DOI:10.21276/sjpm

Saudi Journal of Pathology and Microbiology
Scholars Middle East Publishers
Dubai, United Arab Emirates
Website: http://scholarsmepub.com/

ISSN 2518-3362 (Print)
ISSN 2518-3370 (Online)

Review Article

Health Hazards of Special Stains

Bharadwaj Bordoloi[1]*, Rohit Jaiswal[2], Safia Siddiqui[3], Aanchal Tandon[3]

[1]Post Graduate student, Department of Oral Pathology, Sardar Patel Post Graduate Institute of Dental and Medical Sciences, Lucknow, India

[2]Professor and Head, Department of Oral Pathology, Sardar Patel Post Graduate Institute of Dental and Medical Sciences, Lucknow, India

[3]Senior Lecturer, Department of Oral Pathology, Sardar Patel Post Graduate Institute of Dental and Medical Sciences, Lucknow, India

***Corresponding Author:**
Bharadwaj Bordoloi
Email: bharadwajbordoloi@gmail.com

Abstract: Special stains are dyes that are used for special purpose and are not used routinely in a histopathology laboratory. They are of special interest in research and diagnostic. Most of the special stains are synthetic stains and are composed of harmful chemicals. Continuous and regular exposure to them possesses serious risks to health. Many of the synthetic special stains are found to be genotoxic, mutagenic, immunotoxic and carcinogenic. Their safe handling and disposal is very important from the point of view of health and environmental safety. This review focuses on the effects of the chemical dyes on human health**.**

Keywords: Special stain, dye, health, safety, hazards.

INTRODUCTION

Histology is the study of microscopic structures of cells and tissues and staining is the artificial coloration of a substance to facilitate its examination by the use of a colored organic molecule called dye [1]. Histological staining involves series of techniques applied for staining sections of tissues that help in visualization of the cellular structures under a microscope. Staining is used to highlight important features of the tissue as well as to enhance the tissue contrast. Histological staining is commonly used for pathological diagnosis and in forensic studies [2]. However, there are other several staining techniques used for particular cells and component [3]. The most common and routine stain used in histopathology is a combination of haematoxylin and eosin (H&E). However, it simply cannot answer all the questions that a case poses at the plain diagnostic level, and it is clearly insufficient when one engages in an etiologic, histogenetic, or pathogenetic quest. As a consequence, the pathologists have always searched for additional techniques to probe those questions. Colloquially, these techniques have been referred to as 'special', simply because they are applied only under special circumstances [4].

The term "special stains" has long been used to refer to a large number of alternative staining techniques that are used when the H&E does not provide all the information the pathologist or researcher needs [1]. Special stains are not routinely used. The term "special stains" is of uncertain provenance, but one can be certain that it began to be used after 1876 when H&E was introduced [5]. Special stains use a variety of dyes and techniques to stain particular tissues, structures or pathogens (such as bacteria) to assist pathologists with tissue-based diagnosis [6]. It covers a wide variety of methods that may be used to visualize particular tissue structures, elements, or even microorganisms not identified by H&E staining [7].

Special stains have two broad areas of application: research and diagnostic. In a histopathology laboratory, their use is unavoidable alongside the routine staining techniques. Most of the special stains are synthetic chemicals and many of the risks in a laboratory will relate to chemical hazards. Each chemical, therefore, needs to be considered carefully while using [7].

DISCUSSION

Dyes are classified into natural and synthetic dyes [8]. Haematoxylin, which is obtained from the Mexican tree *Haematoxylon campechianum*, is an example of a natural dye that is widely used in histology and histochemistry, while eosin is a synthetic dye also used in histology [9]. Synthetic dyes are more

efficient but have been reported to be hazardous to human health [10].

Effects of chemical dyes on human health

A wide range of chemicals which are potentially dangerous are employed in pathology laboratories [11]. All chemical substances may be harmful depending on the dose, duration and conditions of exposure. Nearly all the chemicals can be irritants, given sufficient exposure to tissue and can cause reversible inflammation, specially, in eyes, skin and respiratory passages. The alkaline substances, strong acidic substances, dehydrating and oxidizing agents are particularly corrosive and can damage or destroy living tissues. Chemicals that are sensitizers cause allergic reactions in a substantial proportion of exposed subjects. Sensitization may occur at work because of the high exposure level. A prime example here is formaldehyde, which is used universally as a tissue fixative [12]. Special stain, such as modified Gallego Stain, which is used for differentiation of bone, dentine and cementum, contains formaldehyde in one of its components [13].

Many of the stains and dyes used for staining in a histopathology laboratory are extremely harmful to humans and other animals[14]. Toxicity, carcinogenicity, genotoxicity, immunotoxicity are only some of the harmful effects of the standard stains and dyes available to us [15-17]. For example, crystal violet, a common stain used in microbiology labs for staining is a possible carcinogen (as proved by a study conducted on mice) and also very toxic to aquatic organisms [18]. Similarly, there are thousands of dyes and many have been implicated in causing cancers in rats in experimental conditions. All should be handled with due caution when in the powder state, but liquids pose little risk except through skin contact and ingestion. Chemicals including chloroform, chromic acid, dioxane, formaldehyde, nickel chloride and potassium dichromate are known to be carcinogenic [12]. Dyes such as; aniline, auramine O, basic fuchsin, ponceau 2R, and benzidine derived dyes, such as Congo red, diaminobenzidine and chlorazol black E, are carcinogenic [12,19,20]. Benzidine has long been recognized as a human urinary bladder carcinogen and tumorigenic in a variety of laboratory animals. They must be treated accordingly in both handling and disposal [21].

