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To investigate the potential carcinogenic effects of Keratin treatmentand identify underlying mechanisms that may contribute to cancer

Aryan Bhardwaj[1], Ashish thakur[2], Astha sharma[3], Nancy sharma*

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ABSTRACT: The term keratin is generally accepted to refer to the epithelial keratins of soft and hard epithelial tissuessuch as skin, cornea, and nail. Intermediate filament proteins are a key group of cytoskeletal proteins found in animals, crucial for maintaining the structure of cells and tissues . In cancer ,IF proteins can act as diagnostic markers because tumor cells often keep some of their original .If proteins are involved in essential cellular functions such as growth programmed cell death , movement and invasiveness, which affect tumor formation, growth and traits . The review examines 3 IFproteins closely linked to cancer keratins, vimentin and nestin and their relationship to the “hallmarks of cancer”. Cytokeratins CK18 and CK8, found in various adult epithelial tissues, may contribute to cancer development.The most commonly used hair straightening products may contain formaldehyde (FA) or a "hidden" formaldehyde donor (able to break the bonds of the hair permanently at high temperatures, forcing it to remain straight and smooth until new hair grows). Hair straighteners are also causing new onset of asthma. Various methods has been used for analyzing the formaldehyde exposure .

I. INTRODUCTION:

The intermediate filaments consist of a large number of nuclear and cytoplasmic proteins that are expressed in a tissue and differentiation dependent manner. The filaments are highly conserved during evaluation and shows a high degree of conservation among species.(1)The intermediate filament protein family is vital for keeping cells structurally sound and is involved in many cellular activities including differentiation, growth, and stress responses. Keratins, a key group within this family, are important structural proteins found family, are important structural proteins found in epithelial cells.(2) They create networks of intermediate filaments in the cytoplasm and are divided into categories based on their amino acid sequences : type 1(acidic) and type 2 (neutral-basic). These keratin filaments that support cell structure and assist in various cellular functions. (3)

One of the most researched keratins is CK18, a type 1 intermediate filament protein mainly found in simple epithelial tissues like the liver, lungs, and digestive system.CK18 is crucial for the structural stability of these cells and plays a role in progression, and signaling. When CK18 pairs with its type 2 partner CK8, they create stable keratin filaments essential for maintaining cell structure. Studies have shown that CK18 function and stabilities are regulated by its phosphorylation recent research also suggest that posy – translational modifications, like O-GlcN- acylation ,affect CK18 solubility and filament organization.(4)

Keratin 17(K17) ,another type 1 keratin, is usually found in epithelial structures like hair follicles and sebaceous glands .K17 is not typically present in healthy skin but is produced in response to stress, such as skin injuries or viral infections like psoriasis.(5) It is involved in managing skin inflammation and the development of skin appendages .Notably, K17 can move between the cytoplasm and nucleus, suggesting it may impact other cellular processes as well.(6)

The cytoskeleton is a network of proteins that provides structure within the cell’s cytoplasm, Eukayotic cells have 3 main types of cytoskeletal filaments: microfilaments, intermediate filaments(Ifs), and microtubules. Microfilaments are made of 6nm actin chains that twist together. They help the cell resist tension, keep its shape, create extensions, and aid in interactions between cells and their surroundings. Intermediate filaments, which are 10nm in diameter ,are more stable than actin filaments . they help organize the cells’ internal structure and also support cell shape by bearing tension. Microtubules are hollow cylinders with a diameter of 23nm, usually made of 13 proto filaments formed from alpha and beta tubulin. They are important for transporting

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materials within the cell and for forming the mitotic spindle during cell divison.(7)

Unlike actin filaments and microtubules, intermediate filaments are produced by a large group of genes that are specific to different tissues and stages of cell development. They are categorized bases on amino acid sequence of their rod domain. Type 1 and Type 2 IFs are mainly found in epithelial cells and include acidic and basic keratins. Type 3 IFs consist of vimentin, desman and glial fibrillary acidic protein. Type 4 IFs form neurofilaments, while Type 5 IFs are the nuclear Lamins.(8) All intermediate filaments have a central rod domain made of two coiled alpha helices, with varying lengths of head and tail domains.(9)

The cosmetic industry, particularly in capillary aesthetics has seen significant attention due to the high demand for products that treat and beautify hair. Among these hair straighteners are popular for their ability to enhance appearance,social acceptance and ease of maintenance. However, such treatment can alter the chemical structure of hair, raising concerns about safety and potential hair damage. For effective hair straightening, it is crucial to understand the hair's composition and structure, as well as the products and techniques used. This review examines the morphology, chemical structure and risks of hair straightening treatments, alongside their impact on hair health and legal considerations.(10)

Formaldehyde has been used from long time in homemade and commercial hair straightening products to prolong the straightening effects of product.(11) The various number of active ingredients are used in hair straightening products and they vary according to product. Some of them are sodium hydroxide, calcium hydroxide, formaldehyde, pyrogallol, thioglycolic acid and others. The commonly used compound which alters the structure of hair protein is formaldehyde.(12)

