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Highlights

- Export of Green Hydrogen
- Analysis of the Port Environment
- Identifies a Novel Mutation
- Indicators of Overweight and Obesity

Discovering Thoughts, Inventing Future

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Evaluation of Bactericidal Performances on Various Knitted Fabric Treated with the Natural Dye Extraction from Tagetes Erecta Leaves

By Sonjit Kumar Saha, Md. Golam Robbani, Rozina Aktar, Md. Abdul Mukit & Mahbuba Sultana Mukta
University of Fashion & Technology

Abstract- Preventing environmental pollution is the primary goal of obtaining natural dyes from available natural sources.The alkaline extraction process is used to extract dye from Tagetes erecta leaves. In 100% cotton and 60/40 CVC (60% cotton and 40% polyester) single jersey weft knitted fabrics, three different mordants (CuSO4, FeSO4, and K2Cr2O7) are applied simultaneously. At 100°C as well as at room temperature, the dye fixing is done. Various shades of yellow, brown, and light green were produced when the dye was treated with a mordant. Comparing the dye fixed at 100°C to the dye fixed at room temperature, the dye at 100°C displayed dark colors and good fastness qualities.Tagetes erecta dye extract demonstrated significant antibacterial action against gram-positive bacteria (S. aureus), and FTIR was used to identify chemical components and functional groups.

Keywords: *natural dye, antibacterial activity, knitted fabrics, tageteserecta.*

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Evaluation of Bactericidal Performances on Various Knitted Fabric Treated with the Natural Dye Extraction from Tagetes Erecta Leaves

Sonjit Kumar Saha [α], Md. Golam Robbani [σ], Rozina Aktar [ρ], Md. Abdul Mukit [ω] & Mahbuba Sultana Mukta [¥]

Abstract- **Preventing environmental pollution is the primary goal of obtaining natural dyes from available natural sources.The alkaline extraction process is used to extract dye from Tagetes erecta leaves. In 100% cotton and 60/40 CVC (60% cotton and 40% polyester) single jersey weft knitted fabrics, three different mordants ($CuSO_4$, $FeSO_4$, and $K_2Cr_2O_7$) are applied simultaneously. At 100°C as well as at room temperature, the dye fixing is done. Various shades of yellow, brown, and light green were produced when the dye was treated with a mordant. Comparing the dye fixed at 100°C to the dye fixed at room temperature, the dye at 100°C displayed dark colors and good fastness qualities.Tagetes erecta dye extract demonstrated significant antibacterial action against gram-positive bacteria (S. aureus), and FTIR was used to identify chemical components and functional groups.**

Keywords: natural dye, antibacterial activity, knitted fabrics, tageteserecta.

I. Introduction

Natural colors are frequently glossy, smooth, and calming to the eyes. A wide range of colors can be produced using natural dyestuffs. Many mordants are used to develop different colors. The artificial dyes don't create a wide range of colors, including unusual colors. Natural dyes are generally nonhazardous to human health, good for the skin, anti-allergenic, and safe for contact with skin(Saravanan P et al., 2013). Natural colors improve with age, but synthetic dyes deteriorate. The generalization that natural colors bleed but do not deteriorate with other textiles does not apply to turmeric. Since natural colors are frequently mothproof, they can alter synthetic dyestuffs in children's clothing. Natural dyes are utilized in textile dyeing, food coloring, beauty products, dye-sensitized solar cells, histology staining, and pH readings, among other things. (Paramasivam et al., 2022).

Since ancient times, Tagetes erecta flowers have been used traditionally. The leaves are applied to boils and used as an antiseptic including for piles, kidney problems, and muscularpain (Samanta & Konar, 2011). Fever and epileptic fits can be treated with the flower. According to Ayurveda, the juice of Tagetes flowers can be utilized to treat several ailments, including astringent, stomachic, carminative, and scabies, as well as liver problems and eye illnesses.They clean the blood,and flower juice is administered to treat bleeding piles. It is also used to treat colds, arthritis, and bronchitis. It was utilized by the Cherokee as a skin cleanser and to produce a yellow color (Shetty et al., 2015).

Flower juice is occasionally used as a blood purifier and piles treatment in numerous countries. In Brazil, Tagetes erecta flowers and leaves serve as a vermifuge. Flowers and leaf tinctures are used as diuretics and carminatives in Mexico. Marigold relieves stomach aches, joint pain, muscle spasms, anemia, irregular periods, and pain in the muscles and bonesin Mexico and Brazil (Shetty et al., 2009). Internally, Tagetes was used to cure indigestion, cough, colic, and diarrhea. It is also used topically for ulcers, eczema, rheumatism, and painful eyes (Elumalai et al., 2012). When metallic salts are employed as a mordant mix with the fabric and dyes, metal complexes are formed. After mordanting, the metal salts adhere to the fibers and entice organic molecules there where they can bond with the fibers and get bonded to them. After that, it creates coordinating complexes to make a linkage between the molecules of dye and the fiber itself (Patil et al., 2016). A specific mordant that can react chemically with functional groups found in natural dyes and held together by coordinated or covalent interactions, hydrogen bonds, and other interactional forces combines with ferrous sulphate or other types of metallic mordants that are bonded to any fiber substances (Saravanan et al., 2013). In acid-base titrations, the methanolic extract of Tageteserecta flower is employed as an acid-base indicator. Indicators for titration displayed discernible color changes at specific pH intervals (Geetha & Sumathy, 2013). The majority of indicators are organic dyes with synthetic origins. Today, acid-base titrations are chosen using synthetic indicators. The current study can be utilized to investigate the colorant behavior of Tagetes erecta

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flowers and leaves, and additional testing is done on its potential usage as a natural dye (Elumalai et al., 2012).

II. Materials and Methods

a) Materials

100% cotton and 60/40 CVC single jersey knitted fabric in the ready-to-dye stage (scoured & bleached) was obtained from Masco Group (Gazipura 27, Tongi, Gazipur, Bangladesh). In Table 1, the fabric parameters are listed.

Table 1: Fabric Specifications

Fabric structure	Fabric Type	Count (Ne)	Grey WPI	Grey CPI	Finished WPI	Finished CPI	Weight GSM
Plain S/J	100% Cotton	30/s	31	50	34	53	145
Plain S/J	60/40 CVC	30/s	31	50	34	53	145

b) Gathering of Plant-Based Supplies

The Tagetes erecta plant was gathered from Uttarkhan, Dhaka, Bangladesh. The obtained flower samples were transferred to the lab after being firmly packaged in plastic bags. The petals of the flowers were divided and separated from one another. Then, to remove dirt and dust, the petals were briefly surface-cleaned under running water two to three times.

c) Chemicals

For the extraction of natural dye from Tagetes erecta leaves, all scientific-grade chemicals acquired from a local shop (Tongi, Dhaka, Bangladesh) were utilized.

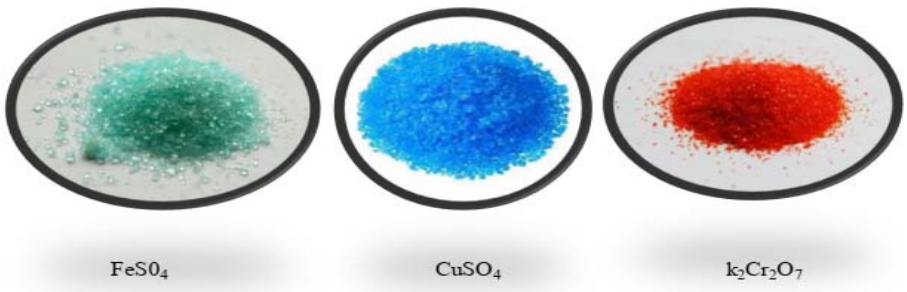


Figure 1: Different Mordants $FeSO_4$, $CuSO_4$, and $K_2Cr_2O_7$

d) Preparation of Dyeextraction

i. *Alkaline Extraction*

100 cc of distilled water dissolved 1gm of Sodium Hydroxide (NaOH). T. erecta petals weighing 10–20 gm were measured and stored in an alkaline solution. Finally, it was exposed to high heat for 30 minutes at 90°C. Whatman no. 1 filter paper was used to filter the dye. The acquired dye extract was kept in the refrigerator (Tripathi et al., 2015).

ii. *Mordant Preparation*

Separately, 1gm of copper sulphate, ferrous sulphate, and potassium dichromate was dissolved in 100 ml of distilled water.

iii. *Fabric Preparation*

Separately, 1gm of copper sulphate, ferrous sulphate, and potassium dichromate was dissolved in 100 ml of distilled water (Ahamed et al., 2017).

iv. The Dyeing Process using a Simultaneous Technique

With this technique, 100% cotton and 60/40 CVC fabrics were dyed using dye extract and several chemical mordants, which were then fixed at 100°C for 20 minutes. The ordinary dye table of the plant dyes was compared (Aglawe et al., 2019).

III. Characterisation

a) Lightfastness

The fabric was left out in the sun for 24 hours. Colorfastness (IS-2454-85) was determined by comparing the color change of the subjected part to the color change of the original material that had not been exposed (Aglawe et al., 2019).

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a) Washing Fastness(IS-687-79)

The colored samples were sandwiched between two white, uncolored samples. These three separate parts were stitched together at the edges. The sandwiched fabric was placed in a vessel with the preheated soap solution (Tide at 55°C in the ratio of 1:50, i.e., 0.5g/25 ml water) and left for 45 minutes. The cloth was then taken out and washed in cold water(Nasrullah et al., 2012).



