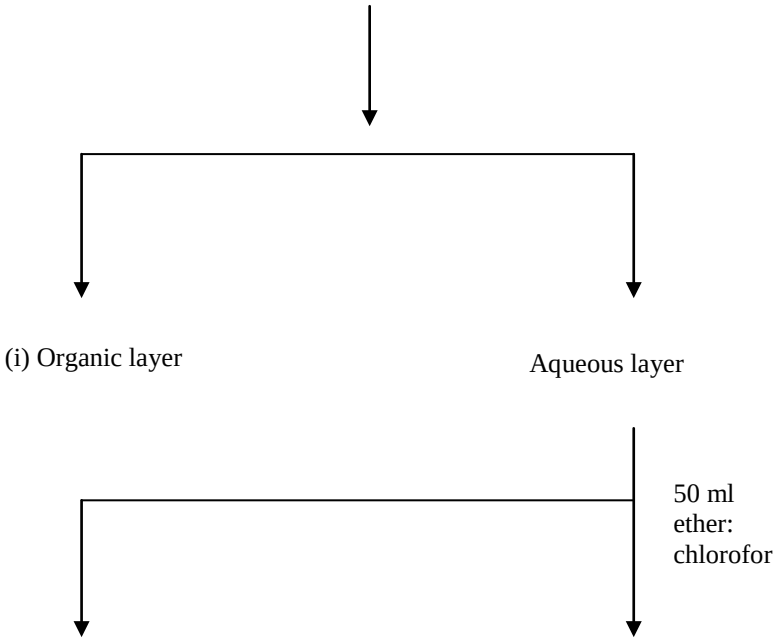


Step II

Three filtrates are pooled + 50 ml of ether is taken in a separating funnel and shaken for 5 min.

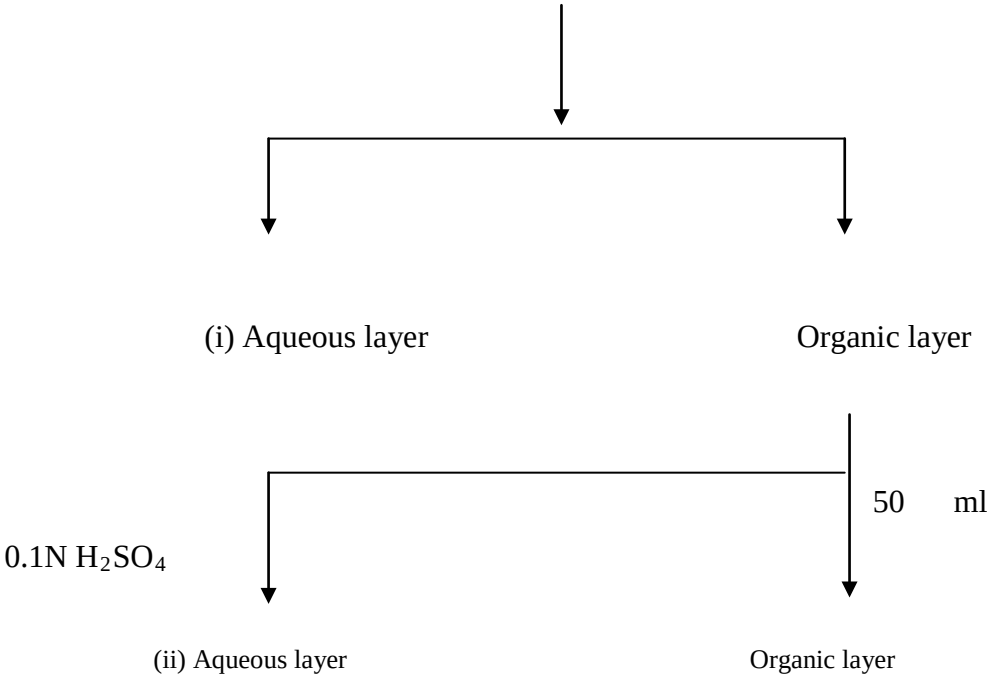


Two aqueous layers are pooled + ammonium hydroxide (to make it alkaline) + 50ml mixture of ether: chloroform (3:1) taken in a separating funnel and shaken.

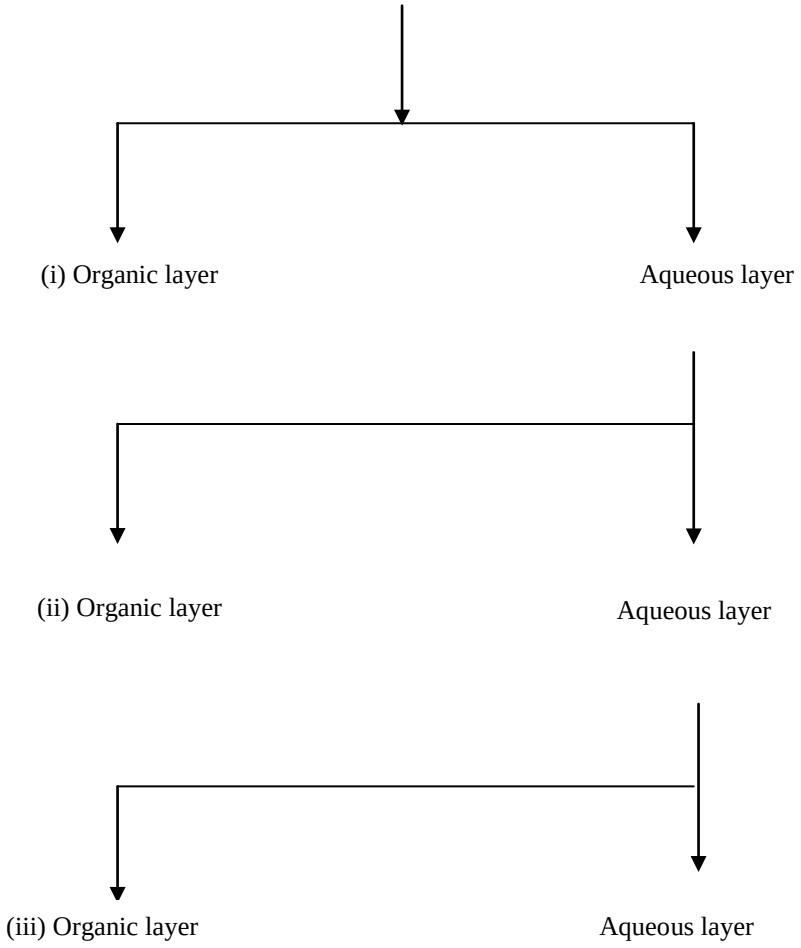


Organic layer (i), (ii) & (iii) are combined ⟶ say (A)

Combined organic layers (A) + 50 ml portion of 0.1 N H_2SO_4 is taken in a separating funnel.



Pooled Aqueous layer + Ammonium hydroxide (to make it alkaline) + 100 ml ether: chloroform (3:1) mixture.



The organic layer are pooled and dried by passing the Na_2SO_4 and evaporating to dryness.

e) Spotting of sample on HPTLC plate, Development & Spraying

HPTLC plates were activated at 110°C for 30 minutes and then cooled at room temperature before spotting. The spotting of standard sample and extracted sample was done manually with the help of fine capillary tube on the HPTLC plate 10x10 cm. The diameter of the spot was maintained small as far as possible. HPTLC plate was then placed in a chromatographic chamber containing different solvent system mentioned in table 1. Then the development plates were taken out of the chamber, air dried and then

sprayed with Draggendroff's reagent. Orange colour spots were observed. Attention was paid to the factors which controlled the reproducibility of result on HPTLC plate.

Results and Discussion

After the HPTLC plates were sprayed with the Draggendroff's reagent, Atropine appeared as orange spot in both sample and standard surrounded by yellow background. The reaction was instantaneous. Colour formation was permanent. R_f value of Atropine extracted from viscera, under experimental conditions was found nearly equal to that of standard used.

All the 41 solvent system were analysed & out of which 20 solvent systems were found to be good to run the sample. The 20 solvent systems which were found as good solvent system are from 1 to 20 in table 1 and solvent systems from 21 to 41 were not considered good solvent system for detection of Atropine. R_f value of sample in different solvent system was in between 0.0062 to 0.887 and the time taken for run of 10 cm by solvent system was in between 27 min to 146 min.

Benzene: Acetone: Ammonia (4.5:4.5:1) resulted high R_f i.e. 0.887 and Cyclohexane: Ethylacetate: Water (5:4:1) took 27 min to run the sample which is the least time among the 20 solvent systems. Therefore according to highest R_f value and time taken by them both of them can be considered as good solvent system. In case any one of 20 solvent systems is not available, other mentioned system can also used for the analysis of Atropine.

Table 1: HPTLC result obtained with different solvent system

S. No.	Solvent system	Time to run	Colour of spot	R_f of standard sample	R_f of extracted sample
1.	Methanol: Ammonia (9.5: 0.5)	33min.	Orange	0.31	0.30
2.	Diethylether: Acetic acid (6: 4)	35min.	Orange	0.16	0.15
3.	Ethyl acetate:Methanol: Conc.Ammonia (8.5:1:0.5)	30min.	Orange	0.36	0.36

4.	**Cyclohexane: Toluene: Diethyl amine (7.5:1.5:1)**	**35min.**	**Orange**	**0.16**	**0.15**
5.	**Chloroform: Methanol (9:1)**	**36min.**	**Orange**	**0.13**	**0.10**
6.	**Cyclohexane: Chloroform: Diethylamine (5:4:1)**	**42min.**	**Orange**	**O.36**	**0.34**
7.	**Chloroform: Isopropanol (1:4)**	**67 min**	**Orange**	**0.18**	**0.16**
8.	**Acetic acid: Ethanol: Water (3:6:1)**	**73min.**	**Orange**	**0.43**	**0.43**
9.	**n-Hexane: Acetone (8:2)**	**105 min.**	**Orange**	**0.41**	**0.40**
10.	**Ethanol: Water: Ammonia (6:3:1)**	**104 min.**	**Orange**	**0.59**	**0.57**
11.	**Chloroform: Ethanol: Water (2:6:2)**	**86min.**	**Orange**	**0.33**	**0.31**
12.	**Chloroform: Methanol: Water (3:5:2)**	**73min.**	**Orange**	**0.39**	**0.39**
13.	**Benzene: Methanol (6:4)**	**110 min.**	**Orange**	**0.16**	**0.14**
14.	**Acetic acid: isobutanol (2: 8)**	**136 min.**	**Orange**	**0.35**	**0.33**
15.	**Methanol: ammonium hydroxide (9.5: 0.5)**	**31min.**	**Orange**	**0.11**	**0.11**
16.	**Isopropylalcohol:Chloroform:Ammonia(4.5: 4.5: 1)**	**76min.**	**Orange**	**0.825**	**0.821**
17.	**Benzene: Acetone: Ammonia (4.5:4.5:1)**	**146 min**	**Orange**	**0.8875**	**0.887**
18.	**Methanol**	**30 min**	**Orange**	**0.125**	**0.123**
19.	**Cyclohexane: ethylacetate: water (5:4:1)**	**27 min**	**Orange**	**0.0625**	**0.062**
20.	**Methanol: isobutanol (6:4)**	**125 min**	**Orange**	**0.1**	**0.1**
21.	**Ethylacetate**	**62 min**	**Nil**	**Nil**	**Nil**
22.	**Benzene**	**35 min**	**Nil**	**Nil**	**Nil**