Structure of the hair fiber

The hair is an appendage derived from the epidermis; it is a keratinized structure formed from the invagination of the epidermis into the dermis. From this invagination, small saccular structures called hair follicles originate. Thus, it can be divided into two major parts; the hair follicle and the hair shaft. The hair shaft extends from its root or bulb. Passing through the various layers of the epidermis, surpassing the stratum corneum and then continuing with a stem. Despite its shine, body, and texture, it is a dead structure.(10)

Hair follicles are essential growth structures of hair, being strongly invaginated into the scalp tissue. At the base of each hair follicle, cells proliferate in up flow. The complex and intertwined processes of protein synthesis, structural alignment and keratinization transform the cytoplasm of these cells into a fibrous material known as hair. Thus, the primary component of hair fiber is keratin (about 65-95%), the remaining constituents being represented by other proteins, water, lipids (structural or free), pigments and trace elements (10-12).(13)

Hair fibers (about 50-100µm) in diameter are not continuous in their entire length, but rather the result of the combination of compact groups of cells within the follicle, from which originate three basic morphological components: a) the cuticle , which is outermost region covering the core of fibers; b) the cortex; which comprises most of the hair volume (75%) and is responsible for sustaining the hair shaft ;and c) the medulla, which is the central area of the hair and is not always present .

The cuticle is composed primarily of keratin and display a stepped structure with five to ten superimposed flat overlapping cells of 0.3-0.5 micrometer thick. The cuticle encircles the cortex, which forms the most voluminous part of the hair fiber and is comprised of microfibrils consist of keratin intermediate filament also known as microfibrils. The cortical cell is spindle shaped about 100µm long and generally 1-6µm thick. The hair appearance importantly depends upon the health of cuticle. When the cuticle is strong and healthy then the hair appears to be strong and healthy. Intact and closed cuticle act as a protective shield against harmful environmental elements. From a cosmetic point of view, the cuticle is very important component of hair fiber, the cortex also has a great cosmetic importance as its optical properties strongly affect the color and shine of the hair fiber.(14)

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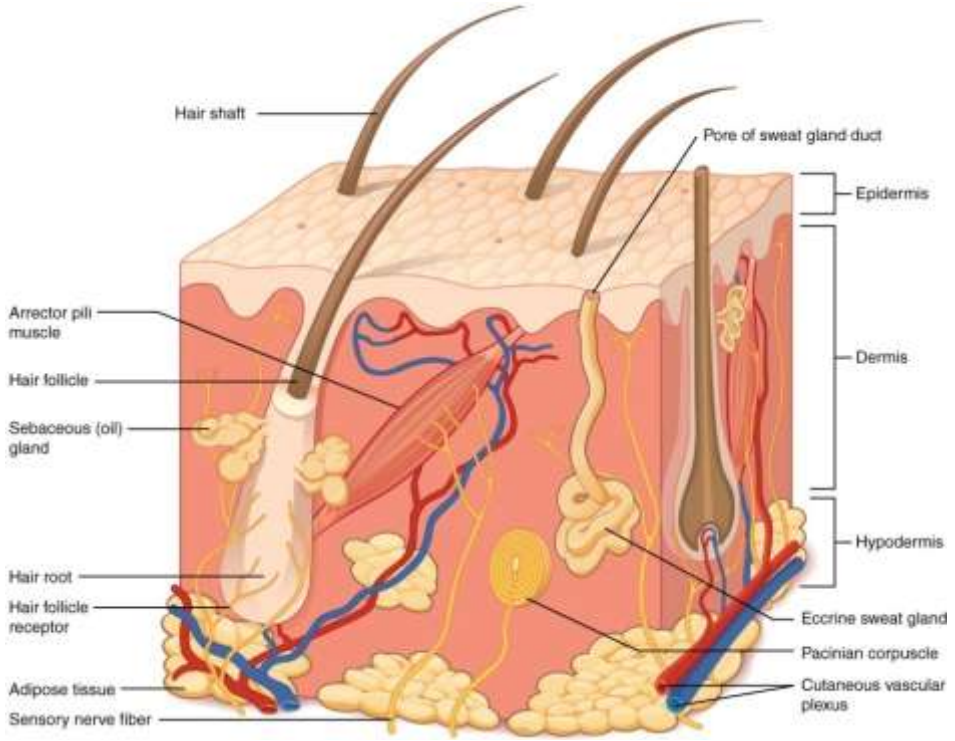