Figure 2: a. Tagetes erecta plant; b. Grinding; and c. Liquid Dye Extraction

b) Evaluation of Antibacterial Assay

The inhibition zone was determined using the Kirby-Bauer disc diffusion test and the antimicrobial efficacy of the experimental samples against the Staphylococcus aureus bacterium. On tryptone soya agar media, fresh subcultures of the bacterial strain (S. aureus) were created. Nine milliliters of tryptone soya broth were inoculated with one loopful of a freshly cultivated colony. From a newly cultured plate, one loopful of a colony was taken and used to inoculate 9 ml of tryptone soya broth. After that, the mixture was overnight kept at 37°C in an incubator to get the desired turbidity of 0.5 Mc Farland Standard (1.5×10^8 CFU/ml). The bacterial culture was sequentially diluted in normal saline to a bacterial cell concentration of 1.5×10^5 CFU/ml. After evaluating the samples, the zone of inhibition (ZOI) was identified (Sun, 2016). Pelletizing knitted cloth into a 13 mm diameter disc on an agar plate produced the sample for the experiment. Samples were then incubated for 24 hours at 37°C. After incubation, the plates were examined to check for the zone of inhibition encircling the treated sample (Gokarneshan, N & Velumani, 2018).

c) Evaluation of Bonding Behavior by FTIR

The materials were analyzed using Fourier transform infrared spectroscopy (FTIR) (IR Prestige 21, Shimadzu, Japan). According to the infrared absorption with a resolution of 4 cm^{-1} and a frequency range of 600–4000 cm^{-1}, spectrum data from the automated spectros copyprogram was used to pinpoint the precise chemical groups that predominated in the sample (Badr et al., 2016).

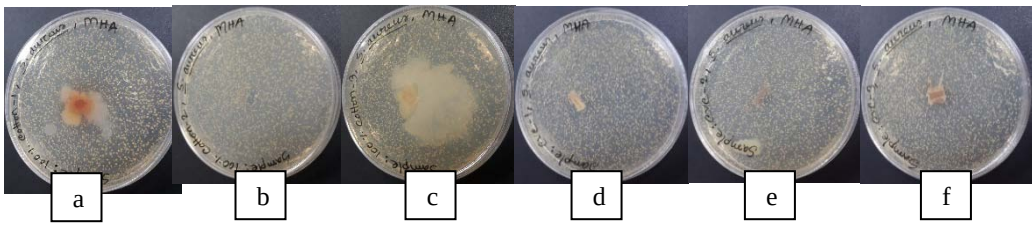


Figure 3: Inhibitory Zone Formation Surrounding Developing Samples (a. 100% cotton with $FeSO_4$; b. 100% cotton with $CuSO_4$; c. 100% Cotton with $K_2Cr_2O_7$; d. 60/40 CVC with $FeSO_4$; e. 60/40 CVC with $CuSO_4$; f. 60/40 CVC with $K_2Cr_2O_7$

d) Measurement of Color Strength

K/S values were used to determine the color strength of the dyed 100% Cotton and 60/40 CVC fabrics. The dyed materials' light reflectances were measured with a Text flash spectrophotometer (Data Color Corp.). The Kubelka-Munk equation was used to calculate the K/S values.

$$K/S = (1 - R)^2 / 2R$$

Where R is the decimal fraction of the colored fabrics' maximum light reflectance (λ_{max}). The absorption coefficient is K, while the scattering coefficient is S (Habibzadeh et al., 2010).

IV. Results & Discussion

a) Evaluation of Light Fastness Properties

Excellent to moderate light fastness at 100°C fixed on 100% cotton and 60/40 CVC fabrics. The alkaline dye-extract of T. erecta with the mordant $CuSO_4$, $FeSO_4$ and $K_2Cr_2O_7$ showed excellent to moderate light fastness at 100°C fixed 100% cotton and 60/40 CVC (Table 2). Likewise, the test specimen was dyed using

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marigold leaf extract and mordanted with iron and copper sulphate resulting in good light fastness. The resistance to dye attack is an inherent property of the dye chromophore, however, the auxochrome can also significantly affect the fastness.

Table 2: Light Fastness Properties (IS-2454-85) of Samples Dyed with Leaves Extract from T. Erecta

Dye Extracts	Mordants	100% Cotton	60/40 CVC
Tagetes erecta dye extract at 100^{0}c	Ferrous sulphate	Excellent	Good
	Copper sulphate	Excellent	Moderate
	Potassium dichromate	Excellent	Moderate

b) Evaluation Of Wash Fastness Properties

At 100°C fixed 100% cotton and 60/40 CVC materials, the washing fastness of T. erecta dye extract ranged from mild to good. At 100°C, 100% cotton and 60/40 CVC fabrics showed good washing fastness with mordant $FeSO_4$ (Table 3). Likewise, the exceptional washing fastness of Cordia Sebestena flower extract dye can be successfully utilized for dyeing silk to achieve a wide range of soft and light colors by combining mordants.Using varied temperatures (50°C, 60°C, and 70°C) and different washing fastness hours (6h, 7h, and 8h) of various chemical mordants, the color fastness characteristics of the colorant derived from the brake of acacia leucophloea on cotton have been examined. In the current study, various mordants were used for dyeing fabrics made of bleached cotton and CVC (Shinde et al., 2010).

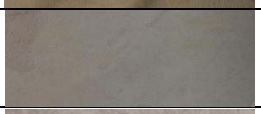
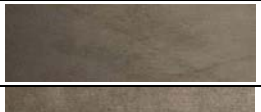
Table 3: Wash Fastness Properties (IS-687-79) of Samples Dyed with Leaves Extract from T. Erecta

Dye Extracts	Mordants	100% Cotton	60/40 CVC
Tageteserecta dye extract at 100^{0}c	Ferrous sulphate	Good	Moderate
	Copper sulphate	Good	Mild
	Potassium dichromate	Moderate	Mild

c) Mordant Optimization using K/S Values and Color Hue Shifts

Various hues of color were obtained from post-mordanted cotton and CVC fabric with ferrous sulphate, copper sulphate,and potassium dichromate as shown in table-4. Different mordants alter the brightness index values, L* values, and K/S values in addition to affecting color shades and K/S values significantly(Jeyakodi & Venkataraman, 2015). The effect ofmordants on color values of cotton and CVC fabrics dyed with leaves ofTageteserectais shown in Figure 4. Table 5 displays the values for L*, a*, b*, and K/S. As can be observed, mordants with higher L* values produce lighter hues, while those with lower L* values provide deeper shades for the sample fabrics. Similarly, green and blue are represented by negative values for a* and b*, respectively. Among the chemical mordants used, the highestcolor value (K/S = 65.203) was obtained with ferrous sulphate,and the lowest color value (K/S = 23.985) with Potassium dichromate.60/40 CVC fabric showed a higher color value than the 100% cotton fabric.

Table 4: Color Produced on Cotton and CVC Fabric by Different Mordants in Post-Mordanting (POM)

S. No.	Fabric type	Mordants	Color Obtained
1	100% Cotton	Ferrous sulphate	
2	100% Cotton	Copper sulphate	
3	100% Cotton	Potassium dichromate	
4	60/40 CVC	Ferrous sulphate	
5	60/40 CVC	Copper sulphate	
6	60/40 CVC	Potassium dichromate	

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Table 5: L*, a*, b*, and K/S Values for Cotton and CVC Fabric Colored with Tageteserecta Leaves, in Addition to others Post-Mordants

S. No.	Fabric type	Mordants	L*	a*	b*	K/S value
1	100% Cotton	Ferrous sulphate	69.817	0.599	19.785	65.203
2	100% Cotton	Copper sulphate	73.247	1.974	27.355	35.837
3	100% Cotton	Potassium dichromate	77.154	1.602	15.379	23.985
4	60/40 CVC	Ferrous sulphate	65.020	0.297	12.510	62.529
5	60/40 CVC	Copper sulphate	74.857	1.865	13.257	39.789
6	60/40 CVC	Potassium dichromate	74.300	0.495	9.741	36.946

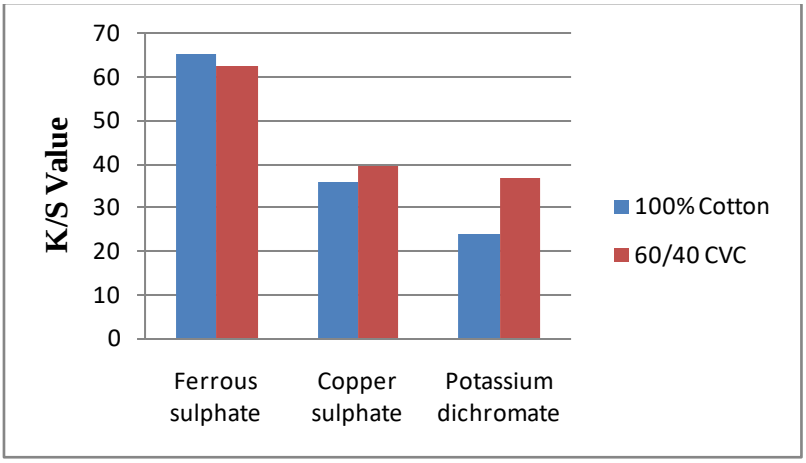


Figure 4: Post-Mordants' Impact on the Color Values of Dyed Cotton and CVC Fabrics

d) Antibacterial Efficacy of the Alkaline Dye Extract from Tagetes erectas

The dye extract showed average antibacterial activity against Staphylococcus aureus bacteria. Among the six samples with three different mordants, 60/40 CVC samples dyed with T. erectas leaves extract along with copper sulphate as mordants showed more effectiveness against S. aureus bacteria where the zone of inhibition was 6±0.54 mm as shown in Table 6. In contrast, ferrous sulphate-containing samples showed less effectiveness against the bacteria. Moreover, 60/40 CVC samples showed higher results of antibacterial efficacy than 100% cotton samples because polyester fiber itself influences bacterial resistance due to its hydrophobic nature (Pekhtasheva et al., 2011).