23.	**Ethylacetate: Chloroform: Acetone (6:2:2)**	**65 min**	**Nil**	**Nil**	**Nil**
24.	**Hexane**	**57 min**	**Nil**	**Nil**	**Nil**
25.	**Hexane: Benzene (5:5)**	**23 min**	**Nil**	**Nil**	**Nil**
26.	**Hexane: Chloroform (5: 5)**	**74 min**	**Nil**	**Nil**	**Nil**
27.	**Ethanol: Acetone (2:8)**	**137 min**	**Nil**	**Nil**	**Nil**
28.	**Cyclohexane: Chloroform (7:3)**	**25 min**	**Nil**	**Nil**	**Nil**
29.	**Benzene: 2 propanol: acetone (6:3:1)**	**31 min**	**Nil**	**Nil**	**Nil**
30.	**Cyclohexane: Ethylacetate (5:5)**	**62 min**	**Nil**	**Nil**	**Nil**
31.	**Ethylacetate: Cyclohexane: Ethanol (5:4:1)**	**27 min**	**Nil**	**Nil**	**Nil**
32.	**Hexane: Ether: Acetone (8.5:1:0.5)**	**16 min**	**Nil**	**Nil**	**Nil**
33.	**Diethyl ether: Ethylacetate (8:2)**	**63 min**	**Nil**	**Nil**	**Nil**
34.	**Benzene: Ethanol (7:3)**	**69 min**	**Nil**	**Nil**	**Nil**
35.	**Ethylacetate: isopropanol (8.5:1.5)**	**12 min**	**Nil**	**Nil**	**Nil**
36.	**Chloroform: acetone (8:2)**	**69 min**	**Nil**	**Nil**	**Nil**
37.	**Hexane: Benzene: Acetone(6:2:2)**	**300 min.**	**Nil**	**Nil**	**Nil**
38.	**Acetone**	**22 min**	**Nil**	**Nil**	**Nil**
39.	**Hexane: acetone (8:2)**	**383 min**	**Nil**	**Nil**	**Nil**
40.	**Acetone: Benzene (5:5)**	**17 min.**	**Nil**	**Nil**	**Nil**
41.	**Benzene: 2-Propanol: Acetone (6:3:1)**	**31 min.**	**Nil**	**Nil**	**Nil**

Conclusion

Due to the time involved in screening Atropine with other analytical methods, such as Gas Chromatography, alternative methods were sought. HPTLC proved to be an excellent choice because it is a simple equipment, takes short time for development, simultaneous analyses of large number of samples are possible, early recovery of separated components is possible, separation effects are superior, early visualization of separated compounds, sensitive as extremely sharp delineated spots are obtained in HPTLC. The main advantage of HPTLC is the low cost. When all the 41 solvent system were analysed, it was found that 20 solvent systems were good to run the sample. If any one of the solvent system is not available, we can choose any one of 20 solvent systems. Beside Draggendroff's reagent, we can also use Iodoplatinate solution as a spraying reagent.

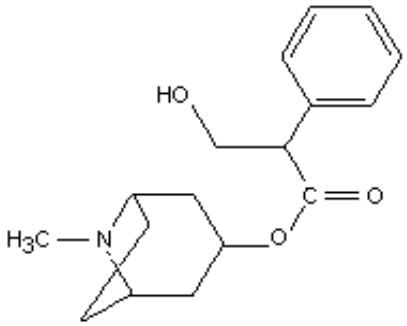


Fig. (a) : Chemical Structure[7]

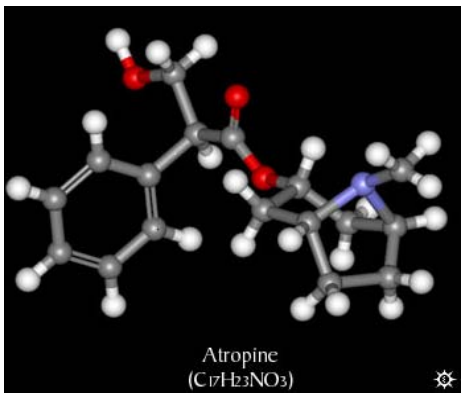


Fig. (b) : Molecular Structure[3]

Fig. (c) : Dhatura Fastuosa[6]

Fig. (d) : Dhatura Stramonium[7]

Fig (e) : Dhatura fruit[8]

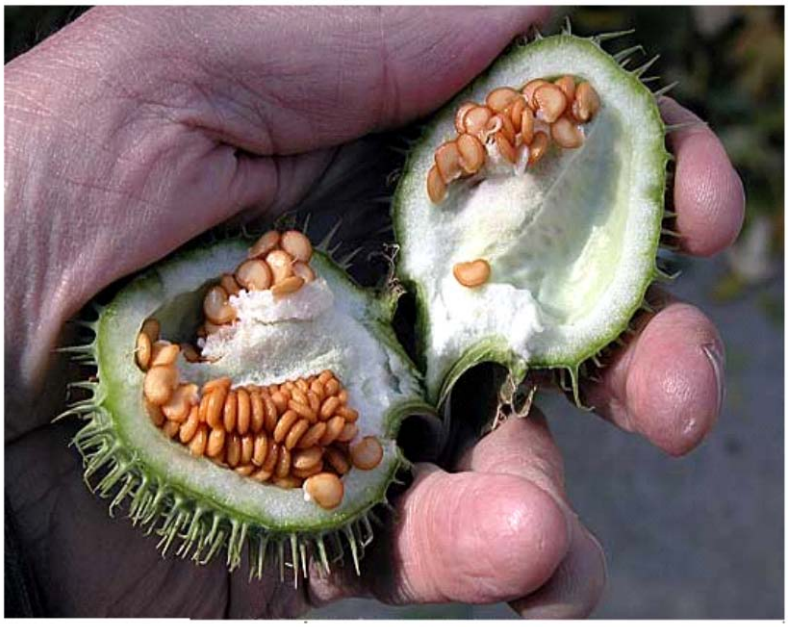


Fig (f) : Dhatura seeds[9]

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CASE REPORT

Obturator artery and vein injury: A rare fatal complication of revision total hip arthroplasty

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Abstract

A 62 year old female had an operation for revision of total hip arthrosplasty. She developed signs of haemodynamic collapse a few hours after the operation. Emergency laparotomy showed a massive retroperitoneal haemorrhage in the pelvis which appeared to arise from lacerated branches of the right internal iliac artery. The right internal iliac artery and vein were ligated but the patient died 3 hours after the emergency laparotomy. The postmortem examination showed a torn obturator artery and vein due to reaming procedure and intrusion of cement from the acetabulum.

Keywords: Vascular injury, hip replacement, hip arthroplasty, obturator artery, obturator vein.

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Introduction

A total hip replacement operation is quite a common procedure done in an elderly patient who has a painful hip due to various causes such as osteoarthrosis, traumatic arthritis, fracture of acetabulum, rheumatoid arthritis, bony ankylosis, among others (Duthie and Ferguson 1973). Even though the procedure is considered safe, it is not without complication. We report a case of a revised total hip replacement surgery with obturator artery and vein laceration due to intrusion of cement substance into the pelvic cavity from the acetabulum.

Case history

A 62 year old caucasian female had an operation for a revised total hip replacement (right acetabular HG socket) after she complained of pain in the hip. The clinical examination showed the presence of marked acetabular erosion and shortening of the right leg. During the operation, the surgeon had "reamed" the acetabulum to 65 mm diameter prior to the application of cement substance and the socket prosthesis.

A few hours after the operation, the patient developed signs of haemodynamic collapse (pale and clammy, blood pressure 60/48 mm Hg). The right hip was swollen but not tense. The abdomen increased in girth. Blood was transfused to maintain the circulation. Emergency laparotomy showed massive retroperitoneal haematoma due to bleeding from branches of the right internal iliac artery. After clamping the right internal iliac artery, its branches were tied. This appeared to control most of the bleeding. Fifteen pints of packed cells were given during the operation. Platelets, fresh frozen plasma and cryoprecipitate were given to combat coagulopathy. The patient remained hypotensive, and arterial blood gases showed mixed respiratory and metabolic acidosis. She developed ventricular ectopic beats, became asystolic and failed to respond to resuscitation. She died about 3 hours after the emergency laparotomy.

Coroner Autopsy

The body was that of an obese, elderly caucasian female. There was a recent surgical incision on the lateral aspect of the right hip, 22 cm in length. There was no apparent haematoma or swelling of the right hip. A 20 cm long midline incision was present on the abdominal wall. Another recent transverse surgical incision 6 cm in length was noted on the right side of the abdomen. Petechial haemorrhages were present on the right lower abdomen. The lower limbs showed marked bilateral pitting oedema to the level on the knees.

Internal examination showed extensive retro-peritoneal haematoma. There was a large amount of blood clot in the pelvic cavity. The previously tied right internal iliac artery and vein were noted. There was an intrusion of cement substance 2x2x1.5 cm with sharp and ragged margins from the anteroinferior quadrant of the acetabulum into the pelvic cavity (fig.2). The obturator artery and vein adjacent to the cement susbstance were lacerated. The bladder and right ureter were not injured. The uterus was absent

(previous hysterectomy). The kidneys were pale but otherwise unremarkable.