Figure 1: Structure of the human skin showing hair as epidermal annex

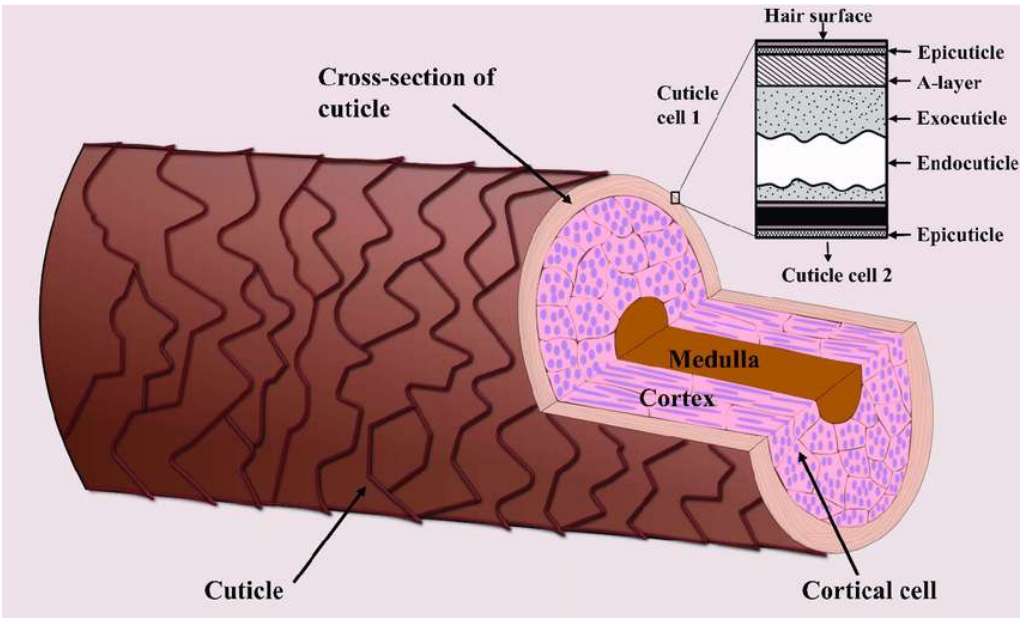

Figure 2: Schematic cross-section of hair fiber showing medulla cortex and cuticle cell layers

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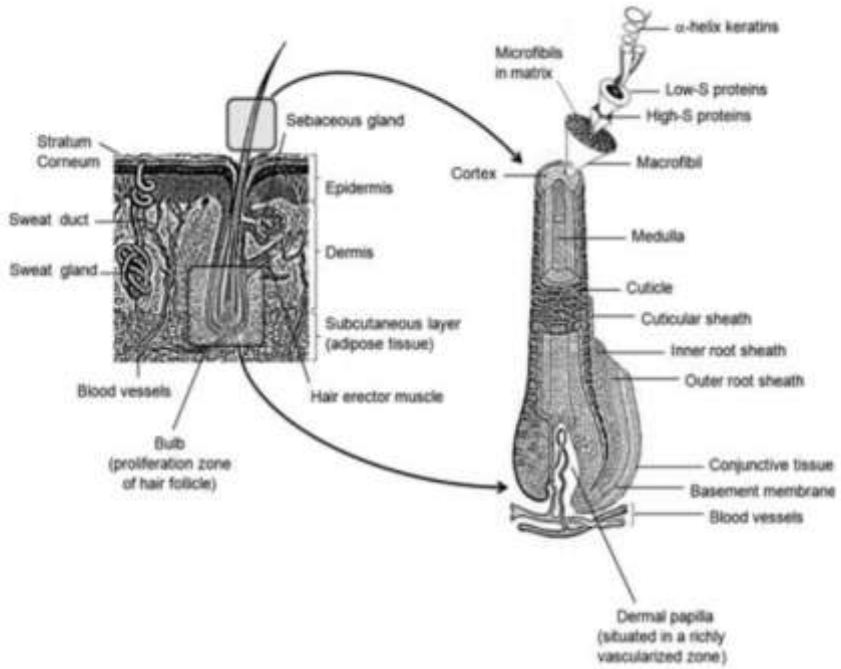

Figure 3: Schematic representation of human hair fiber structure and its insertion into scalp