Table 6: Antibacterial Efficacy of Alkaline Dye Extract from Leaves Of T. Erecta

Sample	Mordants	Bacterial cell concentration (CFU/ml)	Sample weight (gm)	Disk size	Zone of inhibition (ZOI)
100% Cotton	Ferrous sulphate	1.5×10^5	5	7 mm x 7 mm	3± 1.7 mm
100% Cotton	Copper sulphate	1.5×10^5	5	7 mm x 7 mm	4±1.9 mm
100% Cotton	Potassium dichromate	1.5×10^5	5	7 mm x 7 mm	4±1.4 mm
60/40 CVC	Ferrous sulphate	1.5×10^5	5	7 mm x 7 mm	3±0.86 mm
60/40 CVC	Copper sulphate	1.5×10^5	5	7 mm x 7 mm	6±0.54 mm
60/40 CVC	Potassium dichromate	1.5×10^5	5	7 mm x 7 mm	4±1.7 mm

e) FTIR Spectra Investigation of the Dye Extracted from T. Erectas

By using FTIR spectroscopy, developed samples of both 100% cotton and 60/40 CVC were examined to verify the requisite functional groups and internal bondings. Figure 5 depicts cotton fiber's FTIR spectrum, where typical bands of cellulose, lignin, and hemicellulose may be seen whereas in 60/40 cotton-polyester (figure 6) fabric sample, the FTIR spectra emerge the strong peaks like cotton and weak peaks like polyester due to the proportion of fiber composition (Lam et al., 2012). The T. erecta dye extract showed more absorption bands at 3286.7, 2929.87, 1955.82, 1718.58, 1365.6, and 721.38 that were assigned to the alcohol as a functional group, including O-H stretching, C-H stretching, N=C=S stretching, N-H bending, and

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C-H rocking(Altameme et al., 2015). The alkaline dye extract of T. erecta contains several chemical components such as alcohol, amine, alkane, isothiocyanate, and alkyl halides (Fig. 5). There are no discernible variations in the sample of different mordants as shown in the figure.

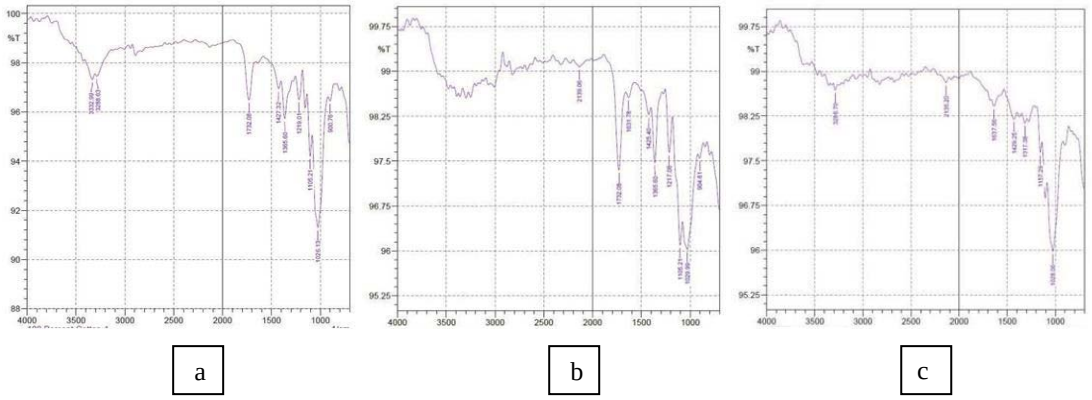


Figure 5: FTIR Band Spectra of 100% Cotton with Mordant a. $FeSO_4$; b. $CuSO_4$; c. $K_2Cr_2O_7$

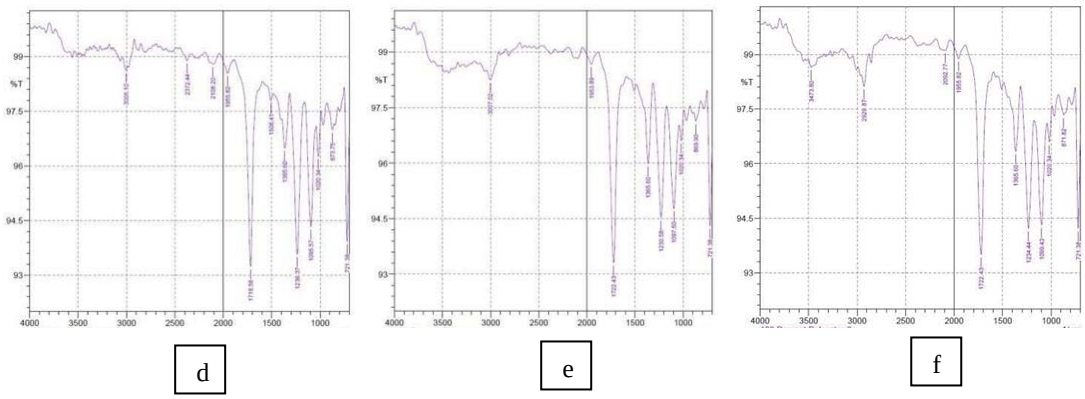


Figure 6: FTIR Band Spectra of 60/40 CVC with Mordant a. $FeSO_4$; b. $CuSO_4$; c. $K_2Cr_2O_7$

V. Conclusion

Natural dye has a great opportunity in the usage of textile coloring due to its nonhazardous characteristics to the environment. The study found that the alkaline dye extract of T. erecta may be successfully used for dying cotton and CVC fabric when combined with various mordants. Dyeing with mordants had good washing and lightfastness, and the dye extract showed antibacterial efficacy. Moreover, the alkaline solution of leaf extracts of T. erecta showed satisfactory color strength and light reflectance. The dye has a wide range of scope in commercial cotton dyeing.

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Analysis of the Port Environment for the Export of Green Hydrogen

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Abstract- The Paris Agreement, signed in 2015 during the 21st Conference of the Parties (COP21) of the United Nations Framework Convention, was an important milestone for the establishment of ambitious and concrete climate goals, especially with regard to the reduction of greenhouse gas emissions effect and energy security. To this end, several countries committed to achieving the goals established by the Paris Agreement are in the energy transition phase from a carbon-based matrix to a cleaner, low-carbon matrix. In this context, green hydrogen emerges as a clean energy alternative, being a favorable option to move towards the current process of decarbonization of global economies (in particular, industries and the mobility sector) and to achieve the goals established in the Agreement. Brazil, in turn, prepared an energy expansion plan to compose its matrix with a 45% share of clean energy by the year 2030, however, with regard to the use of green hydrogen as a direct source of energy or energy vector, the country is still in the incipient stage of research and development (R&D).

Keywords: *green hydrogen, supply chain, port environment.*

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Analysis of the Port Environment for the Export of Green Hydrogen

Chrislaine do Bomfim Marinho [α], Vitorio Donato [σ], Carolina Sacramento Vieira [ρ] & Maíra Silva Andrade [Ꞷ]

Abstract- The Paris Agreement, signed in 2015 during the 21st Conference of the Parties (COP21) of the United Nations Framework Convention, was an important milestone for the establishment of ambitious and concrete climate goals, especially with regard to the reduction of greenhouse gas emissions effect and energy security. To this end, several countries committed to achieving the goals established by the Paris Agreement are in the energy transition phase from a carbon-based matrix to a cleaner, low-carbon matrix. In this context, green hydrogen emerges as a clean energy alternative, being a favorable option to move towards the current process of decarbonization of global economies (in particular, industries and the mobility sector) and to achieve the goals established in the Agreement. Brazil, in turn, prepared an energy expansion plan to compose its matrix with a 45% share of clean energy by the year 2030, however, with regard to the use of green hydrogen as a direct source of energy or energy vector, the country is still in the incipient stage of research and development (R&D). Currently, Brazil has a very diversified energy matrix, concentrating a high installed capacity for generating energy from clean sources (such as wind, solar photovoltaic, hydro, biomass), which has made it possible as a promising region for the development and production of hydrogen. green. As it is a new technology, the production of GH2 presents challenges to be overcome, mainly those related to the supply chain. In this sense, this article aims to investigate the port environment for the storage, transport and export of green hydrogen, in order to develop a methodology to analyze the port environment for H2 export. For this, this study will map and study the port environment in qualitative and quantitative aspects, thus identifying the resistance to the flows that make up the green hydrogen supply chain.

Keywords: green hydrogen, supply chain, port environment.

I. Introduction

Several countries are in the process of transitioning from a carbon-based matrix to a low-carbon matrix, in response to the Paris Agreement, which set an ambitious goal of limiting global temperature rise below 2°C above pre-industrial levels, with the intention of limiting it to 1.5°C. Faced with this challenge, green hydrogen (*GH2*) emerges as a clean energy alternative, being a favorable option to advance in the current process of decar bonization of global economies (especially in the industrial sector). Countries with ambitious goals have shown interest in green hydrogen, produced from electricity generated through renewable sources, based on the electrolysis process (Delgado and Costa, 2021).

The European Union (EU) strongly supports the implementation of a hydrogen-based energy system as one of the main tools to achieve the European Green Deal and the COP21 Paris Agreement. With this, it aims to help the EU reach the carbon neutrality target by 2050, in addition to limiting dependence on hydrocarbons (Díaz-Cuevas *et al*., 2021). Germany, which consolidated its National Hydrogen Strategy in June 2020, has reinforced funding of more than €1 billion to be applied to research into the use of hydrogen under the German Decarbonisation Programme.