The heart weighed 350g and showed no evidence of recent infarction. The coronory artery showed moderate atheromatous change with 60% luminal occlusion of the left anterior descending branch. The heart valves were unremarkable. The lungs were mildly congested and oedematous but otherwise healthy.

The soft tissue around the right hip was removed using 3% NaOH of antiformin solution (Chauhan 1989). The bone appeared thin at the anteroinferior quadrant of the acetabulum. The intrusion of the cement substance was confirmed to come from this quadrant (fig. 3).

The cause of death was given as:

1a. Haemorrhage due to laceration of obturator artery and vein
1b. Right hip replacement surgery.

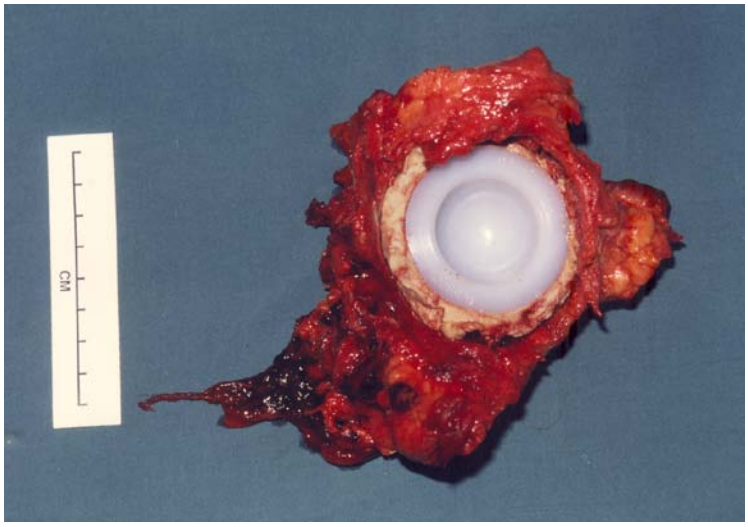

Fig. 1: The socket prosthesis in the right acetabulum held in place by cement substance.

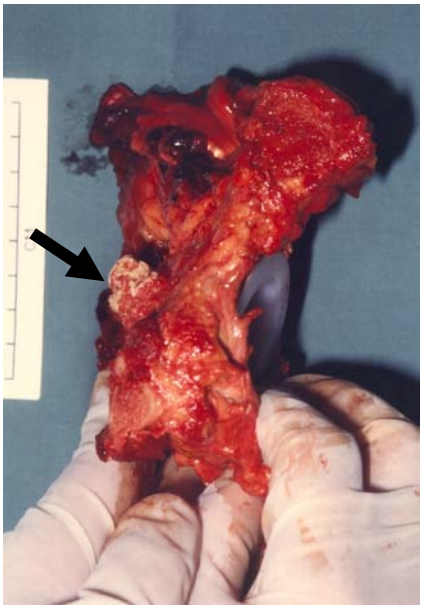

Fig.2: The cement substance (arrow) 2x2x1.5 cm protruded into the medial aspect lateral wall of the pelvic cavity.

Fig.3: The soft tissue around the right hip has been removed using antiformin technique. The cement substance (arrow) is seen protruding from the perforated anteroinferior quadrant of the acetabulum into the medial aspect lateral wall ofthe pelvis. Above it is part of the pubic bone which has been sawn during autopsy.

Discussion

There are various complications of total hip arthroplasty. Harkess (1992) classifies the complications into: nerve injuries, vascular injuries, haemorrhage, bladder injuries and urinary tract complications, haematomas, limb-length discrepancy, dislocation and subluxation, heterotopic ossification, thromboembolism, fractures, trochanteric nonunion and migration, femoral and acetabular loosening, stem failure, infection, osteolysis and miscellaneous.

Vascular injuries as a result of total hip arthroplasty are rare with an incidence between 0.2% - 0.3% (Harkess 1992). However they may pose a threat to the survival of the limb and the patient. Most of the vascular injuries have been reported to occur during revision surgery, and since there is an increase in the number of revisions being performed, the incidence of vascular injuries may increase (Harkess 1992).

Revision of total hip arthroplasty may increase the risk of vascular injury because part of the older bone which holds the older cement in the acetabulum has to be removed by reaming procedure. This is to allow the application of the new cement to hold the socket prosthesis in place. The reaming procedure itself may cause thinning and perforation of the acetabulum and so injure some vessels that can be found on the other side. In such a case, the haemorrhage occurs during the operation.

The cement substance applied to the perforated acetabulum may protrude on the other side of the acetabulum. After the cement substance has hardened, it may injure vessels when its sharp and ragged edges impinge or rub against the vessel wall. However, the haemorrhage occurs much later after the operation.

Review of the literature showed 4 cases of vascular injury in relation to total hip arthroplasty. Two involved the external iliac artery (Tisnado et al 1981 and Akizuki et al 1984), one the superior gluteal artery (Lozman and Robbins 1983) and one the iliac vein (Saenz-Martinez et al 1992). There was no reported case involving the obturator vessels.

Wasielewski et al (1990) have devised a clinically useful system for determining safe areas for placement of the screws. The system is based on a line drawn from the anterosuperior iliac spine through the centre of the acetabulum and a second line drawn perpendicular to this, creating four quadrants. Screws or intrusion of cement through the anterosuperior

quadrant may cause injury to the external iliac artery and vein. Those passing through the anteroinferior quadrant may injure the obturator nerve and vessels. In our case, the reaming procedure and intrusion on the cement substance could be the reason for the injury to the obturator vessels. Screws placed through the posterosuperior and posteroinferior quadrants do not emerge within the pelvis but may pass into the sciatic notch and endanger the sciatic nerve and superior gluteal vessels.

Harkess (1992) recommended retroperitoneal exposure and temporary clamping of the lilac vessels when there is excessive bleeding during surgery to prevent additional blood loss and to preserve the patient's life and limb. This was done in this case during emergency laparotomy to secure haemostasis. However, the patient succumbed to vetricular arrhythmias despite vigorous resuscitation. Haemorrhagic shock in this patient worsened the condition of the myocardium already compromised by coronory atheroma (60% luminal occlusion of the left anterior descending branch).

Postmortem examination showed the lacerated obturator artery and vein quite clearly to the naked eye. However, we would like to recommend postmortem angiography and venography or injection of coloured polyester resin into the internal iliac artery and vein (Ludwig 1979). These procedures may allow the pathologist to locate torn vessels in the clump of haematoma within the soft tissue that might not be seen macroscopically. Furthermore, the pathologist may document the finding on an x-ray film or a cast of the shape of the lacerated vessels.

During postmortem examination, normal variations of the branches of internal iliac artery have to be considered. In 20% - 30% of people, the obturator artery is replaced by an enlarged pubic branch of the inferior gastric artery (Warwick and William 1973). This descends almost vertically to the upper part of the obturator foramen, therefore it may escape any intrusion of cement substance at the anteroinferior quadrant of the acetabulum. The obturator artery may also originate as a bifid from the external and internal iliac artery (Warwick and William 1973) in which may increase the chance of it being injured when there is intrusion of cement substance into the pelvis.

In conclusion, even though injury of the obturator artery and vein is rare, it may occur in a revision of total hip arthroplasty because it involves a reaming procedure. The outcome may be fatal which justify the Coroner’s investigation to determine the cause and manner of death, especially to exclude the possibility of medical negligence.

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CASE REPORT

Age estimation for an adult illegal immigrant detainee at the Sungai Buluh Prison: A Case Report

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Abstract

The Forensic Identification Centre was approached by the police with letter from the Prison Authority and a Court Order to establish the age of a convicted prisoner. The prisoner had almost completed his prison term and was waiting to be whipped as part of his sentence. Two methods were selected which were clinical evaluation and radiographic method. His age was estimated to be 50.9 years and he therefore was exempted from being whipped and has been deported back to his home country.

Keywords: Age estimation, dental, radiographic, prisoner, whipping.

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Introduction

Age estimation is one of the responsibilities of forensic odontologists. It could be needed in single case on its own, in several related cases especially in children or part of dental identification of unknown deceased. Age generally refers to a period of existence and normally used in the context of the length of time a person has lived. This is usually called chronological age and differs from skeletal age and dental age. Skeletal age indicates the stage of skeletal maturation while dental age indicates the stage of dental development. The changes that occur in skeletal and dental tissues in the course of time are indications of individual's biological development.

Tooth mineralization stages are less affected by nutritional and endocrine status therefore developing teeth provide a more accurate indication of chronological age than developing bones[1]. In forensic odontology this dental development is measured as an indicator of age, which could be used as estimated age. Age estimation from teeth is also frequently used because teeth are well preserved long after all the organs and even the bone become disintegrated[2] in deceased person and unlike bone, teeth can be examined directly in living individuals.

Dental age estimation can be divided into two periods in life. The first is when the teeth are developing in the maxilla and mandible up to the age of twenty years. This dental development can be divided into the crown and root formation of the teeth and their eruption or emergence into the oral cavity. Comparison of the developmental stages with tables for all the different teeth present in the jaws could be used as a scientific statistical method. When all the teeth are fully developed as in adult, a method called regressive age related changes might be used as a scientific method[3]. Dental age estimation can be performed in the living as well as in dead persons. Generally the same methods could be applied. However, in dead people it is seldom possible to get information about the living conditions and diseases of the individual[3].

Various methods have been devised to estimate dental age of a person and these are through visual examinations, radiographs and extraction and preparation of single teeth. In adults the scientific methods normally used are extraction and destruction of a single tooth as in Gustafson's and Solheim's methods[4], extraction non-destruction of a tooth[5], periapical radiographs[2,6], panoramic dental radiographs[7] and a combination of clinical and orthopantomogram[8]. The earlier two methods required that the teeth being extracted and later prepared for the estimation of age therefore can only be used for the deceased. Another method[6] is also only suitable for deceased person as the canines must be extracted and the periapical x-rays were taken extra-orally. For living person, the other methods[2,7,8] are more suitable.

The age of Malaysian citizens are quite easily determined. For children below twelve years old their age can be confirmed through their birth certificate, which is also a necessary document for their school registration and immunisation record. As for adults and children aged twelve years and above they are required to have an Identity Card. This card is used as an official document whether for banking, hospital, school and for court procedures. Identity card has the details of the owners' name, address, fingerprint and date of birth therefore their chronological age is easily verified.