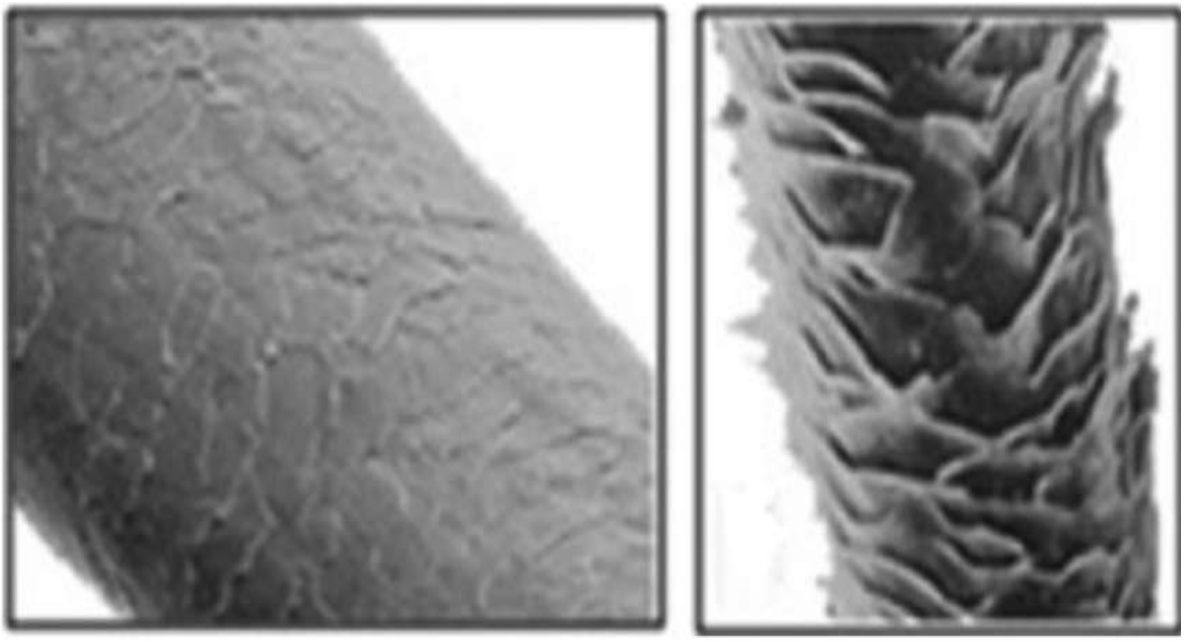

Figure 4: Cuticles scales closed and open

II. LITERATURE REVIEW :

Keratins

Keratins are a group of more than 30 proteins that make up the cytoskeleton. They are known as intermediate filaments because their diameter ranges from 7 to 11 nm, which is between microtubules (20–25 nm) and actin microfilaments (5–6 nm). These proteins are large polymers made of long chains of amino acids connected by various interactions. Keratin fibers are formed from these long chains that are tightly woven together through strong covalent bonds, including disulfide bonds, as

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well as weaker interactions like hydrogen bonds, electrostatic forces, and Vander Waals forces. When water is present, hydrophobic bonds also play a role.

Research using electrophoresis has classified hair keratins into two groups: type I and type II. Type I keratins are acidic and have molecular weights between 40 and 48 kid, while type II keratins are basic to neutral, with molecular weights ranging from 58 to 65 kid. Heid et al. (1986) identified eight main hair keratins, four from each type, with additional keratins discovered later. These keratins are organized in pairs of type I and type II chains and differ from those found in the skin by having a higher cysteine content (7.6% compared to 2.9%) and forming disulfide bonds between adjacent cysteines, which creates a stronger and more resilient structure.(15)

Intermolecular Bonds, stability and strength of hair fiber

Disulphated Bonds

The elevated cysteine concentration in the protein that constitutes human hair significantly influences the physical characteristics of the fibers. Adjacent cysteineresidues are interconnected, formingcystine, which acts as a bridge between two proteins or different segments of the same protein. In hair fibers, the prevalence of these cross-links (0.8 mM g^{-1}) andtheir vulnerability to oxidation or reduction are crucial for various chemical alterations of hair, which in turn impact the physicochemical properties of the hair fibers.

Coulomb Interactions

The substantial presence of acidic and basic side chains (1.6 mM g^{-1}) leads to the formation of Coulomb interactions that remain relatively stable in aqueous conditions but can be easily disrupted by acids and alkalis.

Hydrogen Bonds

Despite being relatively weak and easily disrupted by water, hydrogen bonds are the most abundant (approximately 4.6 mM g^{-1}) within the hair fibers. These interchain bonds, formed between the amide groups along the polypeptide chain, are vital for maintaining the stability of the α-helix structure of keratins.(10)

What determines the curl of the hair fiber?

Hair evaluations show no significant biochemical differences among ethnic groups, but some structural differences are noted. All hair types share common features in morphology, chemical makeup, and molecular structure, yet the shape of hair varies widely across different ethnicities. This leads to the classification of hair into three main groups: African, Caucasian, and Asian, which helps identify specific traits like color and curl pattern. Methods for classifying hair based on curvature, regardless of ethnic background, have been outlined in other studies and aid in standardizing terminology in hair science. Despite the variability in the data, the amino acid composition of hair fibers remains consistent, with overlapping concentration ranges that do not significantly differ by ethnicity. Additionally, all hair fibers, regardless of ethnicity, contain a high level of cystine disulfide bonds, which play a crucial role in the fiber's stability. (10)

Shape of the hair follicle:

The shape of hair fibers is determined during their development, particularly during the keratinization process, when the fibers harden. It makes sense to think that the shape of the hair follicle in the keratinization area influences the hair fiber's shape. This means that the growing fiber takes on the shape of the follicle, where it hardens. If the follicle is curved in the keratinization area, the resulting hair will be very wavy. Conversely, if the follicle is straight, the hair will be straight. The shape of the hair's cross-section and its growth are closely linked to the hair follicle's shape and its location on the scalp. The hair's cross-section is typically elliptical, which can be closer to circular. Just like a thin strip is easier to twist than a thick rope, hair with a flat, thin cross-section, like that found in African hair, tends to be curled or crimped, forming rings of a few millimeters in diameter. In contrast, hair with a thicker, cylindrical cross-section, like Asian hair, remains straighter.(16)

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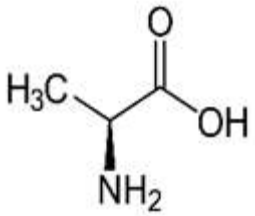
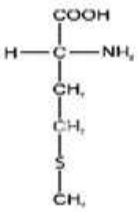
Table 1: Ranges of amino acid composition in the whole cosmetically unaltered hair fiber and of human hair from various ethnic origins