Elsewhere in the world, China is emerging as a hydrogen-producing powerhouse, expecting hydrogen to make up 10% of the energy share by 2050 in order to meet its ambitious climate targets: zero carbon by 2060 and "peak carbon" by key sectors by 2030. Australia is investing significantly in the development of the hydrogen industry, implementing a range of policy initiatives and funding R&D projects. In 2018, the CSIRO Organization (Commonwealth Scientific and Industrial Research Organisation) published the National Hydrogen Roadmap, which set out the blueprint for the development of the Hydrogen industry in Australia and outlines numerous actions across the hydrogen value chain. Soon after, in 2019, the COAG group (Council of Australian Governments – Energy Council Hydrogen) of the Government of Australia publishes the National Hydrogen Strategy report, which also establishes an action plan to develop the hydrogen industry. Numerous countries have recently implemented or are initiating and developing hydrogen strategies in order to satisfy their considerable energy demand.

Brazil does not demand, nor does it have current plans to replace fossil fuels, however, its role is extremely important for the *GH2* transition in other countries, since multinationals such as Engie and Shell have been creating pilot projects in the Complexo do Pecém (Ceará) and Porto de Açu (Rio de Janeiro), precisely because of the contribution of renewable energies (such as wind and solar photovoltaics) in the country – a key point for understanding Brazil's role in the *GH2* production chain. The country has large areas with high solar irradiation and favorable wind speeds that help make wind and solar photovoltaic energy viable economic alternatives for the world stage. Other factors include lower investments and lower cost of grid

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integration relative to the global average due to the large share of hydropower (De Lara and Richter, 2023).

In this scenario, European countries gain access to the renewable energy that projects need for *GH2* production, and Brazil, in addition to exporting sustainable fuel to other countries, expands a wide range of economic opportunities, as it will open a new market with global suppliers, and may in the future be a leader in *GH2* exports due to the energy matrix of renewable origin, also being a new source of employability and income for Brazilian citizens (Matos and Bitencourt, 2023).

For this reason, it is of great importance to develop studies on *GH2* in Brazil, as it is a clean energy alternative, but still with challenges to be overcome, especially those related to the supply chain (transport and storage). In this context, this article aims to evaluate the conditions and viability of Port Terminals for the storage and disposal of hydrogen, knowing that the production of its "green" form will occur in the near future and that developing the mapping of the port environment it is fundamental. For this purpose, qualitative and quantitative criteria will be considered, thus identifying the resistance to the flows that make up the green hydrogen supply chain.

II. Green Hydrogen Supply Chain

Supply chain is defined as the management of a network of organizations connected from the point of origin (upstream) to the point of consumption (downstream), in the different processes and activities that produce value in the form of products and services for the final consumer (Asgari *et al*., 2016). Chopra (2011) explains that the supply chain encompasses all stages involved, directly or indirectly, in fulfilling a customer order. That is, it does not only include manufacturers and suppliers, but also transport, storage of products, productive assets, forwarding to distributors and availability to retailers where the final customer will make the purchase.

The supply chain of the *GH2* industry encompasses the activities of receiving (raw material - RM), production (with the manufacture of the main raw material, *GH2* and inputs, renewable energy and catalysts), storage and distribution of the produced hydrogen. The hydrogen chain is analyzed in three stages, namely: Upstream stage, Midstream stage (intralogistics – "producer") and Dowstream. The Upstream stage comprises renewable energy sources, energy generation equipment such as wind turbines and solar photovoltaic systems, water treatment systems and energy distribution networks. The Downstream stage constitutes the transport of H2 and, finally, the Midstream stage is characterized by being the production and storage stage of *GH2*.

According to Donato (2012), the same analysis is carried out on the supply chain of the oil, gas and biofuels industry and which should be used to define the green hydrogen supply chain, and as a proposal cites the same classification of logistics for oil, gas and biofuels:

i. *Upstream Stage:* it concerns the inputs, the way in which the raw material and inputs arrive at the company. In this sense, it involves issues such as the relationship with suppliers, crucial for an assertive purchase of raw materials, aiming to meet the customer's needs.
ii. *Midstream Stage:* It is the stage where the raw material is transformed into a finished product, encompassing activities such as preparation, packaging, production line supply, maintenance support, removal of packaging residues from the line and the like.
iii. *Downstream Stage:* it is how the product or service will reach the customer, such as defining the means of storage, displacement and delivery. At this point, it is essential to value punctuality, quality and adequate quantity.

An important factor that should characterize the *GH2* Supply Chain is that it be a chain centered on low carbon emissions, not just in the production of H2, but throughout the chain, especially in means of transport. Caglayan *et al*. (2021), employs a green approach for the fully renewable use of the European energy system by 2050, for which he foresees the use of gas pipelines to transport liquid hydrogen. This approach is being considered in defining the *GH2* chain in Europe. Lamb et al. (2019), present the possibility of transporting and storing H2 in ammonia (NH3) and identify advantages of this method such as the absence of carbon, high efficiency of ammonia production and use of existing infrastructure around the world.

III. Port Environment

Ports are defined by Valentine and Gray (2002) as complex organizations with a variety of inputs and outputs, embodied in different physical, logistical, access and legal aspects. This makes analysis and direct comparison between apparently homogeneous ports difficult. For Bichou and Gray (2005), ports are multipart and complex organizations in which institutions and functions normally intersect at several levels.

According to Tolley and Turton (1995), ports are a component of the physical distribution of goods and offer a sea-land interface for export and import activities. This sea-land interface is directly related to the port environment analysis process, which involves different actors in the logistics chain, such as: i) shipowners - define the ports of call on each route; ii) shippers (exporters/importers) - define the ports of loading and

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unloading in their operations; iii) cargo agents - support and assist exporters and importers in the port process.

With regard to export and import criteria, the analysis of the port environment takes into account the "green mode" agreed between the parties and it is clear that the analysis varies according to the perspective of the actor to be considered.

The original port sites, commonly located adjacent to city center areas, have become obsolete. This was also marked by changes in the spatial relationships between Porto and the urban core. Numerous opportunities for converting port facilities to other uses (seaside parks, housing and commercial developments) have been created.

In the current stage of increasing specialization of cargo handling, both in export and import, the increase in the size of ships and the growing demand for space for cargo handling and storage in port areas, resulted in the implementation of port activity in places far from the urban coastal facilities. From the initial port location, the ports were modernized, as a result of the evolution of maritime technology and improvements in cargo handling technology.

In this context, three major phases identified so far in the port development process involve reconfiguration, expansion and specialization. Colonetti (2017) mentions that the port environment can be analyzed in four phases: Hinterland, Umland, Vorland and Foreland.

For the purposes of this work, the analysis of the port environment will be carried out, classifying it in criteria. The criteria considered are diverse and can be classified into two types: quantitative criteria and qualitative criteria. Quantitative criteria are those that can potentially be measured and compared objectively, and are grouped into three major phases: Hafen Stadt; Umland and the Vorland. Qualitative criteria include subjective influences, such as Hinterland, Foreland, Inland and Gotland that can be developed between users and ports. All will be detailed below:

a) Quantitative Criteria

Hafen stadt: is the proximity of a city to a port (urban port). In this case, the road distance is considered.

Umland: it is the physical environment of the Port, that is, its facilities, infrastructure and other factors that characterize its operation (quality of services, tariffs, etc.). In this case, the number of operational installations is considered.

Vorland: it is related to a greater or lesser distance of Porto from the main shipping routes. Considering that the main commercial maritime routes are in the Northern Hemisphere, it can be assumed that the ports in the Northeast of Brazil have a higher Vorland than the other national ports. In this case, the nautical distance is considered.

b) Qualitative Criteria

Hinterland: it is the region that surrounds a port center, from which cargoes come, that is, the port's business potential generator (the area of influence is a subjective criterion as it depends on the radius to be considered).

Foreland: it is the scope of commercial relations of a Port. It is the ease and competence of a Port to relate with its partners.

Inland: this term refers to the location of a Port in relation to the coastal region, that is, if it is located inland (located in a river region) or on the sea coast.

Gotland: this is the concept where a direct land connection is established between a land cargo terminal (related to inland areas, also known as dry ports) and the coastal port.

IV. Resistance to Logistics Flows

According to Ammer (2013), the path of least resistance to a flow is the easiest method, way, or course of action. The path of least resistance is described as the physical path that provides the least opposition to the movement of a given object or entity, among a set of alternative paths (Neto, 2004). In physics, the path of least resistance is preferably taken by objects moving in a system. For example, water flowing downward follows the path of least resistance, since it is pulled downward by gravity.

In the early 20th century, the first works on the study of flows of goods began to appear, and in the thirties, more precisely in 1927, Ralph Borsodi published the book "The Distribution Age", in which he addressed the topic of physical flows of materials, under the name "distribution". For Rogers and Tibben- Lembke (2001), physical transport flows can be either forward or reverse. There is, therefore, an interesting parallel between the flows studied by physics and the flows of product distribution.

The load driving systems are accelerated by the driving force of the conveyor units, but due to flow resistances, they eventually reach an average speed that is inversely proportional to the channel resistances. As mentioned by Neto (2004), resistance to logistical flows in transport channels is the set of restrictions or inhibitions, both physical, economic, and legal, that affect the performance of the logistical flow and that occur during circulation on transport routes.

In attempts to implement an export flow of *GH2*, companies are running into logistical difficulties. Scholars call these difficulties in implementing a flow as resistance to logistical flows. The more steps the chain needs for its development, the greater the resistance to flows.

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The number of levels in the supply chain defines the resistance to the logistical flows of the chain under analysis, as shown in Table 1.

Table 1: Number of levels x level of resistance to flows

Number of Chain Levels	Resistance Level	Description
<= 3 levels	Low Resistance	Chain with a low number of levels greateroperational ease.
> 3 < 5 levels	Medium Strength	Chain with a medium number of levels, attention to communication between the links in the chain.
> 5 levels	High Resistance	Highly resistant chain, provision must be made for communication between the links in the chain.

Source: Marinho et al., 2023

What will classify the level of resistance to logistical flows will be the fluidity of communication between the links. Thus, the greater the number of levels identified, the greater the number of contacts between the links, therefore, the greater the resistance to flows.