Recently, the Government of Malaysia has started to introduce a chip-based visa to foreign workers, students and long-staying visitors to this country[9]. Individuals' personal data and their thumbprints are kept in this document.

Malaysia is a heaven for people from neighbouring countries to come and work in the constructions, plantations and manufacturing industries and a number of these immigrants come illegally or do not have valid personal documents. And unfortunately, some do involve in crimes as well as flouting the Malaysian immigration law. When this occur, the immigrants will have to face the law of the land and among them are prison terms and whipping. Whipping has age limit requirements. Upon conviction, occasionally these immigrants claimed that they were older than what is stated in their personal document that they have with them.

In this case report, we presented a case where we were requested by the Prison Director in Kuala Lumpur to estimate the age of an illegal immigrant sentenced to whipping.

Case Report

An illegal immigrant had been convicted and sentenced to six month jail and one stroke of the rattan. The convict had almost completed his prison terms when he claimed that he was fifty-five years old, which is older than what was stated in his personal document. In this document and the court papers his age was written as forty-five years old. He therefore demanded that he should not be whipped because he is above the permitted age. Therefore, a Court Order was obtained for his age to be determined. We were requested by the Prison Director from Sungai Buluh Prison to estimate the age of this convict. Two Police Officers accompanied the prisoner to the hospital so that the procedures to estimate his age can be done at the Forensic Identification Centre.

Consent

The prisoner was read his right either to accept or refuse the examination and the following procedures to estimate his age. The procedures were explained to him and he was encouraged to ask questions if he did not understand or wanted further information. Following the acceptance of the procedures he was asked to sign the prepared consent document and both the left and right side of his thumbprints and the rest of the fingerprints were recorded.

Examination (Clinical)

General questions regarding his medical history were enquired. He has remarkable health. Extra-oral and intra-oral examination was done. Extra-orally, his temporomandibular joints and muscles of mastication showed no sign of trauma, clicking sounds or pain and he had normal mouth opening. Intra-orally, the muscles of mastication were also free from pain. Generally, his oral hygiene was not excellent with plaque and calculus present and marked tobacco staining could be seen on these teeth. No marked attrition or erosion on the teeth and no dental appliances in the oral cavity. Charting of the teeth including retained roots, fractured teeth, restorations and caries were done. The morphology of his teeth, gingival condition, periodontal ligament recession and attrition were examined. The visual appearance of his face was also noted.

Photographs and dental impressions

General photographs were taken from the front, right and left side of the face. Smiling photographs were also taken. Following that, cheek retractor was placed and the dentition in the maxilla and mandible was photographed in occlusion as well as when the teeth are apart (**Fig. 1**). Close-up view of the upper and lower teeth and their respective jaws were done using dental photo mirror.

Fig. 1: Anterior close-up view of the dentition

Silicone impression materials were used to take two sets of the upper and lower impressions of the teeth. This was followed by bite registration. Dental models were constructed from these impressions and the bite registration was used to guide the occlusion of the teeth extra-orally. Photos were also taken of the dental models (**Fig. 2**).

Fig. 2: Dental model of the maxillary arch

Radiographs

Extra-oral and intra-oral radiographs were taken for this case. The extra-oral x-rays are the orthopanthomogram and right and left lateral skull. Periapical radiographs (Fig. 3) of the anterior and posterior teeth of the maxilla and mandible were taken intra-orally using paralleling technique. The extent of the dental health and disease could be observed from the orthopanthomogram x-ray. From clinical observation and examination of the radiographs it was determined that the teeth that are selected for age estimation are free from caries, dental restorations or periapical lesions.

Age Estimation

The teeth selected for age estimation were the permanent maxillary central incisor, lateral incisor and second premolar. The method used for the estimation of age follows the work of Kvaal et al. (1995)[2]. Periapical radiographs of the selected teeth were taken and calculations using the formula as prescribed[2] were based on these. We calculated the age estimate

on each tooth as well as a combination of all three teeth. The basis for this method is by measuring the size of pulp from dental radiographs to estimate the chronological age for adult individual. From the calculation, the average age when all three maxillary teeth were combined together as one is 50.9 years. When the age estimation was calculated on individual teeth the estimated age were between 49 years to 51 years old.

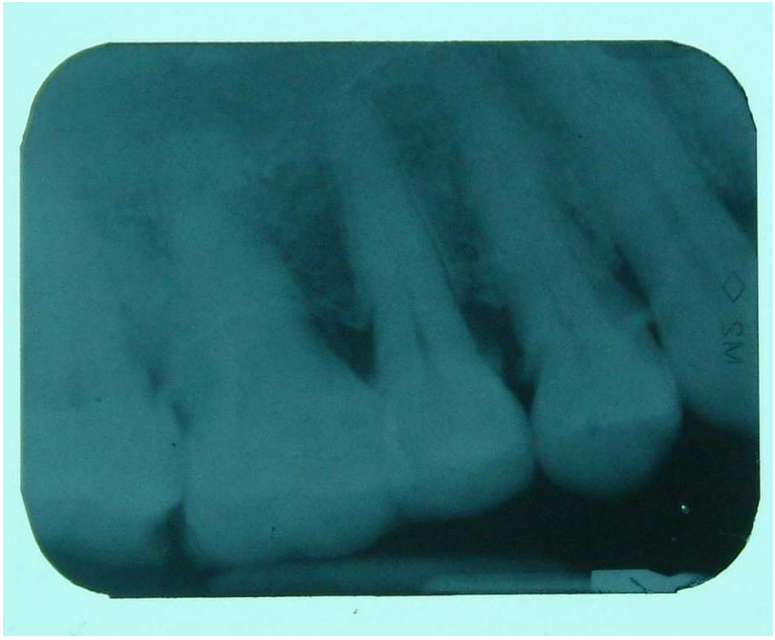

Fig. 3: Periapical x-ray of the upper right side of teeth using paralleling technique

Discussion

Age estimation is employed as one of the several means being used in the investigation to establish the identity of unknown human remains, and used as an aid in population palaedemography in archaeological work. It can also be used in living human to establish their age when they do not have an acceptable identification document as in the case of adopted children and refugees[2]. The age estimation technique proposed by Kvaal et al. (1995)[2] is a non-destructive method which can be employed on living individuals and on the unknown dead. Although the authors[2] proposed that the same principle can be adopted on panoramic radiographs, the technique was not tested or established yet at the time of this case being done.

Age plays a big role in medicolegal practice. In Malaysian Law (Penal code) Section 82 of Penal Code (Act 574)[10], stated that nothing is an offence which is done by a child under ten years of age. In Section 83 of the Penal Code (Act 574)[10], it was stated that nothing is an offence which is done by a child above ten years of age and under twelve, who has not attained sufficient maturity of understanding to judge of the nature and consequence of his conduct on that occasion. In the Criminal Procedure Code (Act 593)[11] it is stated that no sentence of whipping shall be executed by instalments, and none of the following persons shall be punishable with whipping:

a) females;
b) males sentenced to death;
c) males whom the court considers to be more than fifty years of age.

Consent in the context of practising medicine concerns[12] with situations in examination of the living patient for the purpose of diagnosis and subsequent treatment, examination of the living person for medico-legal purposes and postmortem examination and removal of tissues for transplantation. Conscious, mentally sound adults normally give consent themselves as in this case. In our centre, 'Informed consent' is practiced. Informed consent is basically obtained after a reasonable explanation of the proposed procedures to the patient so that he is enabled to make an informed decision whether or not to submit[12]. Informed consent has today emerged as a significant ethical and legal standard of medical and dental practice[13].

Age estimation has become increasingly important in forensic odontology and has been used to identify unidentified corpses[7] and could also be applied to living individuals without proper birth record or any personal document. The demand for age estimation in living individuals has increased in multicultural society where legal and illegal immigration is rising as can be seen in Malaysian experience.

The medico-legal reasons to do age estimation in living individuals can vary. It could be for employment, marriage, social benefits or to differentiate the juvenile from the adult status in criminal law cases[14]. In this case report, age estimation was needed to determine whether the person was deemed legally fit by the Malaysian Law to be punishable with whipping.

Various methods have been advocated to estimate the age of the adults and these scientific methods are called regressive age related changes. These could be in the form of clinical evaluation, morphological or radiographic technique. The clinical evaluation comprised of evaluation of age-related

changes visible in the mouth such as degree of attrition, periodontal recessions, as well as estimation of visual appearance[15]. The morphological methods are calculation based on apical root dentine translucency[5], peritubular dentine deposits[16], regressive changes such as attrition, periodontal recessions, secondary dentine, cementum apposition and apical translucency[17]. Solheim[4] formulated an age estimation calculation based on attrition, periodontal ligament recession, secondary dentine, cementum apposition, root resorption, apical translucency, tooth colour and root surface structure (surface roughness) while a number of authors used radiological methods[2,7,8,14]. The morphological methods are usually done by extraction and destruction of the teeth selected for the estimation of age and therefore more suitable for deceased persons therefore not suitable for living individuals as in this case.

It is imperative that the forensic odontologist involved should select the most suitable scientific methods being used. In this case we used clinical evaluation[15] and radiographic methods[2]. A well trained pathologist and especially forensic odontologist should be able to give a good estimation of age through inspection of a person's teeth[18]. Periapical x-ray taken intra-orally was opted for this living person to estimate his age. Periapical x-rays are inexpensive, fast, routinely used in dentistry[6] and they are also fairly easy to be studied and measured. In this case we took the x-rays using paralleling technique.

Kvaal et al.[2] stated that there is no statistically significant difference in their measurements between teeth from the right and the left side therefore we selected two teeth from the upper right quadrant (second premolar and lateral incisor) and one from the left quadrant (central incisor). The teeth selected follows strictly the conditions put forward by Kvaal et al. (1995)[2] where the teeth must be in normal functional occlusion and free from any manifestation of pathological insults such as caries, attrition, abrasion, erosion or trauma and dental restorations.

Other authors[7] used this method[2] of radiological calculation on orthopanthomogram in adult and claimed that the age estimations are comparable to those based on the original technique if at least the selection criteria are respected and good quality orthopantomographs with clear radiological image are used. Another group[8] calculated the age estimates based on a formula using a combination technique of clinical and orthopantomograms. Their formula for the calculation included DMF-T index, clinical findings at tooth crown and alveolar bone level. They stated that it was strongly not recommended for accurate age estimation as a single method but can only be used to support other methods of age estimation. Radiological age estimation method[6] using maxillary and mandibular

canines being exposed to periapical x-rays were claimed to be rather accurate. However, this method can only be used for deceased person as the teeth need to be extracted.