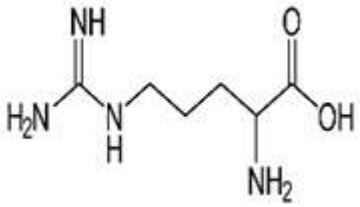
No.	Amino Acid	Africoid	Caucasoid	Mongoloid
1	Alanine	44.3	45.3	45.9
2	Arginine	52.1	55.9	57.2
3	Aspartic acid	58.5	53.6	58.0
4	Cystine	3.3	2.3	3.0
5	Glutamic acid	150.0	139.0	143.0
6	Glycine	120.4	115.3	117.2
7	Histidine	56.2	59.1	56.5
8	Isoleucine	7.2	8.3	7.3
9	Leucine	23.3	20.8	24.4
10	Lysine	54.9	55.8	58.2
11	Methionine	16.9	23.7	18.7
12	Phenylalanine	1.7	4.6	2.6
13	Prolyne	13.9	14.8	14.4
14	Serine	74.6	76.2	72.0
15	Threonine	114.5	124.7	113.8
16	Tryptophan	68.4	67.6	67.2
17	Tyrosine	19.1	19.1	18.9
18	Valine	41.1	44.2	50.2



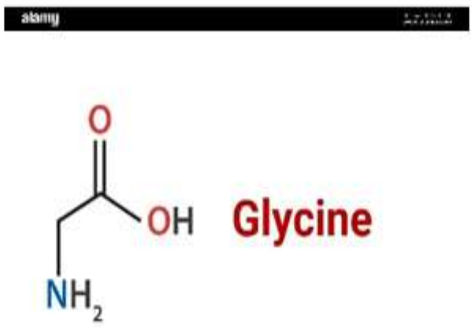
Alanine



Methionine



Arginine



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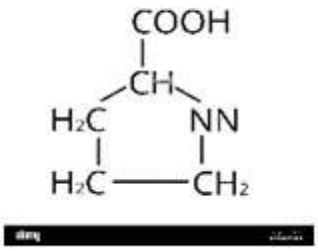
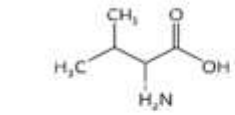
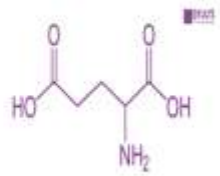
Proline

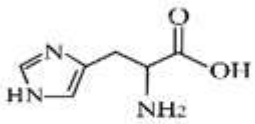


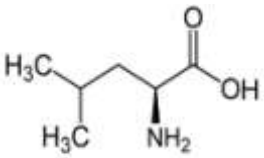




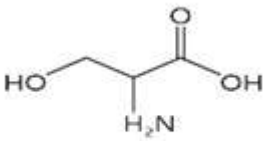
Glutamic acid

Histidine





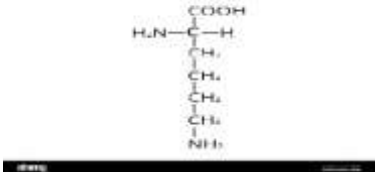
Leucine



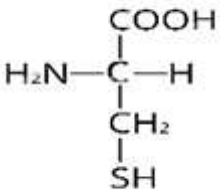
SERINE

Tryptophan

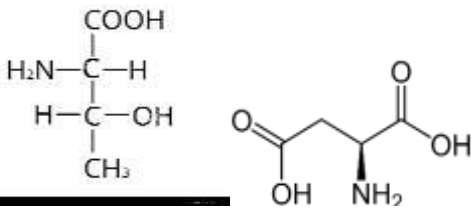
Lysine



Cysteine



Threonine



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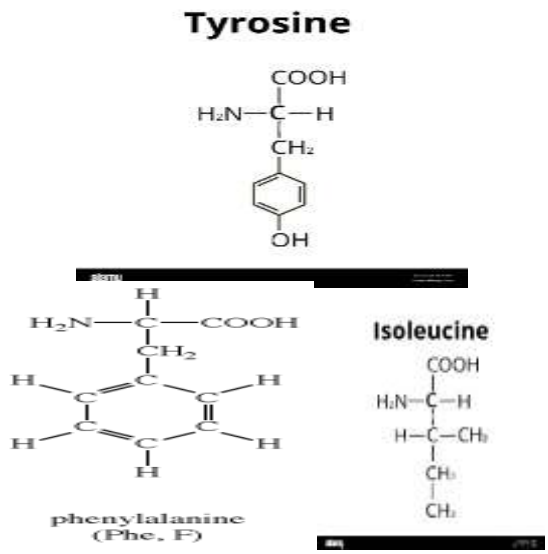
Aspartic acid



- The ranges of composition of amino acids were assembled from the results of Robbins and Kelly (1970) [21], Ward and Lundgren (1955) [22] and Clay et al. (1940) [23], obtained from Robbins (2002) [24]. Whole fiber results approximated by cortex analysis
- Data obtained from Wolfram (2003) [9].
- Cysteines (Cyst) are usually determined by quantification of their oxidation product, cystic acid, generated by treatment with performic acid.
- Cystine (Cs) is a dimeric amino acid formed by the oxidation of two Cyst residues that are covalently linked through a desulphated bond. It only forms after the protein chain have been synthesized and the protein starts to fold.
- Both Cyst and Cs residues can be oxidized to cystic acid.