This happens because the restrictions or inhibitions, both physical, economic, and legal, that affect the performance of the logistic flow, and that occur during the circulation in transport routes, tend to increase according to the number of actors that are involved in commercial negotiations within the chain. As a consequence, a delay in inter-network communication occurs, and thus, promotes a reduction in the flow speed of logistics channels. Thereby, the fewer steps the chain needs for its development, the lower the resistance to flows.

V. Methodology

Port environment analysis is the exercise of evaluating each stage of the process, assuming the premise that the Port is a business environment and not a non-profit state enterprise. This process can be analyzed from the perspective that a series of products is being delivered to customers. The analysis process requires an evaluation of each step, from the analysis of the potential load generator in the region, to the moment the company buys the product (port service) to the moment the company receives/ships the loads.

From the traditional port, with side piers adjacent to the city center, the ports adequacy for export/import of H2 becomes a product of the evolution of port technology with improvements in loads handling and safety. After carrying out the analysis of the port environment, three main conclusions can be identified in the Port under analysis: reconfiguration, expansion and/or specialization. The three conclusions portray well the stage that the Port is in, especially the traditional ones. However, the proposed model presents some weaknesses to explain the adequacy of the contemporary port environment.

The analysis of the port environment begins with the mapping of the process steps that should not be reduced or neglected, in order to reduce the time needed to perform the analysis without sacrificing product quality or service level. A conceptual perspective on the port environment for H2 exports could be made by analyzing the seven criteria for the port environment analysis.

a) Criteria for the Analysis of the Port Environment

i. *Hinterland*

When analyzing the port hinterland for the *GH2* industry, one must analyze the scope of load generation in the region where the Port is installed, taking as an example the generation of similar products such as petrochemicals, petroleum products, natural gas production, grains, cellulose and other products that, if produced in large scale, make the hinterland of a region viable.

In the analysis of a port's hinterland, the verification of how the loads will be received must also be taken into account. The analysis of the land coverage, the rail connections, and the availability of good roads and pipelines are fundamental for the development of the port hinterland.

ii. *Hafen Stadt*

This criterion analyzes whether a city effectively has an urban port in operation. It defines the interaction, safety, proximity, and interconnection of the city with the Porto. In the stadthafen analysis, the risk that the loads may exert on urban settlements must be considered.

iii. *Umland*

The better structured a region is, the higher the quality of the service provided tends to be. To this end, the physical environment of the region must be analyzed, i.e., the facilities and infrastructure of other factors that characterize logistics operations (quality of services, tariffs, etc.). This criterion considers the infrastructure (roads, railroads, pipelines, airports, docks, draft, etc.), the superstructure (handling equipment, terminals, warehouses, etc.), the operation costs (tariffs and transport expenses), and the quality of the services provided. It is necessary to verify the existence of this logistical infrastructure, since it will act in the flow of the final product.

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iv. *Vorland*

The vorland is related to the logistics coverage area, that is, it is related to a greater or lesser distance from the main transportation routes. When planning a certain supply or flow route, the closest points that have the capacity to receive/offload the loads are chosen as the stopping point. Considering that the main trade routes are close to the coastal region, we can assume that the metropolitan region of Salvador has a higher vorland than the other regions.

v. *Foreland*

It is the scope of commercial relations in a region, that is, the projection in which the region maintains commercial ties, it is the set of markets reached through connections and partnerships. The foreland represents the commercial coverage area of a micro-region in relation to the others. This aspect is related to the set of markets reached, through installed companies, with which there are regular commercial exchanges.

vi. *Inland*

This criterion analyzes the location of a port in relation to the coast, i.e., if it is in a fluvial region, located located inland. This argument takes advantage of the advantages of intermodal transport and the improvements in transhipment efficiency of port terminals. These inland ports are integrated with the hinterland services of coastal ports through transport services by land, barges, or smaller ships. This is particularly the case along the Rhine/Scheldt delta, where fluvial barge ports act as feeders to delta ports such as Rotterdam and Antwerp.

vii. *Gotland*

This is the concept where a direct land connection is established between a land load terminal and the coastal port. These are the inland areas, also known as dry ports. Land terminals tend to have more space available where it can provide a variety of logistics services, such as consolidation and deconsolidation, for load shipped from coastal ports that are normally congested. The developed hinterland favors Port to havea good gotland.

b) Analysis of the Port Environment

Logistics for the oil, gas and biofuels industry classifies the stages of transport, storage and distribution into upstream, midstream and downstream stages. Table 2 brings an analysis through a framework, carried out in the logistical stages of the H_2 production chain.

In Table 2, it is evident that the transport only occurs in the upstream step. Storage, on the other hand, occurs in three stages and distribution is a process that occurs only in the downstream stage.

Table 2: Framework for Analyzing the Logistical Complexity for H_2 Production

logistical steps / Location	Upstream			Midstream	Dowstream	
Acquisition	Selection of suppliers	Transport selection	Route selection	Receipt and conference	Storage	Preservation
Transport	Transport selection	transport of raw material	Transportof inputs	Internal	-	-
Storage	Selection of suppliers	Raw material	Inputs	intermediate storage	GH_2 Storage	-
Distribuition	-	-	-	pipeline	Pipeline	Transporte long haul

Source: Author, 2023

Table 3 presents the analysis criteria of the port environment versus the level of the green supply chain. This analysis is necessary for the movement to understand how the port environment will be influenced by the GH_2 movement.

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Table 3: Framework for Analyzing the Port Environment: Analysis Criteria x GSC Levels

Green Supply Chain Levels		Level 3 (physical)	Level 2 (physical)	Level 1 (physical)	Level 0 (physical)	Level 1 (physical)	Level 2 (Physical)	Level I 3 (virtual)
Analysis of the port environment	Hinterland	Proximity to the RM supplier	Proximity to the supplier of inputs	stock management	Buffer need	customer proximity	-	Use of SCM software
	Hafen Stadt	access lead time	Risk in loading	storage risk	Risk of keeping a buffer	Risk in loading	Trans. risk.	Use of risky software
	Umland	Infrastructure for RM	Infra. for inputs	Infra. to store PM and Supplies	Infra. For buffer	Infra. for storing GH2 in port area	Infra. to drain the GH2	Use of ERP software
	Vorland	Lead Time for accessing RM and Inputs	Lead Time. obtaining RM	Lead time inputs	Infra. for Port	Lead Time to store	Transport Lead Time	Use of ERP software
	Foreland	Supplier management	Relationship with suppliers	Relationship with transp.	Relationship with port partners	Relationship with the consumer market	Relationship with transporter	Use of SCM software
	Inland	-	Infra. for access	Distance from the port to the urban center	Port	ease of movement	-	Use of ERP software
	Gotland	-	Infra. for access	Land connection between Porto and the customs area	coastal port	Land connection between coastal port and customer	-	Use of ERP software

Source: Marinho et al. (2022)

Table 4, on the other hand, shows the resistance to existing flows in the movement of GH_2 in relation to each criterion of analysis of the port environment.

Table 4: Framework for Analysis of CS Resistance Levels for GH_2

Resistance to Flows	Location	Upstream				Downstream		
	Chain Level	Level 3 RM supplier	Level 2 RM Transport	Level 1 Supply inputs	Level 0 Port	Level 1 GH_2 Storage	Level 2 GH_2 Transport	Level 3 Virtual
CO_2 emission		yes	yes	yes	yes	yes	yes	No
Use of fossil fuel in transportation		yes	yes	yes	yes	yes	yes	No
NON-renewable energy consumption at SC		yes	yes	yes	yes	yes	yes	No
congestion		yes	yes	yes	yes	No	yes	No
legislation		yes	yes	yes	yes	yes	yes	No

Source: Author, 2023

VI. Results and Discussion

The supply chain for GH_2 must be mandatory green, as it makes no sense to define a product that will be classified as green in a conventional supply chain.

a) Green Logistics Chain Analysis for a Port Environment

To analyze the port environment for the export of green hydrogen, a framework was developed that simultaneously correlates the following concepts:

- Criteria for analyzing a Port: evaluation of the following criteria: Hinterland, Stadthafen, Foreland, Umland, Vorland, Inland and Gotland.
- Analysis of resistance to flows: identification of bottlenecks and other impediments to GSCflows.
- Green supply chain analysis: identification of GSC levels by stage: Upstream, Midstream, and Downstream.

The complexity of the GSC is analyzed at each stage of the production process and presented in Table

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2. With the information displayed in Table 3, it is possible to classify the length of the chain according to the number of existing levels (the more levels, the longer the chain), within each criterion related to the Port environment.

The analysis criteria for the port environment must vary according to the perspective of the actor (link in the chain) to be considered. In this way, with the Framework, the user will carry out quantitative analyzes on the route factors; of cost; and of service; and qualitative such as flexibility and ease of use, marketing efforts of the port, commercial maturity of the port, personal contacts and level of cooperation that can be developed between the user and the port.

In the end, the analysis of the various frameworks that make up the port environment will present the user with a more comprehensive view of the flows that offer resistance to the export of H2 in a given port, identifying the dimension of the physical environment (related to the infrastructure for the storage of H2 and cargo handling, the potential area of greatest influence, the commercial scope and the most optimized routes, in relation to distance (shorter supplier-port and port-customer mileage) and logistical costs (which is influenced by the commercial maturity of the technologies used in the transport and storage of H2).

The analysis model proposed for the green hydrogen supply chain, presented in Table 3 and Table 4, demonstrates that this chain has seven levels, with an intermediate level. The model also proposes that the user use Table 4 to compare the levels of carbonization present along the chain under study.