At the moment we haven't any extensive study of age estimation for the Mongoloid's group of people which is one of the main populations in Southeast-Asia. However, we have started to develop a database for the estimation of age for Malaysian population.

The age of the convict was estimated to be 50.9 years and the report was sent to the Prison Authority. We were later informed by the Prison Director that the sentence was amended whereby the prisoner was granted not to be whipped. He has also been deported to his home country since then.

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CASE REPORT

Signet ring cell carcinoma of the breast presented with widespread metastases: A sudden death case report

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Abstract

Signet ring breast carcinoma is an unusual neoplasm. We presented a 54-year-old Chinese lady with a history of cough, orthopnea, multiple nodules over the left chest and abdomen and bilateral leg swelling for the past one month. Clinically, she was discovered to have generalized lymphadenopathy, and hepatosplenomegaly. At autopsy, she was found to have 2 masses in the left breast, 0.4cm apart and diagnosed as signet ring carcinoma of the breast. Sections of the 2 breast lumps showed singularly distributed malignant cells, mainly showing vacuolated cytoplasm with some are signet ring shaped separated by fibrous septa. Some of the cells are present within the vascular spaces. The distance between the 2 lumps was 0.4cm. Osseous metaplasia was seen. The malignant cells showed presence of mucin with Alcian-blue-Periodic Acid Schiff (ABPAS) stain. Immunohistochemically, they were positive for cytokeratin, CK7 and EMA. These cells are negative for ER, PR, c-erbB-2, LCA, CK20, ALK protein, S-100 protein and HMB 45. Also she had metastases to multiple lymph nodes (left axillary, submental, supraclavicular), lungs and pericardium. Pleural effusion and ascites were present. The stomach showed no growth. She died after 2 days in the ward.

Keywords: breast carcinoma, signet ring, sudden death, cytokeratin, fracture vertebra.

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Introduction

Breast carcinoma is a common malignancy [1]. It can metastasize to any sites, the most common are the bone, lung and liver. However, signet ring breast carcinoma is an unusual neoplasm. It was first recognized in 1976 and was suggested as a variant of infiltrating lobular carcinoma.[2,3] It often metastasizes to gastrointestinal tract, like the stomach[4,5]and female genital tract, like endometrium and also cervix.[6,7]
A rare case of signet ring carcinoma of the breast with extensive metastasis in 54-year-old lady is presented.

Case Report

Clinical History

Patient was a 54-year-old Chinese lady who presented to Hospital Universiti Kebangsaan Malaysia with a history of cough with whitish sputum, worsening effort tolerance, orthopnea, bilateral leg swelling and multiple nodules over the left chest and abdomen for a duration of one month prior to the admission. Her condition became worst 2 days before the admission. She denied any chest pain or any history of change in the bowel habits. There was no significant past medical history except for thyroidectomy in which the cause was unknown. In the ward, clinically she was discovered to have generalized lymphadenopathy, hepatosplenomegaly with multiple subcutaneous nodules. The only significant investigation was the chest X-ray that revealed left middle zone lung consolidation. Her condition became worst and succumbed to death as dated above (21/02/2002).

POSTMORTEM EXAMINATION

External examination

The body was of a well-built female with a weight of 52kg and a height of 1.55meter at autopsy with hospital clothing. She had short curly hair with ankle oedema. Left supraclavicular lymph nodes and skin nodules on the chest were felt on palpation. Rigor mortis was fully established and hypostasis lividity was present at the back. No external mark of injury noted.

Internal Examination

Head

The scalp was opened in the usual manner. The skull and meninges were healthy. There was no haemorhage or bruising of the scalp. The brain weighed 1008gm with no gross abnormality seen. The vertebral and basilar arteries both showed atherosclerotic plaques however, the lumen was still patent.

Chest

The chest wall and the ribs were unremarkable. There were 3 left axillary lumps, each measuring 1cm in diameter. There was left supraclavicular matted lymph nodes measuring 4x4x2cm. Left submental lymph node was found measuring 2x1x0.5cm Two nodules were found over the left breast and each measuring 5x5x2cm and 1.5x2x0.6cm. The distance from each mass was about 0.4cm. Cut sections of each of the masses showed that the borders were ill-defined with diffuse whitish appearance.

The right and left pleural cavities showed presence of bloody fluid measuring 400mlls and 600mls respectively. The oesophagus and trachea were normal. The right and left lungs weighed 460gm each. Cut sections of the lung showed no obvious tumour.

The pericardium showed irregular surface. The heart weighed 280gm. All the coronary arteries were patent. No atherosclerotic plaque seen. The myocardium showed no focus of fibrosis or necrosis.

The mediastinum was filled with whitish pale mass attached to the trachea and aorta. The mass measured 3x2cm.

Abdomen

The abdomen was mildly distended. On opening the peritoneal cavity, there were multiple masses on the peritoneum measuring about 2 cm in diameter each. The ascetic fluid was straw coloured and measured about 300mls.

The stomach showed no growth. No thickening of the wall was noted. The large and small bowels were unremarkable and on sectioning no tumour was seen. The liver weighed 1500gm and appeared yellowish in colour. Serial sectioning of the liver showed homogenous surface with no mass found. The spleen was enlarged weighing 160gm and cut sections showed no gross abnormalities. The pancreas weighed 100gm and appeared normal. The right and left kidneys weighed 180 and 140gm respectively with no gross abnormality noted. The right adrenal gland, which was enlarged, weighed

20gm and cut sections showed diffuse whitish appearance. The left adrenal gland was unidentifiable.

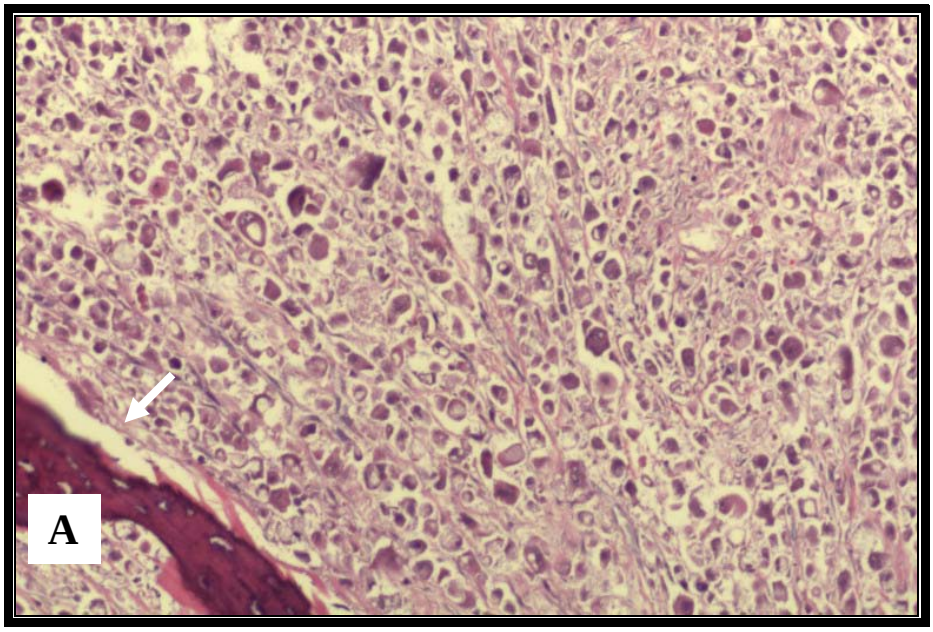
The uterus with bilateral ovaries and fallopian tubes weighed 123gm and the uterus measured 11cm from fundus to cervix, 5cm from cornu to cornu and 2.5cm antero-posteriorly. Cut section was unremarkable. The left and right fallopian tubes both measured 3x4x0.4cm. The left ovary measured 2x1x1cm and cut section was unremarkable. The right ovary measured 4x2x0.5cm and cut sections showed presence of bony material.

Microscopic Examination

Brain
Sections of the cerebrum, brain stem and cerebellum were unremarkable. The basilar and vertebral arteries showed presence of atherosclerotic plaque.

Left breast
Sections of the 2 breast lumps showed singularly distributed malignant cells, arranged in single-file pattern, and mainly showing vacuolated cytoplasm with some are signet ring shaped separated by fibrous septa (Fig. 1A & B). The malignant cells had round vesicular nuclei, some possessing prominent nucleoli and nuclei are wrinkling hyperchromatic nuclei. A few nuclei show marked nuclear pleomorphism. Some cells have eosinophilic cytoplasm. Occasional intracytoplasmic lumen with a central mucoid secretion is seen (Figure 1B). There was hardly any mitosis. Some of the cells are present within the vascular spaces. The distance between the 2 lumps was 0.4cm. Osseous metaplasia was seen (Fig. 1A). The malignant cells showed presence of mucin with alcian blue-periodic acid-Schiff (AB-PAS) both before and after diastase treatment (Fig. 1D). Immunohistochemically, they were positive for cytokeratin (CK), CK7 and epithelial membrane antigen (EMA). These cells are negative for estrogen receptor (ER), progesterone receptor (PR), c-erbB-2, leucocyte common antigen (LCA), CK20, ALK protein, S-100 protein and HMB 45.