Table 2: Variation in growth and cross section of hair, according to ethnicity

Hair type	Growth	Shape	Diameter (μm)	Characteristic
African	0.9 cm per month	Flat oval	44-89	As hair grows almost parallel to the scalp, it grows curled
Caucasian	1.2 cm per month	Almost oval	47-74	Hair grows at an oblique angle to the scalp and is slightly curved
Asian	1.3 cm per month	Almost circular	71-92	The mode as the follicle is implanted causes the hair to grow straight and perpendicular to the scalp

Figure 8: Classification of hair samples (a)Straight, (b) Wavy, (c) Curly

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Straightening

Hair straightening can be done in two main ways: mechanically by pressing or chemically with relaxers. This method is especially popular among black individuals.(17)

Pressing: Pressing was created by Madame C. J. Walker in the late 1800s when she introduced the hot comb. This method gives temporary straightening by altering weaker hydrogen bonds. Today, only a small number of people still use pressing. The original process involved applying an ointment to the hair and then using a hot metal tool to press it. Over time, the technique has improved with better tools like combs, flat irons, and curling irons, as well as the use of electric heaters. Pressing oils have also been enhanced to keep hair soft and not greasy.Before starting, the hair is washed and dried, then an oil or petrolatum-based ointment is applied. A hot metal comb is then used from the roots to the tips, section by section, until the hair is straightened. After that, a hot curling iron is used to curl the hair. The hot comb can reach temperatures between 300–500°F. However, this process can lead to hot comb alopecia.(18)

Chemical Relaxing: Chemical relaxing, also known as pantheonization, is now the preferred method for straightening very curly hair and is commonly used in certain ethnic groups. Hair relaxing is similar to permanent waving, but instead of curling, it permanently straightens curly hair. This is done by breaking disulfide bonds with an alkaline reducing agent, then straightening the hair with a comb during the process to rearrange the disulfide bonds into new polypeptide keratins. Finally, an oxidizing agent is used to set these new bonds.

For permanent waving, liquid solutions are more effective because they allow for even application despite the rollers. This issue does not arise with relaxing treatments.(19)

Conditioning

Hair conditioners were created to bring back the manageability, shine, and softness that sebum usually provides. They became popular after shampoos with effective cleaning properties were introduced, which remove too much sebum from the hair. Conditioners are also used to help hair recover after chemical treatments like curling, straightening, and coloring, as well as after physical damage from drying, brushing, and styling. These activities can often weaken the hair, making it brittle and porous.

Conditioners help make hair easier to manage by reducing static electricity and friction between hair strands, which can cause tangles. They lower static by adding positively charged ions to the hair, which balance out the negative charges from combing or brushing. Friction is minimized by smoothing the cuticle layers, helping them stick better to the hair shaft. Smooth cuticles reflect more light, making hair shinier and softer.There are various types of conditioners, including instant conditioners, deep conditioners, blow-drying lotions, and hair glazes.

Instant Conditioners: Instant conditioners are the most widely used. They are applied right after shampooing and left on for a short time before rinsing. These conditioners typically contain water, a conditioning agent, lipids, and thickeners. The conditioning agents can be cationic detergents, film formers, orproteins. Cationic agents, which are positively charged, are attracted to the negatively charged damaged hair and are often used for treated hair. Film formers, made from polymers like polyvinylpyrrolidone, fill in gaps in the hair shaft and smooth it out. They also carry a positive charge, which helps reduce static. Protein conditioners, such as silk protein and hydrolyzed animal protein, are believed to bond with keratin, helping to repair damaged hair.

Deep Conditioning: Deep conditioners are like instant conditioners, but they come in cream form instead of lotion or liquid and are more concentrated. They are typically applied to the hair for 20 to 30 minutes, and using heat can help them work better. These conditioners are mainly for very dry hair and can be used as a protective step before chemical treatments like coloring or waving. By repairing damaged hair, they help ensure that the treatment is applied evenly.

Blow Drying Lotions: Blow drying lotions have similar ingredients to instant conditioners but do not contain oil, allowing them to stay on the hair. They are used after towel-drying the hair and before styling.

Hair Glaze: Hair glazes, also called hair thickeners, coat the hair shaft to make it appear thicker. They usually contain proteins and come in liquid form. They are applied to towel-dried hair before styling and are left in.

Coloring

Hair coloring is popular among both men and women. There are many products and methods available, and the chemistry can differ based on the

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type of dye used. Hair dyes are generally categorized by how long the color lasts: gradual, temporary, semi-permanent, and permanent.

It's fascinating to see the range of natural colors that can be achieved by adjusting the amount of melanin, although creating a specific shade often requires various coloring agents. While hair coloring options are abundant, there is still much to discover about the biochemistry behind it. Natural dyes, like henna, which gives an orange-reddish hue, are commonly used, but other plant-based dyes, such as chamomile, are rarely chosen. In some Asian countries, extracts from nutgall, logwood, and Brazilwood are used to darken gray hair. Natural coloring has a limited color palette and can be unpredictable in intensity. Most people prefer synthetic dyes because they offer a wider range of colors and more consistent results.