The user must fill in the framework according to the possibility of using carbon at each level and infer whether it meets the total or partial environmental requirement, or if the item does not fit. From there, it is possible to identify the level of carbonization of the chain under study and infer whether this chain is in the process of being decarbonized.

b) Influence of the Concepts used in the Analysis of the Port Environment

First, the concepts used seek to explain the recent increase in port terminals that act mainly as transshipment centers in extensive hub-and-spoke maritime and collection and distribution networks. The increase in the availability of loading has triggered changes in the size of ships, in the schedule of regular services and in the structure of liner shipping. The carriers and alliances have redesigned their liner shipping networks by introducing new types of end-to-end services, round-the-world services and pendulum services, especially on major east-west trade routes. As a result, a new generation of terminals has emerged along east-west shipping routes in unlikely places, far from the immediate hinterland that has historically guided port selection. These locations were selected to serve the market flow of the continents and for transshipment at transit points on trade routes.

The port terminal depends heavily, sometimes completely, on traffic flows that are generated at a distance by the interaction of widely places separated and stimulated by the location or intermediation of the port en route. The model does not provide a basis for explaining the emergence of hub terminals in 'offshore' or island locations with limited or non-existent site hinterlands.

VII. Conclusion

This work aimed to analyze the validity of the analysis model of the Port environment for the export of H2. That is, to verify the opportunity to carry out the export of a new fuel in order to allow an energy transition at a lower cost or with less environmental impact, given the elimination of carbon emissions in CS, as this chain is green.

The logic behind the analysis of the port environment is that the more flexible a port is, the more likely it is to respond to customer needs. Several other benefits can be identified with companies that undergo an analysis of the port environment. These benefits include the analysis of whether the Port environment generates enough load to supply large ships.

The analysis of the port environment through the mapping of the port process, through the upstream, midstream and downstream stages, allows the identification of resistance to logistical flows, since a Port with resistant logistical flows implies high management costs, which can make operations unfeasible. Another way to identify resistance to flows is by measuring the length of the supply chain, and the longer the length (number of levels) the greater the resistance to flows in this chain.

With the process mapping, a graph is assembled that identifies all the participants in the chain, including the suppliers of inputs and raw materials that the company uses to manufacture or produce the product. Participants' interactions are also mapped, such as how raw materials arrive at the H2 production process.

An assessment of the three frameworks and the interactions between the players helps the evaluator determine where changes can be made to improve flow. Port environment analysis works hand in hand with supply chain management as product or information flows from one point in the process to another. Logistic analysis and analysis of the port environment allow business managers or owners to determine how to speed up the transfer of goods and information at each point in the

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process. This speeds the product to market, which makes the business money.

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Whole Exome Sequencing Identifies a Novel Mutation in ADGRV1 Responsible For Usher 2C Syndrome in a Large Inbred Family

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Abstrack- *Introduction:* High-throughput sequencing facilitates the diagnosis of Usher syndrome and other conditions involving deafness and blindness that are genetically related, with improvements not only in accurate diagnosis, time savings, and genotype-phenotype correlation. Advances in genomic sequencing also makes it possible to approach isolated or remotely located populations with community genetics methodologies.

Keywords: *usher syndrome, ADGRV1, usher syndrome type 2C, autosomal recessive inheritance, inbreeding.*

GJSFR-G Classification: *FOR Code: 1103*



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Whole Exome Sequencing Identifies a Novel Mutation in *ADGRV1* Responsible for Usher 2C Syndrome in a Large Inbred Family

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Abstract- Introduction: High-throughput sequencing facilitates the diagnosis of Usher syndrome and other conditions involving deafness and blindness that are genetically related, with improvements not only in accurate diagnosis, time savings, and genotype-phenotype correlation. Advances in genomic sequencing also makes it possible to approach isolated or remotely located populations with community genetics methodologies.

Material and methods: A remotely located and highly endogamic family with Usher syndrome with four affected members were ascertained with USH, by one ophthalmologist and several undergraduate medical students. Eye fundus diagnostic and audiometry tests were made to the index patient. Following an informed consent adapted application, several family members were sampled for WES analysis. The sequencing and detection of variants was also performed from the sample of affected patients and from five healthy relatives, by means of a multigenic panel based on exome.

Results: A pathogenic variant in the ADGRV1 NM_032119.4 c.6819dup T gene in homozygosity was identified in those affected. This variant is a new mutation for this gene and it causes an early stop codon NP_ 115495p.Ala2274Cysfs*4 that coincides with the familial segregation pattern of those affected.

Conclusion: Whole exome sequencing allowed to identify a new pathogenic variant for Usher Syndrome type 2C in an inbred family located in a remote region of Colombia.

Keywords: usher syndrome, ADGRV1, usher syndrome type 2C, autosomal recessive inheritance, inbreeding.

I. Introduction

Usher syndrome (USH) is a rare autosomal recessive condition characterized by degenerative vision, sensorineural hearing loss and, sometimes, vestibular dysfunction [1]. USH is classified into four main types and at least 16 genes have been implicated in their etiology.

High-throughput sequencing (HTS) and whole exome sequencing (WES), are the preferred molecular diagnostic tools for USH diagnosis, as well as for other retinal dystrophies and hearing loss disorders in which genetic heterogeneity is present [2]. HTS has made it possible to identify candidate genes, pathogenic variants, genotype-phenotype correlations, rare undiagnosed diseases and information on the phenotypic spectrum of the disease [2].

This article presents as WES was performed to evaluate and precisely diagnoses a highly inbred family group affected by Usher type 2C syndrome, those who had not been able to be adequately studied, because they are located in a place of Colombia with scarce health resources, near a low complexity hospital, in a context of community genetics.

II. Materials and Methods

a) Subjects

The index case (III,2) was ascertained by blindness and hearing loss into the hospital departmental San Vicente de Paul de Garzón (Huila), Colombia, the regional hospital facility located from the family's habitual residence. A genealogy was constructed showing that there were other affected members in the place of origin (III,1 deceased, III,4, III,6 and IV,1), including an inbreed union between siblings with an affected child (Figure 1A). A team was then organized with the ophthalmologist, undergraduate medical students with the support from geneticists located in a molecular genetics' laboratory in the capital city of Colombia. The team went to the nursing home and Pitalito (Huila), and evaluate the rest of the affected and healthy relatives. In summary, three more patients were identified, evaluated and sampled, so, they did not have any access to other additional functional tests.

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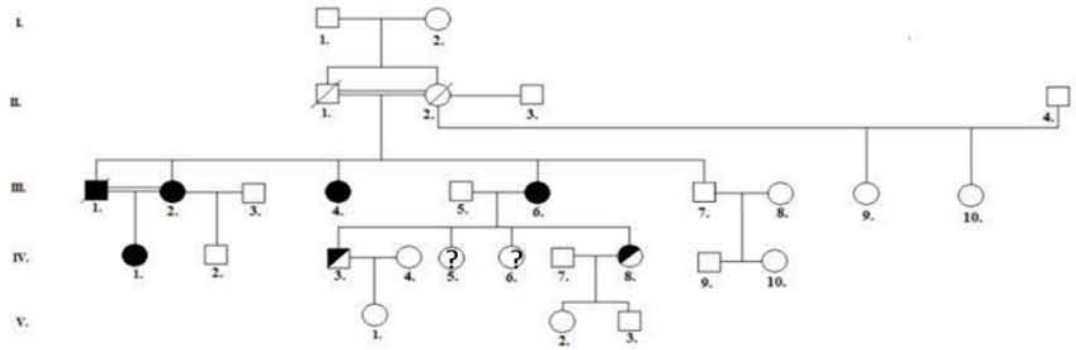


Figure 1A. Genealogical Tree



Figure 1B. Index Patient right eye funduscopy

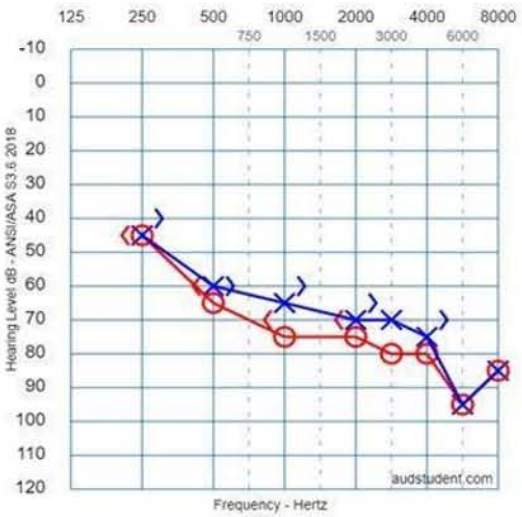


Figure 1C. Index Patient Tonal Audiometry

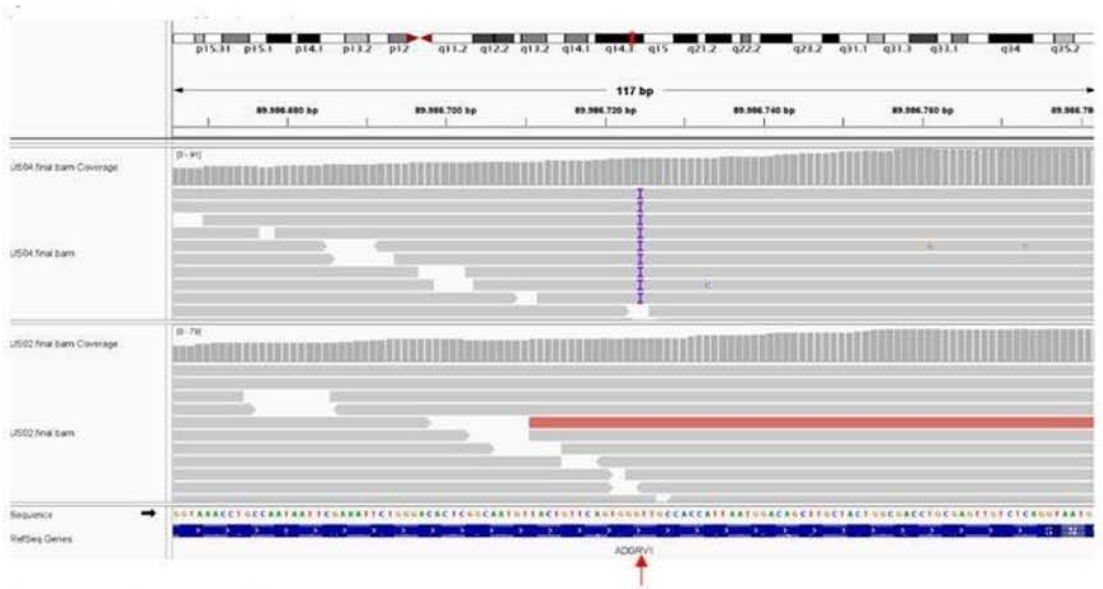


Figure 1D. BAM Archives

Figure 1B. Index patient right eye funduscopy. Pink optic disc, with 0.2 excavation, with defined edges, attenuated retinal vessels of central emergence, speckled pattern with granulation of the retinal pigmented epithelium, presence of bone spicules towards the equator, clear vitreous. Figure 1C. Tonal audiometry of the index case. Right ear: anacusis. Left ear: severe sensorineural hearing loss. Figure 1D. BAM file showing the patient homozygous for the mutant allele (US04) and homozygous for the Wild Type allele (US02).