Mediastinum
Sections showed similar malignant cells as above with an area showing tubular formation. These malignant cells showed presence of mucin with ABPAS both before and after diastase treatment. Immunohistochemically, they were positive for cytokeratin, CK7 (Fig. 1E) and EMA. These cells were negative for ER, PR, c-erbB-2, LCA, CK20 (Figure 1F), ALK protein, S-100 protein and HMB 45.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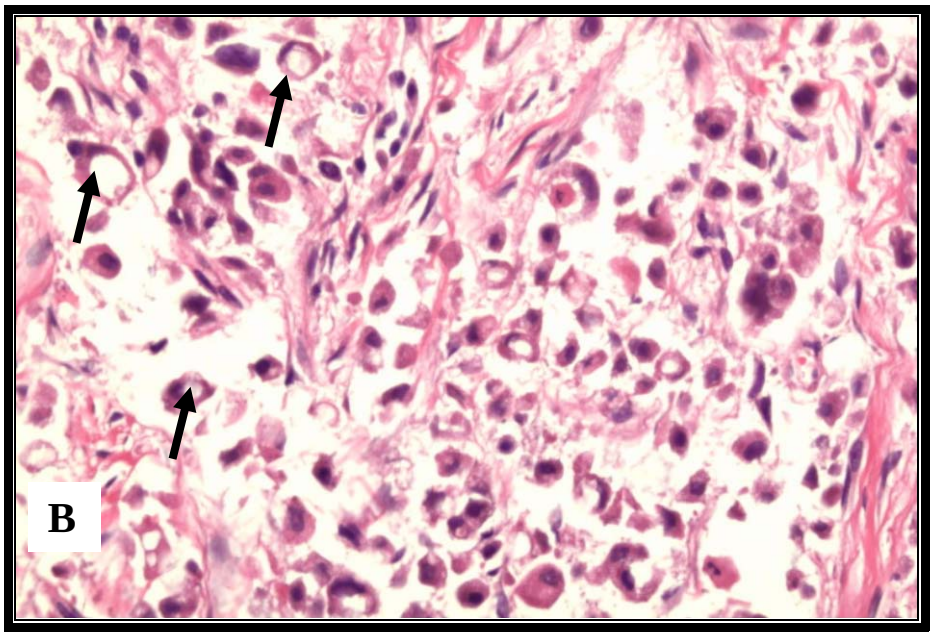
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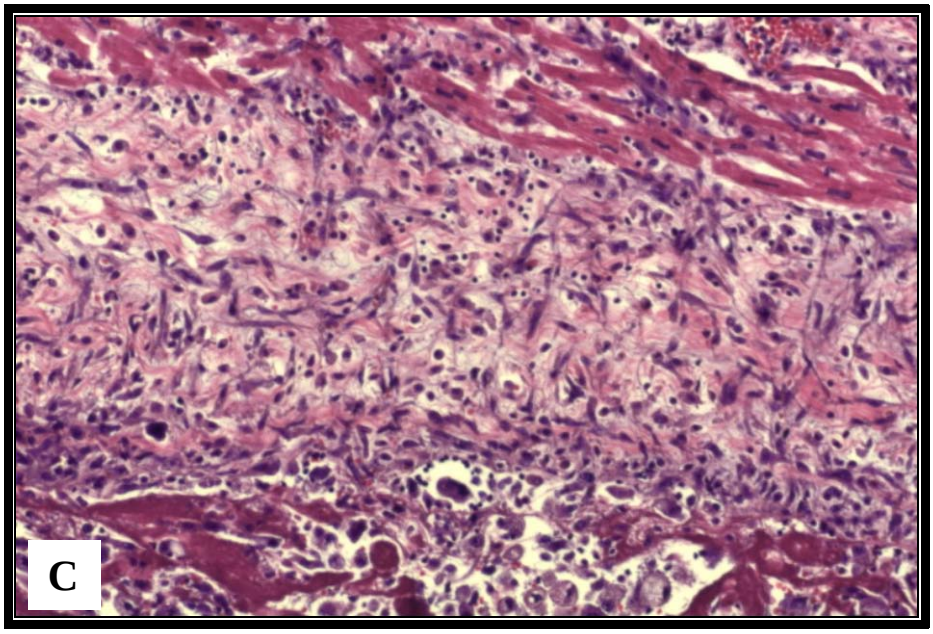
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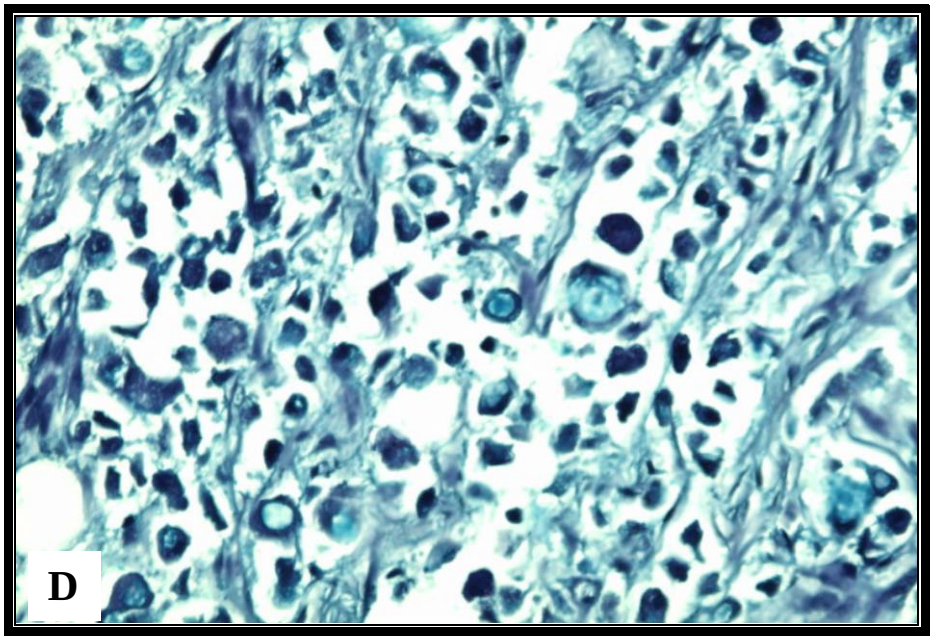
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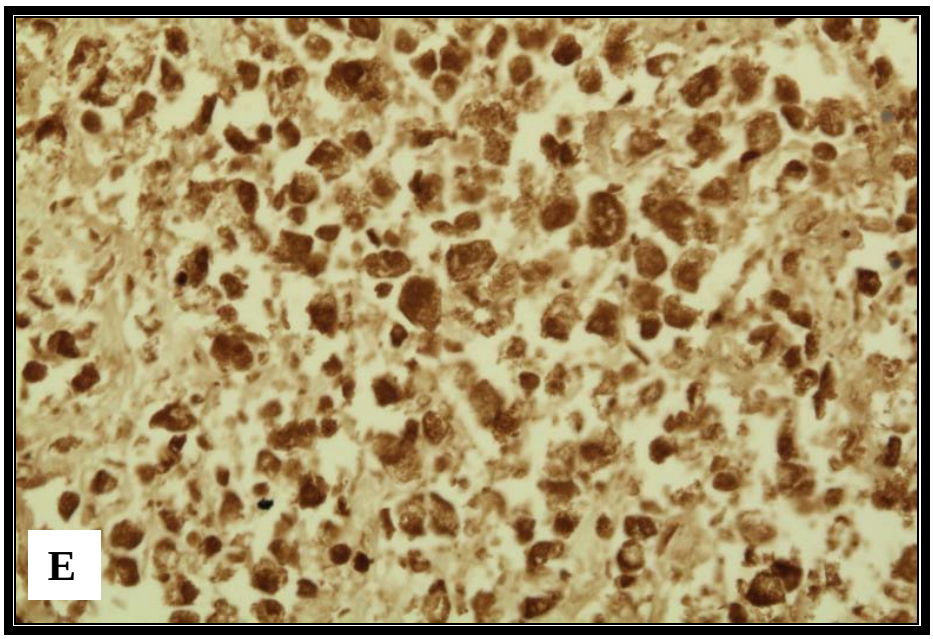

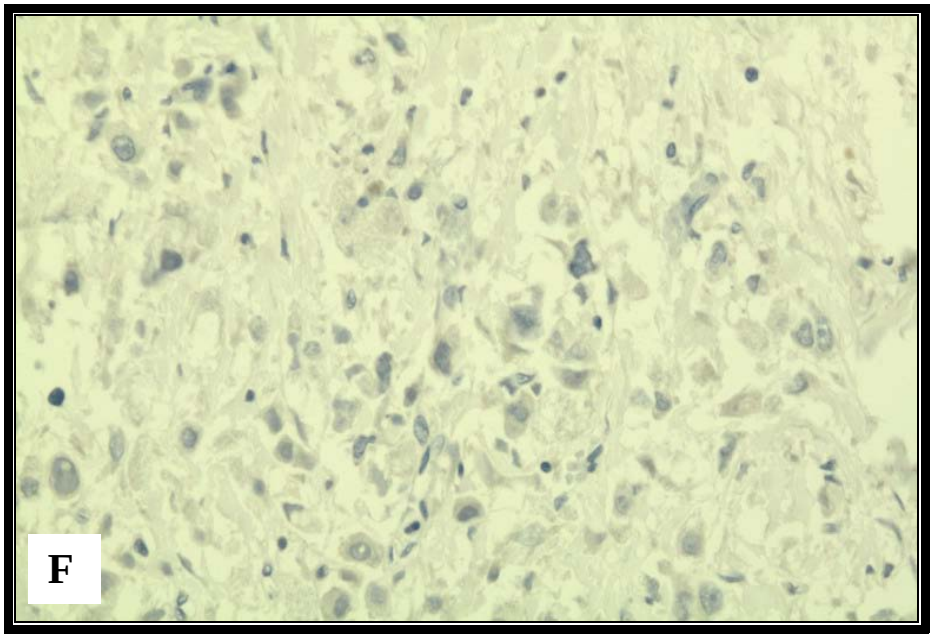

Fig.1: A. Breast with signet-ring type of malignant cells and osseous metaplasia (↓) [Haematoxylin & Eosin (H&E),x100]. **B**. Signet ring cells (↓)

infiltrating the stroma of the breast (H&E),x400). **C**. Tumour cells↓(metastases to the pericardium of the heart (H&E,x100). **D**. Signet ring cell carcinoma (↓) stained with Alcian-blue periodic acid-Schiff positive after diastase treatment (x400). Note the single file formation in (A) and (D). Immunohistochemical stain showed the tumour cells metastases to the mediastinum are positive (brown) with cytokeratin (CK) 7 **(E)** and are negative with CK 20 **(F)** [x400].

Lymph nodes
Sections from the lymph nodes, i.e, left supraclavicular, left submental and left axillary showed presence of similar malignant cells with the one in the axilla showing bony metaplasia.

Lungs
Sections from both of the lungs showed that there were multiple foci of malignant cells with similar features in the breast. They were filling the alveolar spaces as well as around the airways and in the vessels.