According to FDA guidelines, some products may include a warning and instructions for a skin-patch test before use, especially if they contain coal tar derivatives. There have been concerns about the safety of coloring agents, but current data indicates that hair dyes are generally safe.

Gradual hair coloring involves metallic dyes like lead, bismuth, or silver salts. This method can only darken hair (to brown-black) and has a limited range of shades. The metal particles are thought to react with cysteine in the hair cuticle, forming metal sulfides, which gradually build up on the hair strands.

Temporary dyes are water-soluble and acidic, made of large molecules that cannot enter the hair shaft. They sit on the surface and wash out easily, usually after one shampoo unless the hair has been chemically treated. Treated hair is more porous, allowing for better dye absorption, leading to longer-lasting color. These dyes are generally safe and rarely cause skin irritation.

Temporary dyes come in various forms like rinses, gels, mouses, and sprays. They serve multiple purposes, such as creating special effects, enhancing natural or dyed colors, removing yellow tones from gray hair, and covering small amounts of gray (up to 15%). People with completely gray hair can also achieve good results with these products. However, those with a lot of gray mixed with colored hair may need permanent dye for better coverage.

A range of colors is available, including red, blue, yellow, brown, purple, and green. Light purple or blue rinses are often used to brighten yellowish gray hair to a platinum or white shade, while yellow and brown shades help cover gray. Many users find they only need to apply the dye weekly, even though it fades after the first wash. Rinses are applied after shampooing and rinsed out immediately, while gels, mousses, and sprays are applied to towel-dried hair and left on. Using dye on chemically treated hair or the wrong shade can lead to unexpected results. It's usually best to start with a shade that is slightly lighter than the natural color.

Semi-permanent dyes are primarily synthetic. While natural dyes like henna are sometimes used, they have largely been replaced by synthetic alternatives. These dyes consist of low-molecular-weight coal tar dyes and may include other components.

Permanent hair coloring is very popular, making up 70% of hair dye sales. These dyes can lighten or darken natural hair and effectively cover gray hair. Once applied, the color stays until the hair grows out, requiring touch-ups every 4 to 6 weeks for new growth. This process cannot be reversed easily. If someone is unhappy with the color, they must wait for it to grow out or use a different dye, which can further harm the hair.

Using permanent dyes can damage hair. The color comes from a chemical reaction inside the hair shaft. This type of dyeing involves three key ingredients: primary intermediates, couplers, and oxidants. Primary intermediates create color when oxidized, with common agents being ortho- and para-aminophenols and phenylenediamines. Couplers react with these intermediates to form the final dye, with typical couplers including phenols and meta-aminophenols. Hydrogen peroxide is usually the oxidant used.For those wanting a much lighter blonde, a two-step process is needed. First, the hair is bleached with a mix of potassium and ammonium persulfate and hydrogen peroxide. After reaching the desired lightness, a toner or dye is applied for the final blonde shade. This method can be very damaging, so only the new growth is typically treated in future dyeing sessions.Permanent dyeing uses alkaline solutions to help the chemicals penetrate the hair cuticle. Ammonia is often used to achieve a pH of about 9 to 10. The dye is left on for 20 to 40 minutesbefore being rinsed out. There are also lower pH options available that are less harmful to the hair, but they may not be as effective.(20)

Health risks caused by chemical straightening:

Chemical relaxants are commonly used by people with curly hairs because they make hair

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easier to manage and improve its appearance.This process of hair smoothening has very much of side effects.Itcan cause problems like itching, burns and scars on scalp and also thinning of hair, discoloration, hair loss and also may be chances of allergic reactions due to chemicals.Hairdressers and salon workers are at significant risk for asthma due to the use of formaldehyde in hair straightening products and the heating process undertaken during the blow-drying and flat-ironing stages because of the release of free formaldehyde (FHO). The form of formaldehyde in these products is called methylene glycol also known as formaldehyde.(10)

Materials and methods: -

Case study:

Case 1

A woman hairdresser in her early 30s took a 1–2-hour course in 2010 to learn how to use a hair straightening product. After completing the course, she could purchase a bottle of the product, which could provide 10 to 15 treatments depending on the length of the client's hair. In 2010 and 2011, she performed up to two treatments each day. The process involved applying the solution, blow drying, and using a hot iron. During the heating stage, she noticed a white cloud of smoke, which produced fumes that irritated her eyes, nose, and throat. As she continued these treatments, her throat became more irritated, and she started experiencing sinus pain. After six months of using the product, her severe sore throat, chest pain, and lightheadedness persisted. In 2010, she visited her doctor, who observed wheezing and referred her to a specialist. The pulmonologist diagnosed her with chemically induced asthma, despite her having no previous asthma history. She tried a few more treatments but eventually had to stop, even avoiding the salon when others used the formaldehyde-based product. Now, she relies on a long-acting steroid inhaler and a rescue inhaler due to her ongoing asthma. She works part-time because of her breathing issues. Before her lung problems, she could exercise easily, but now she gets out of breath with minimal effort. She experiences asthma attacks when exposed to various chemicals that previously did not affect her. Additionally, she has a daily dry cough and her medical records show an enlarged thyroid. There are no other known environmental or work-related causes for her asthma, and she has denied using persulfate.(21)