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b) Diagnostic Tests

All clinical evaluations, procedures and diagnostic tests had the approval and signature of an informed consent, accompanied by a witness. Given the location of those affected, III,2, III,4, III,6 and IV,1, only diagnostic eye fundus tests were performed on (III,2, III,4, III,6 and IV,1), which data was shown (Figure 1B) and/or audiometry on III,2 (Table 1).

Table 1: Main Usher Syndrome Type 2A (USH2A) Clinical Features

USH2A AFFECTED	AGE	SEX	USH MAIN CLINICAL FEATURES			DEBUTING AGE
			BLINDNESS	HEARING LOSS	VESTIBULAR	
US04 (III-4)	68	F	+	+	-	CHILDHOOD
US05 (III-6)	67	F	+	+	-	CHILDHOOD
US06 (IV-1)	25	F	+	+	-	CHILDHOOD - ADOLESCENCE
US07 (III-2)	52	F	+	+	+	CHILDHOOD

c) Exome Sequencing and Data Analysis

Genomic DNAs from four affected individuals (US04, US05, US06, US07) and four healthy relatives (US01, US02, US03, US08) was extracted from whole blood using standard procedures. Sequencing libraries were prepared using 1.0 μg of genomic DNA per sample and the Agilent SureSelect Human All Exon kit (Agilent Technologies, CA, USA) following manufacturer's recommendations. Briefly, genomic DNA was fragmented by hydrodynamic shearing to generate 180-280 bp fragments. Next, overhang ends were converted into blunt ends and 3' ends were adenylated. Then, DNA fragments were ligated to adapter oligonucleotides, and these were enriched by PCR. Exon capture was performed by hybridization using biotin labeled probes and purification by the AMPure XP system (Beckman Coulter, Beverly, USA). Finally, captured libraries were enriched in a PCR reaction and index codes were added to each sample. Sequencing was performed in an Illumina HiSeq 2500 equipment generating paired-end reads. Library preparation and sequencing was carried out by Novogene (Beijing, China).

Raw reads were trimmed, and mapped to the human reference genome (GRCh37) using the Burrows-Wheeler Aligner v0.7.8-r455 (BWA) [3] (doi: 10.1093/bioin formatics/btp698). Duplicate reads were marked in BAM files with Picard v1.111 and variant calling was performed using the GATK v3.8 [4] (doi: 10.1101/gr.107524.110). The percentage of reads mapped to the reference genome for all the samples was above 99.9% and the fraction of the targeted region covered with at least 10X was above 95%, for all samples. Variant call format (VCF) files were annotated and analyzed using the VarSeq v2.2.3 software. Variants were filtered using a multigene panel: *ADGRV1, ARSG, CDH23, CIB2, CLRN1, CRTAC1, ESPN, HARS1, MYO7A, PCDH15, PDZD7, USH1C, USH1G, USH2A* and *WHRN*; minimum allele frequency (MAF)<0.01 according to gnomAD genome and exome variant frequencies v2.1 [5] (doi:10.1038/s41586-020-03174-8); protein effect, including missense, indels, frameshift and splicing site variants; and family segregation, assuming an autosomal recessive inheritance model.

III. Results

The pedigree identifies five members who manifested USH, two of them were identified as healthy carriers of the mutation for the *ADGRV1* gene (Figure 1A). Four of the patients were interviewed to identify the clinical signs as shown in Table 1.

WES showed a new pathogenic variant which was identified in those affected with USH in *ADGRV1* NM_032119.4 c.6819dupT gene in homozygosis, which is a new mutation for this gene and it causes an early stop codon NP_115495p.Ala2274Cysfs*4, that coincides with the pattern of familial segregation in those affected (Figure 1C).

There were no other pathogenic or likely pathogenic variants identified in ADGRV1 and other Usher known genes; in addition, no modifier alleles were identified in other genes in the whole exome sequencing analysis. Five homozygous ADGRV1 NM_032119.4 c.6819dupT NP_115495p.Ala2274Cysfs*4 affected patients in the present family showed the same phenotype with blindness and hearing loss, even though there were limitations of resources for the detailed visual and hearing diagnosis, for which only two affected patients were tested because of their isolated geographic settlement.

IV. Discussion

Usher syndrome (USH) is a clinically and genetically heterogeneous hereditary condition which is classified into four different main types, USH1, USH2, USH3 and USH4, depending on the age of onset, severity, progression of the symptoms and the presence or absence of vestibular dysfunction [1]. USH also presents genetic heterogeneity with at least 16 genes involved and further into different subtypes: USH1 is caused by the mutation of six different genes: *MYO7A*, *USH1C*, *CDH23*, *PCDH15*, *USH1G* and *CIB2*; USH2 is caused by the mutation of five genes *USH2A*, *ADGRV1,*

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WHRN, *PDZD7* and *DFNB31*; USH3 is related with mutation of two genes, *CLRN1* and *HARS* and USH4 is caused by mutations in *ARSG* gene. In addition, *ABHD12* gene is related with hearing loss, retinitis pigmentosa, cataracts and ataxia; *CEP78* and *CEP250* genes mutations causes two forms of cone-rod dystrophy with hearing loss [6]. This genetic heterogeneity implies difficulties in obtaining an accurate diagnosis in USH and also in no syndromic hearing loss and no syndromic retinitis pigmentosa forms [6], and the adequate correlation between the genotype and the phenotype. USH also has digenic, biallelic and polygenic inheritance, which adds difficulty to establish the precise diagnosis [6].

USH and other related conditions and their clinical and genomic heterogeneity requires a multidisciplinary team supported by complex laboratory equipment at fixed laboratory facilities, and sophisticated analysis to establish a precise diagnosis, to understand genotype-phenotype correlations and other particular issues for a rare disease. Genomic medicine helps us to shortcut those questions and WES analysis is ideal in cases with wide clinical and genetic variability, particularly in cases with real or relative geographical isolation or when it is impossible or difficult to evaluate several patients at the same time and collect all data and diagnostic tests of a sensory type (ophthalmological and auditory). In the present inbreed familial case, genomic analysis by means of WES in a community context, through the taking of blood samples from those affected and relatives, quickly allowed obtaining the accurate diagnosis of USH2C in a short period of time, highlighting how DNA sequencing technologies are simplifying and accelerating genetic diagnosis.

USH type 2 (USH2) is the most frequent subtype and to date, three causative genes have been identified: *USH2A, WHRN and ADGRV1*, respectively causing USH2A, USH2B, USH2C and USH2D. The. ADGRV1 gene has mutations in 6 to 19 % of subtype 2 cases [7].

In the present study, a highly inbred family with five affected individuals of both genders with USH2C was identified: nine people were clinically examined: four women were affected, with three of them presenting progressive sensorineural hearing loss and retinitis pigmentosa; one, in addition, presented vestibular alterations evidenced by vertigo and gait impairment (Table 1), with which the diagnosis of USH2C was proposed. In addition, attention was paid to a first degree inbred union in which the parents and the daughter showed homozygosity for the ADGRV1 and USH2C gene mutation with USH2C. Directed exome sequencing study with an Usher syndrome panel rapidly showed a new pathogenic variant in the *ADGRV1* gene, confirming not only the accurate diagnosis of the Usher Syndrome subtype, but also the number affected for a family in a small village of Colombia; a fact that surely as a result of geographic isolation, facilitated the intrafamily unions observed in the genealogy.

The *ADGRV1* gene codifies an adhesion protein that bines to the V1 receptor of the G- protein which is part of the superfamily of polypeptides coupled to G-protein and expressed in the central nervous system. The ADGRV1 protein functions as a transmembrane receptor with seven domains whose function is conducting the normal stereociliary development in the ear [8]. In the eye, the protein is involved in the growth of retinal photoreceptors [7]. Mutations in this gene have been related to four phenotypes: Usher syndrome type 2C with autosomal recessive inheritance, such as the present familial case; Usher syndrome type 2C, along with a polymorphism in the PDZ7 gene in biallelic digenic inheritance, autosomal dominant isolated sensorineural deafness and familial febrile seizure syndrome type 4 of autosomal dominant inheritance [9-12] *ADGRV1* NM_032119.4 c.6819dupT gene in homozygosis, which is a new mutation for this gene and it causes an early stop codon NP_115495p. Ala2274Cysfs*4. WES analysis rapidly showed they differ because in USH type I, hearing loss is congenital and profound, with absence of vestibular functions and progressive retinitis pigmentosa with onset in childhood; USH type II has moderate to severe sensorineural hearing loss and retinitis pigmentosa with onset in the second decade of life; on the other hand, USH type III presents progressive sensorineural hearing loss and retinitis pigmentosa with onset in the second decade of life and variable vestibular dysfunction

Next-generation sequencing identified a new pathogenic variant in the gene ADGRV1 NM_032119.4 c.6819dupT in homozygosity which caused an early stop codon NP_115495 p.Ala2274Cysfs*4, which predicts the synthesis of a short and possibly aberrant protein that affects the function of the G- protein complex.