Heart
Sections from the irregular area of the pericardium showed presence of malignant cells admixed with fibrin (Figure 1C). The myocardium and coronary arteries were unremarkable. No infarction or atherosclerotic plaque seen.

Peritoneum
The masses from the peritoneum showed similar malignant cells as in the breast surrounded by mature adipocytes.

Intestines
The mucosa was autolysed. No evidence of malignancy seen.

Liver
Sections showed intact vascular relationship with extensive centrilobular necrosis. No evidence of malignancy seen. Intrahepatic bile cholestasis and steatosis were noted. There were mild chronic inflammations of the portal tracts.

Spleen
Sections from the spleen showed congestion.

Kidneys
Sections of both kidneys were unremarkable.

Pancreas
Sections from pancreas showed unremarkable acini.

Right adrenal gland
Sections only showed presence of malignant cells with large areas of necrosis. These malignant cells were arranged in cords, nests as well as tubular formation. The cells showed hyperchromatic and some showing prominent nucleoli. Immunohistochemically, these cells were positive for cytokeratin and CK7. They were negative for CK20, LCA, chromogranin and synaptophysin.

Female genital tract
Sections of both ovaries were unremarkable except that there was osseous metaplasia of the right ovary. The endometrium was atrophic. The cervix was unremarkable. No malignancy seen.

Cause of Death

The cause of death was given as: "Advanced signet ring carcinoma with osseous metaplasia."

Discussion

Signet-ring cell carcinoma cells (SRCC) contain entrapped mucin or mucins that push the nucleus to one side, creating their characteristic morphology.[8] This distinct morphology change, when present in more than 50% of tumor cells, is defined as SRCC. Signet-ring cell carcinoma can arise in various organs, including breast, stomach, colorectum, lung, and skin.[1,8,9,10]

Signet-ring carcinoma cells (SRCC) of the female breast ranged from 33 to 87 years with a median of 59 years and represented 2% of the total breast cancers in this large series.[11] It showed an unusual metastatic pattern with a propensity to involve serosal surfaces and mimicking gastrointestinal disease or retroperitoneal fibrosis and metastasized to any part of the body. These tumors were associated with a poor prognosis, with 60% of their 24 patients died of disease at 7 years.

Signet ring cell carcinomas of the breast are of 2 types.[1] One type is related to lobular carcinoma and is characterized by large intracytoplasmic lumina which compress the nuclei towards one pole of the cell. Their invasive component has the targetoid pattern of classical lobular carcinoma. The

other type is similar to diffuse gastric carcinoma, and is characterized by acidic mucosubstances that diffusely fill the cytoplasm and dislodge the nucleus to one pole of the cell. This type of signet ring cell carcinoma can be seen in association with the signet ring cell variant of DCIS (ductal carcinoma in situ).

In this present study the patient had 2 masses 0.4 cm apart, and no masses or lesion was seen in the stomach, intestine or lung. Invasive lobular carcinoma may present with multicentric tumours.[1] However, the main differential diagnosis is signet ring cell carcinoma metastatic to the breast from gastrointestinal tract or lung.[8,9]

Immunohistochemical workup using predominant differential pattern of cytokeratins (CK) in the tumour cells since some subsets of CKs are unique to certain tumour type. CK7 and CK20 can be used to identify SRCCs of breast and lung which are positive for CK7 and negative for CK20, while colon and some stomach are negative for CK7 and positive for CK20.[9,12] Another immunohistochemical profiling may aid in directing the workup of metastatic SRCC of unknown primary is by using mucin. Mucins are high-molecular-weight heavily O-glycosylated glycoproteins produced by secretory epithelial cells. Specific types of mucin are individually referred to as MUC and designated with a number representing. Mucin profiling of MUC1 shows strong staining of SRCC of the breast and may be useful to identify estrogen receptor / progesterone receptor-negative breast carcinomas.[8] Another study had neoplastic cells of the breast negative for both estrogen and progesterone receptors, used immunohistochemical staining with GCDFP-15 (gross cystic disease fluid protein-15) to differentiate primary gastric signet ring cell carcinoma from metastatic mammary signet ring cell carcinoma.[5]

In the present case, it is similar to lobular carcinoma in its arrangement of single-pattern growth and it is positive for CK7 and are negative for CK20, ER, PR, c-erbB-2. There is no DCIS component.

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CASE REPORT

Differentiation of the third from the first trimester degenerated retained placental tissue in forensic practice using reticulin stain

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Abstract

A 27 year old female, gravida 2 para 1, had a spontaneous vaginal delivery to a healthy baby boy. The placenta was delivered and recorded as 'complete' in the labour room case note. The mother and the child was discharged 'well'. Ten weeks later the mother was admitted to another hospital which she complained of vaginal bleeding since the time of the delivery. Dilatation and curettage revealed some degenerated tissues. A pathologist who examined the specimen and the obstetrician who performed the curettage believed that the degenerated tissue was some retained placenta from previous delivery. Another obstetrician suggested the possibility of the degenerated tissue originated from a product of new conception (missed abortion). The authors were consulted for second opinion. Even though the tissue was severely degenerated, the authors managed to show the presence of vasculo-syncytial membrane and prominent placental vessels by using reticulin stain. This confirmed the placental tisssue was that of the third trimester. It therefore confirmed that the curetted tissues originated from retained placenta of the previous delivery and not from the product of new conception.

Keywords: Placenta, reticulin, chorionic villi, abortion, product of conception.

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Introduction

Determination of gestational age of placental tissue or product of conception is not commonly done in histopathological examination of a curettage specimen. This explains why it is not included in most of histopathology reports of curettage specimens. Even though it is not an important issue in most gynecology or obstetric practice, it may become a crucial factor to determine accurate diagnosis as illustrated in the following case.

Case Report

A 27 year old female, gravida 2 para 1, had a spontaneous vaginal delivery to a healthy baby boy (Apgar score 10). The placenta was delivered by a controlled cord traction technique and was recorded as 'complete' in the labour room case note. The mother and the child was discharged 'well' and was 'advised' for a follow up.

The patient did not turn up for the follow up but was admitted to another hospital with a complaint of vaginal bleeding since the time of the delivery.

There was a discrepancy between the history written in the labour room case note of the former clinic and the history given by the patient. According to the patient, there was some problem in the delivery of the placenta. Another doctor delivered the placenta by introducing his hand into her womb (i.e. manual removal of placenta). She had a heavy vaginal bleeding which soaked two of her sarongs. She was given intramuscular injection twice. She was discharged after the vaginal bleeding stopped. She had intermittent vaginal bleeding two days later until the admission to the present hospital (about ten weeks from the time of the delivery). She went to two other clinics for treatment but she continued to have vaginal bleeding despite taking some medicines prescribed by doctors from the two clinics.

Dilatation and curettage was performed at the second hospital admission. Histopathology examination revealed some degenerated tissue with chorionic villi which was consistent with a product of conception. There were numerous foci of dystrophic calcifications.

When an obstetrician suggested the possibility of the degenerated tissue originating from a product of new conception (missed abortion), the pathologist who examined the specimen and the obstetrician who performed the curettage reviewed their diagnosis. They believed that the degenerated curettage specimen was some retained placental tissue from previous delivery.

They insisted that the presence of numerous foci of dystrophic calcifications was consistent with a term placenta rather than an early trimester trophoblast. Three other reasons which supported the above diagnosis were: (a) the patient was not likely to have sexual intercourse between her delivery and the time of curettage because of vaginal bleeding, (b) even if she had sexual intercourse, the continuous vaginal bleeding would have made conception rather unlikely, and (c) as the patient was breast-feeding her baby, ovulation would probably not have occurred and therefore conception would not have been likely. Despite these reasonings, they could not confirm their believe conclusively.

Consultation for Second Opinion

The diagnosis of the tissue originating from previous delivery was challenged by the patient and an independent second opinion (the authors) was called for.

The authors stated that, the presence of vasculo-syncytial membrane is considered as an irrefutable evidence of a term placenta in contrast to an early trophoblastic tissue. Other indicators of a term placenta are: (a) a large, well developed and more centrally located vessels in the chorionic villi, (b) the absence of 2 layers cytotrophoblast-syncytiotrophoblast, (c) the presence of syncytial knots, (d) the presence of dystrophic calcifications, (e) less prominent of Hoffbaeur cells, (f) more compact stromal villi, and (g) terminal chorionic villi of less than 40 μ in size.

Most of the indicators mentioned above were not seen in the curettage specimen due to severe degeneration and necrosis after being left in the uterus for about ten weeks after the delivery. Therefore, the authors applied reticulin stain to the specimen. The result showed well developed vasculo-syncytial membranes and more centrally located vessels in the chorionic villi of the degenerated specimen.

Apart from the numerous dystrophic calcifications, these changes confirmed the curettage specimen was that of a term placenta and without any doubt whatsoever originated from the previous delivery.