Azo dyes, an important group of synthetic colourants, pose toxicity (lethal effect, genotoxicity, mutagenicity and carcinogenicity) to aquatic organisms as well as animals. Azo dyes are aromatic hydrocarbons, derivatives of benzene, toluene, naphthalene, phenol and aniline. The chronic effects of these dyes have been studies for decades and their environmental and health effects are increasingly becoming subject to scientific scrutiny [21].

Toxic materials are capable of causing death by ingestion, skin contact, or inhalation at certain specified concentrations. Some countries use the term ‘poison’ when referring to these materials. Toxic chemicals pose an immediate risk and some are so dangerous that they are designated as ‘highly toxic’. Among the more familiar toxic substances are the cyanides and heavy metal salts which cause acute or chronic poisoning. Osmium tetroxide, chromic acid, and uranyl nitrate are highly toxic [12,19,20, 22]. The vapours of osmium tetroxide are extremely dangerous. All contact should be avoided with vapours. They are corrosive to eyes and mucous membranes. The containers should not be opened in air [12]. Osmium tetraoxide, which was earlier used for detection of unsaturated lipids, is no longer used in view of its toxicity [12].

Chemicals causing specific harm to select anatomical or physiological systems are said to have target organ effects. Their effects are not immediately evident, but are cumulative and frequently irreversible. Solutions containing heavy metals such as mercury, lead, arsenic etc may have target organ effects and are injurious to health on long term exposure [12]. One of the stains containing mercuric chloride is Golgi-Cox stain, which remains a key method to study neuronal morphology *in vivo* [23]. Similarly, reagents used in Millon reaction for tyrosine contains mercuric sulphate. Mercuric solutions may be severe skin and eye irritant, may have target organ effects on reproductive, urogenital, respiratory, gastrointestinal and fetal systems following ingestion and inhalation. They are also severe environmental hazards [12].

Physical risks of chemicals/dyes

Many of the organic solvents, used in routine and special stain techniques, have flash points below 21°C and they are highly flammable. Explosive chemicals are rare in histology, examples of common explosive chemicals that are used in special stain techniques are picric acid and silver solutions. Picric acid is an important constituent of Masson’s trichrome and van Gieson’s stains. Picric acid is toxic by skin absorption and explosive when dry or when complexed with metal and metallic salts. Silver solutions are commonly used in von Kossa stain and Masson Fonatana stain. Silver solutions may become explosive upon aging and they should never be stored after use. Silver salts and solutions are skin and eye irritants. Ingestion will cause violent gastrointestinal discomfort. They are also serious environmental hazards. Sodium iodate, mercuric oxide and chromic acid are some of the examples for oxidizers [12].

Risk assessment

In the risk assessment, it should be standard to identify how the material should be stored or transported safely, how it can be used safely along with its safe disposal. It should also include the steps to be

taken in case of an emergency situation including the medical treatment of the affected person [7].

Chemical exposure has 2 dimensions, both of which need to be considered. One is concentration- the more concentrated a toxic material is; the more likely it is to cause damage. This is particularly the case with volatile materials. Materials that can be breathed in. The second aspect is length of exposure[7]. Manufacturers usually provide data hazard sheets (DHS) with their products and before using any chemical, one must read the DHS and the level of precautions that has to be taken [7]. A file of DHS should be kept in secure location, and employees must be given reasonable access to it[12]. If data hazards sheets are not readily available, one must access the safety information available in online databases. The exposure is related to time; thus, time-weighted average (TWA) levels indicate the maximum concentration averaged over a complete working day (this may allow higher levels for short periods of time provided they are balanced by lower levels at other times); the short-term exposure limit (STEL) indicates the time-weighted average for a 15 min period, which controls the levels during the peak times; and the ceiling limit of ceiling exposure value gives a value that must never be exceeded, even for a brief period of time. These phrases and codes are often specific to one country or even one regulatory authority within a country, so other terms may be found such as threshold limit value, permissinle exposure limits and maximum allowable concentration [7].

Even for substances with no known significant hazard, exposure should be minimized. Unless known otherwise, assume that any mixture will be more toxic than its most toxic component and all substances of unknown toxicity are hazardous. The permissible exposure limits (PEL) of the Occupational Safety and Health Administration (OSHA) should be observed [22,24].

CONCLUSION

Special stains are indispensable in a histopathology laboratory and they provide immense help to a histopathologist. Use of synthetic, potentially harmful dyes may be limited but cannot be eliminated completely until and unless a suitable alternative comes up. However, safe handling of most of the hazardous chemicals can be achieved with minimum effort and equipment, but a few chemicals are too dangerous to be used and they must be eliminated or reduced to the smallest quantity possible. As toxicology of most chemicals is not well known and dangerous effects of exposure accumulate subtly, it is prudent to limit exposure, avoid and eliminate their uses and look for safer alternatives.

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