Case 2

The study took place on a single day in June 2011 at a 260-m3 hair salon located in downtown Chicago, Illinois. The treatment area was approximately 140 m3 and featured 14 hair styling stations, referred to as chairs, arranged in two rows of seven along the walls. The research involved testing four different hair smoothing products that are available for professional use: Coppola Keratin, Complex Blonde Formula, GlobalKeratin Javelin, Optimized Functional Keratin, La Brazilian Keratin Treatment with Collagen, and Brazilian Blowout Acai Professional smoothing solution. Each product was applied in a random order but only one at a time. The products were used on professional human hair wigs, which were about shoulder long, following the manufacturers' guidelines. Although the instructions varied slightly between products (like the duration of tasks), the overall application process included the following steps:

Preparatory work: The hair was washed and dried until it was damp.

- Task 1: Application. This involved sectioning the hair with a comb and clips, then applying around 2 ounces of the product with a brush. This step took about 10 minutes on average, but one product required a 20-minute processing time after application according to the manufacturer's instructions.
- Task 2: Blow-Dry. The hair was dried using a handheld blow dryer and a round brush, taking about 9 minutes on average.
- Task 3: Flat-Iron. This step began with sectioning the hair again using a comb and clips. Each section was flat-ironed multiple times with both a small and a large flat iron, both set to 450°F.

Two 25-mL samples of three different hair treatment products were gathered in glass vials before starting the treatments. However, since there was only a small amount of the Coppola Keratin Complex available, only 10 mL of that product was collected for analysis. All the samples were then sent to a laboratory to identify and measure the levels of formaldehyde, methylene glycol, propylene glycol, and cyclePenta siloxane. The formaldehyde was identified and measured using a modified NIOSH 3500 method. For the other substances, gas chromatography and mass spectrometry were used along with chemical standards for identification and quantification.(22)

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Case 3

The study includes employees of both sexes that is male and female in 2 districts of Brazil which was approved by Research Ethics Committee at the Universidad Feevale.50 were enrolled in these studies with proper consentsbreaking down as 6,12,6,14,5 and 7 workers from salons named as A,B,C,D,E,F .The samples were taken from 19 November 2013 to 16 September, 2014 .Samples were collected on same days when hair straightening procedures are conducted and environmental formaldehyde concentrations were also determined. Urine samples were also taken before starting of work shift and after work shift of 8 hours of each participant. A questionnaire of participants was given to participants to collect their personal as well as professional information such as: age, weight, smoking, allergic symptoms or whether they haveworn personal protection equipment (PPE) or not.

The concentration of formaldehyde in each product has been assayed using High performance liquid chromatography.Blood samples were also taken for comet assay by venous puncture and collected in tubes containing heparin which were wrapped in aluminum foil and stored under refrigeration and protected from light .(23)

Methods:

Instruments and accessories

The analytical instrument used in the study of a Shimadzu gas chromatography coupled to single quadrupole mass spectrometer equipped with a hp-5ms capillary column containing 5% diphenyl and 95%dimethylpolysiloxane

Reagents solution and samples

The solvents used were HPLC grade acetonitrile,derivation reagent 2,4-dinitrophenylhydrazine, perchloric acid HCLO4.
All hair straightening cream samples are collected at various salons in the city of JoaoMon evade in Brazil sample was enumerated 1-3(first collection period august -steptember2011),4-7(second collection, period June- December 2012) and 9-11 (third collection period may- june2013)

Sample and sample preparation

Initially,75mg of DNPH was weight and dissolved in 50ml of acetonitrile acidified with approximately 0.5ml of 9.2m HCLO4 to provide solution.1 next ,200µL of this solution was diluted in 9.8 ml of acetonitrile. Solution 2 was then used to solubilize 300.00g of cream sample. finally ,1000µL of solution 3was diluted in 9ml of acetonitrile, forming the final sample which was injected into the GC- MS. this procedure was also performed with all samples

Optimization studies for the determination of formaldehyde in creams

Two procedures were evaluated for determination of formaldehyde in the sample creams quantification was achieved by either using external calibration or by the use of the standard addition method
Calibration procedure using the method of analyte additions:

Although the use of an external calibration is common, it is very important to evaluate the difference in sensitivity between traditional calibration standards and calibration standards prepared in the samples with added analyte to check for compatibility thus, for the 11 sample of creams that were evaluated, the influence of the sample matrix was also studied

Calibration curve

5 standards were prepared with different concentration of formaldehyde-DNPH

Test for analyte recovery

The first attempts at GC-MS analysis of unreacted formaldehyde were in good accordance with earlier studies and indicated the need for derivatization the reaction the forms formaldehyde-DNPH .(24)

III. CONCLUSION

In conclusion, the exposure is not only due to presence of formaldehyde but there is also role of other additives which act in conjunction synergistically with formaldehyde to produce mutagenicity . It is possible to give appearance to hairs with cosmetic products but these issues can also be considered and understanding the morphology and to make treatment properly by avoiding the side effects.

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