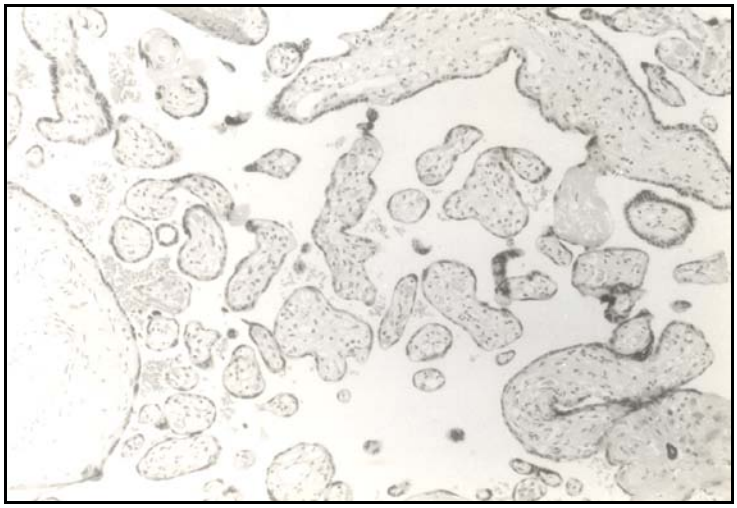

Fig. 1: Appearance of a first trimester chorionic villi with relatively thick syncytiotrophoblast and well-defined cytotrophoblastic layer. (H&E stain, x100)

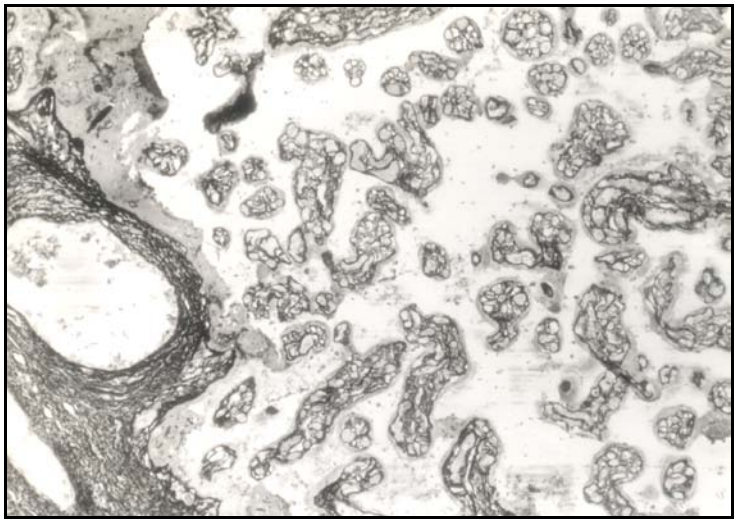

Fig. 1: Chorionic villi with vasculo-syncytial membrane of third trimester/full term placenta (Reticulin stain, x100)

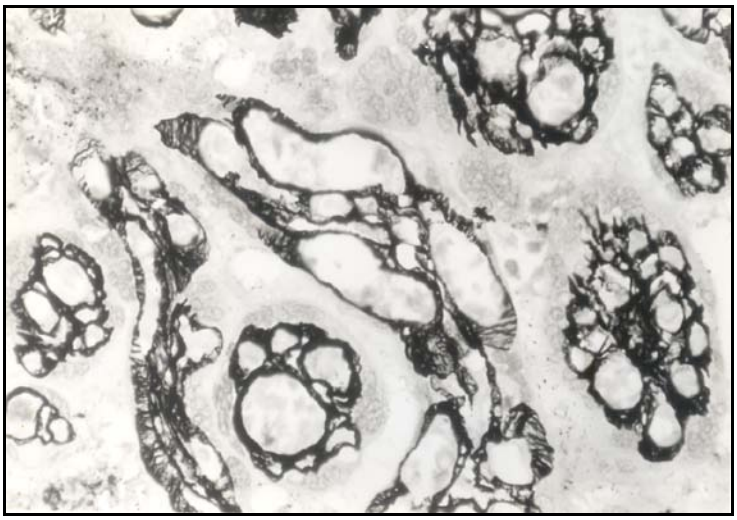

Fig. 3: Vasculo-syncytial membrane of full term placenta at higher magnification (Reticulin stain, x400)

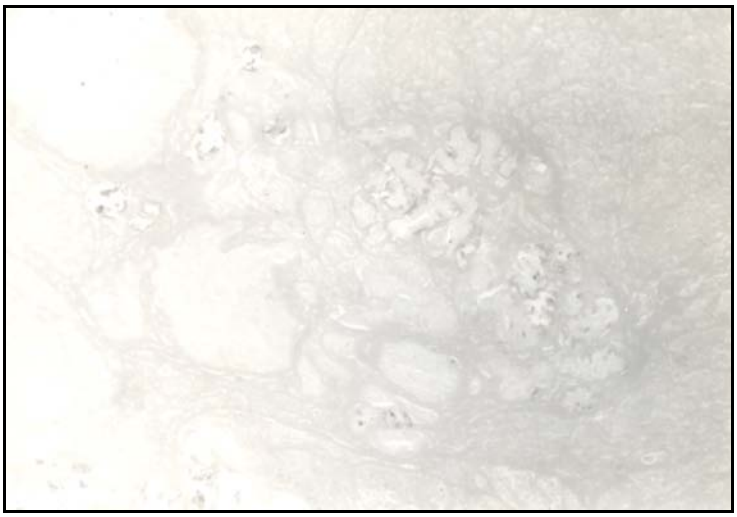

Fig. 4: Severely degenerated tissue of chorionic villi which was consistent with a product of conception. (Faded slide of degenerated tissues from curetted specimen, H&E x40)

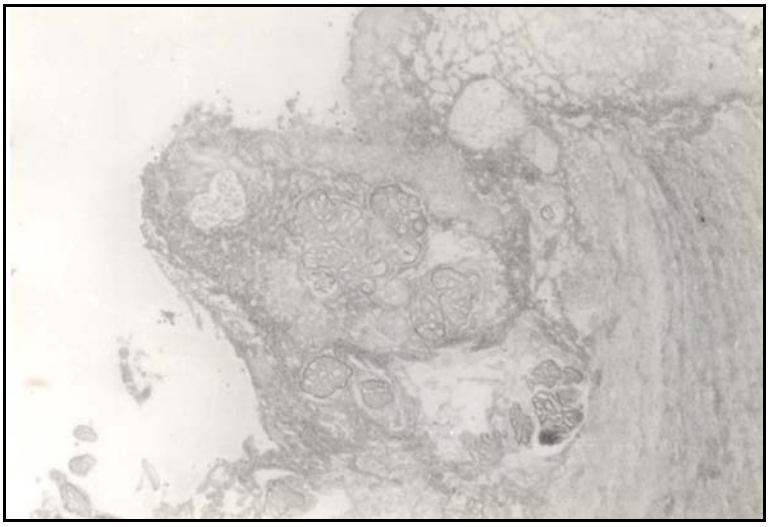

Fig. 5: Some degenerated tissue of chorionic villi which was consistent with a product of conception. (Reticulin stain x40)

Fig. 5: Well developed vasculo-syncytial membranes and more centrally located vessels in the chorionic villi of the degenerated specimen. (Reticulin stain, x400)

Discussion

The placenta is a temporary organ required for the development of the embryo and fetus. It allows for the exchange of metabolic products between the fetus and the mother. Any change or malfunction of the placenta, may affect the well being of the foetus. The morphology of the placenta changed with the gestation of the foetus. This can be seen in the gross as well as the histological appearance.

The understanding of the normal histology of placental structure is of paramount importance especially to solve cases such as in this case.

Raymond (2006) has discussed the histology of placenta comprehensively. He described that the mature placenta is composed of four distinct units of structure-function: 1) chorionic plate and its contiguous vascularized fetal connective tissue, 2) interhemal villous trophoblast and the adjacent intervillous space, 3) basal plate and the underlying maternal uterine vasculature and deciduas, and 4) tripartite placental membranes consisting of amnion, chorion, and decidua.

The chorionic plate (or fetal surface) consists of the fibrous connective tissue supporting the large muscular arteries and veins that distribute fetal blood flow from the umbilical cord to a family of 20-30 large villous trees (Boyd and Hamilton 1970; Benirschke and Kaufmann 2000). The umbilical cord is a squamous epithelial lined conduit normally measuring between 40-80 cm at term that conducts fetal blood from the umbilicus to some location on the chorionic plate (or occasionally to the adjacent placental membranes). It contains paired arteries that spiral around a central vein; all surrounded by a hyaluronate rich matrix (Wharton’s jelly) which provides considerable protection from external compression. The two arteries are connected at or just before their insertion site into the chorionic plate by an interarterial anatomosis (Hyrtyl's anastomosis). Villous trees emanate from the underside of the chorionic plate branch multiple times as they conduct fetal blood through a succession of smaller arteries and veins until they reach capillaries that abut the trophoblastic interhemal membrane in the terminal villi. These conducting villi are sometimes referred to as primary, secondary, and tertiary stem villi with the last representing the arteriolar level at which blood flow to the gas exchanging terminal villi is ultimately regulated.
Interhemal villous trophoblast consists of a single multinucleated layer of differentiated syncytiotrophoblast with underlying basement membrane and occasional basally located cytotrophoblast stem cells. Each stem cell is the progenitor for 80-100 fused syncytiotrophoblastic cells and these large syncytial sheets form a mosaic covering the entirety of the villous tree

(Simpson, Mayhew and Barnes 1992). Turnover of syncytiotrophoblast occurs via clustering of nuclei in syncytial knots followed by apoptosis and shedding into the maternal circulation (Mayhew and Barker 2001).

The basal plate consists of 80-100 anchoring villi, a similar number of perpendicularly oriented perforating maternal arteries, and tangentially oriented draining maternal veins. Uniting these elements into a coherent anatomic structure is intermediate trophoblast, which arises from cytotrophoblast stem cells on the underside of the anchoring villi. Closely related endovascular trophoblast is normally present throughout the wall of basal plate arteries and on the placental aspect of the large maternal veins.

The placental membranes at first glance appear distinct from the first three compartments. While this is certainly true in terms of function the anatomic differences are minor. The membranes form by involution of the placenta and retain all of its layers. The fetal surface of the membranes is covered by amnion and consists of chorionic connective tissue and occasional chorionic villi, albeit without fetal blood vessels. The villous trophoblast coalesces as the intervillous space is obliterated to form a third distinct morphologic variant of trophoblast known as chorion laeve or epithelioid trophoblast. This noninvasive trophoblast layer is supported by underlying maternal decidua, which lacks the remodeling seen in the basal plate.

To summarise the histology characteristics of a term placenta by other authors, i.e. it consists of vasculo-syncytial membrane, a large, well developed and more centrally located vessels in the chorionic villi, the absence of 2 layers cytotrophoblast-syncytiotrophoblast, the presence of syncytial knots, some dystrophic calcifications, less prominent of Hoffbaeur cells, more compact stromal villi, and terminal chorionic villi of less than 40 μ in size (Wynn 1975; Wigglesworth 1984; Gersell, Kraus and Riffle 1987). The above mention features does not present in early trophoblastic placenta.

The general pattern of vasculo-syncytial membrane and the more centrally located vessels in the chorionic villi of a term placenta can be revealed by reticulin staining. The reticulin stain such as Gordon Sweet's method is a silver impregnation method that demonstrates reticular fibers in any organ including placenta.

In this case, the authors managed to demonstrate general pattern of vasculo-syncytial membrane and the more centrally located vessels in the chorionic villi of a term placenta by reticulin staining even though the curetted tissues were severely degenerated after being retained in the uterus for about ten

weeks. These changes cannot be seen or very difficult to be demonstrated with the routine haematoxylin & eosin stain.

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