In summary, a highly inbred family, located in a remote part of Colombia was identified with a pathogenic variant in the ADGRV1 gene which causes USH2C in a fast way and with few resources of medical infrastructure. In addition, a new mutation was found for this gene. Emphasis is given to the usefulness of next-generation sequencing for a rapid solution and diagnosis of difficult cases.

V. Conclusions

Next -generation sequencing is a useful tool to identify mutations and in the rapid and accurate diagnosis of rare diseases such as Usher Syndrome type 2C in remote places, that also was able to be discovered because this family had a highly inbred factor between them, which allowed a new mutation of a recessive syndrome to take place in their genomics.

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Methods: Descriptive, prospective, cross-sectional and correlational study, probabilistic sample of 428 adolescents from three regions of Venezuela. Variables: Sex, age, weight, height, WC, BMI, WHI, WWCI and BFP.

Results: Averages, by sex, similar and non-significant, increase with age and moderate to very high correlations (0.65 0.99) in both sexes. BMI/WHO classifies Excess 11.2% and BMI/CCSS 7.4%; in addition, by sex BMI/WHO classifies a higher percentage of the female sex in Excess (16.2%) than BMI/CCSS (4.8%), the WHI, WWCI and BFP indicators classify the same percentage of the total sample in the Normal category (80.3%; 79.6% and 79.8%); as well as in the Excess category (14.5%; 15.5% and 15.0%). The WHI classifies similar percentage in Excess; the WWCI and cl BFP classify in the category Excess a higher percentage in the male sex (19.1% and 52.7%). For the WWCI and BMI indicators the average is higher in the male sex and for the WHI and BFP indicators higher in the female sex.

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Comparison of Anthropometric Indicators of Overweight and Obesity, in Adolescents from Three Regions of Venezuela

Gerardo Jose Bauce[1]

Summary- The objective is to compare BMI, WHI, WWCI and BFP, measured in adolescents, to propose a combination to assess overweight and obesity.

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Conclusion: BMI is more widely used; the WHI and WWCI on assessing risk or excess; correlates moderately with BMI, 0.61 and 0.62, in both sexes; the BFP behaves better, classifies with percentages similar to those of BMI/CTE, in Excess, in the two sexes; correlates highly with BMI, 0.99 and 0.89 for male and female, can make up a Scale.

I. Introduction

The Body Mass Index (BMI) is used as a screening tool to identify possible weight problems of children and adolescents [(1).] On the other hand, the Centers for Disease Control and Prevention (CDC) and the American Academy of Pediatrics (AAP) recommend the use of BMI to detect overweight and obesity in children from 2 years of age [(2)] The Waist-Height Index, has also been suggested as an indicator of overweight and obesity, in children and adolescents, Matos-Imbert et al [(3).]in a study involving 118 patients, conclude that WHI has predictive value to detect alterations of SBP, PAD and obesity, in children and adolescents. Likewise, Currilem-Gatica et al [(4)], point out that WHI is a measure that has been incorporated, by correlating with indicators of cardiovascular risk in children and adolescents.

The Body Fat Percentage (BFP) represents a very useful indicator, if it is considered that BMI has the particularity of not providing information about the distribution of body fat, according to Moreno-González [(5).] This is an aspect of relevance, considering that abdominal fat and the distribution of fat in the body, represent a different risk and is the one that is associated with greater risk of cardiovascular disease, type 2 diabetes mellitus, cancer, among other diseases, in addition, the measurement of fat mass has been important given the growing problems of overweight and obesity, particularly in children and adolescents [(6)].

Bauce [(7)] has proposed the Weight-Waist Circumference Index (WWCI) as a useful indicator for diagnosing overweight and obesity in children and adolescents. Likewise, Bauce et al [(8)] when evaluating the ROC curve for the WWCI, conclude that it is an indicator with high discriminative capacity.

According to figures published by the WHO, the number of children and adolescents, aged between 5 and 19, who are obese, has multiplied by 10 worldwide, in the last four decades, and if this trend continues, according to a study carried out by the Imperial College of London and the World Health Organization, by 2022 there will be more children with obesity [(9)].

In the case of adolescent children, considered as such those who are between 10 and 19 years old, the WHO defines overweight as the BMI for age with more than one standard deviation above the median established in child growth patterns; and obesity as a BMI greater than two typical deviations above the median established in infant growth patterns [(9,10).] It also refers to the fact that BMI is considered the most universal, cheap and minimally invasive anthropometric indicator; although there is some reluctance when classifying a child as obese is required, if a direct measure of body fat is not taken into account [(11)].

In addition, according to UNICEF, in times of pandemic due to covid-19, it is estimated that some 370 million children in the world have lost access to essential meals since the closure of schools; and if no action is taken, the prevalence of childhood wasting can increase by up to 14%, equivalent to 6 or 7 million children with

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this condition [12]. In addition, overweight and obesity in adolescents is a problem that increases in many countries, which in the long term generates serious effects, such as exposure to an increased risk of type 2 diabetes, cancer and cardiovascular diseases [10].

In Venezuela, data from the Food and Nutrition Surveillance System (SISVAN) and the National Institute of Nutrition (INN) report an increase in overweight in children aged 7 to 14 years, starting in 2000; and in a study conducted by the INN between 2008 and 2010, their results indicate that in children aged 7 to 12 years, 17.6% are overweight and 9.8% are obese; in children aged 13 to 17 years, 12.0% overweight and 9.3% obese [13]. On the other hand, the Comprehensive Treatment Center for Obesity (CitoGroupVe), reveals that the obesity rate fell by a third between 2010 and 2017 due to the economic difficulties of recent years; such a decrease in the rate of obesity from 2011 to the present, went from 24% to 11%, while that of overweight went from 30% to 25%, and that of morbid obesity went from 1.74% to 0.6%, although it does not report on the group that includes children and adolescents [14].

Therefore, this study is of interest to compare indicators of overweight and obesity in adolescents, in order to evaluate them using Body Mass Index (BMI), Waist-Height Index (WHI), Body Fat Percentage (BFP) and Weight-Waist Circumference Index (WWCI), and suggest the one that is most effective in diagnosing overweight and obesity in this age group.

II. Materials and Methods

Descriptive, cross-sectional, prospective and correlational study, based on a sample of 428 adolescents, aged between 13 and 19 years, educational institutions from three regions of Venezuela; of which 56.3% are male and 43.7% female. The variables Age, Weight, Height, Body Mass Index (BMI), Waist-Height Index (WHI), Body Fat Percentage (BFP) and Weight-Waist Circumference Index (WWCI) are considered. Informed consent was requested from the representatives of these adolescents, according to the World Medical Association, WMA (2013), related to the Declaration of Helsinki [15]; we talked with the teachers of the institutions, to agree on the day of the visit, at which time we proceeded to make the measurements and obtain the necessary data.

The anthropometric indices were obtained by applying the following formulas:

Anthropometric Index			Reference
BMI	Quetelet formula		16
WHI	Carbajal-Azcona		17
BFP	Deurenberg et al	PGC = 1.2 x BMI + 0.23 x age - 10.8 x sex - 5.4 Male Sex=1 Female Sex=0	18
WWCI	Bauce et al		19-20

Criteria used for classification

Children and adolescents were classified, according to the curves of BMI percentiles for age of the CDC [16] and the Caracas Cross-Sectional Study [17], in order to compare a national reference with an international one.

Classification according to the CDC (2015) [16], you have to malnutrition, BMI < p5, (equivalent to Deficit); Healthy weight, p5 ≤ BMI < p85, (equivalent to Norm3); Overweight p85≤ BMI < p95 ; and Obesity BMI ≥ p95 (equivalent to Excess BMI ≥ p85).

The values taken as a national reference, according to the Caracas Transversal Study (CTS); are: Deficit, BMI < p.3; Normal: p.3 ≤ BMI < p.97; Excess: BMI ≥ p.97 [17].

For the WHI, the values WHI< 0.50 Normal and WHI ≥ 0.51 Risk [18] were considered, and for the WWCI the values corresponding to the percentiles.

For the Body Fat Percentage (BFP), the values suggested by Moreno [19] were taken into account, for the male sex: Low (BFP< 10%), Normal (10% ≤ BFP< 20%), Overweight (20% ≤ BFP< 25%) and Obesity (BFP ≥ 25%); for females: Low (BFP< 20%), Normal (20% ≤ BFP< 30%), Overweight (30% ≤ BFP< 35%) and Obesity (BFPC ≥ 35%).

To evaluate the Weight-Waist Circumference Index (WWCI), as it is a new indicator, the 85th Percentile was assumed as the reference value, which is close to the value of the mean and was classified according to the following categories: Deficit (WWCI< P5); Normal (P5 ≤ WWCI< P85); Overweight (P85 ≤ WWCI< P95) and Obesity (WWCI≥ P95) [20-21]; and their equivalents Deficit (WWCI< P5), Normal (p5≤ WWCI< P85) and Excess (WWCI ≥ P85).

In addition, adolescents were classified, according to the indicators, with the same criteria; that is, according to the value of the percentiles and categories suggested by the WHO-CDC [22], to classify BMI; in such a way to compare under the same criterion the classification of the four indicators.

Descriptive statistical measures (mean, deviation, percentage), association (correlations, Chi square) and linear regression, and comparison of means and percentages, by sex and age group, were determined using the Student's t-test for independent samples.

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III. Results

The quantitative analysis, based on the results, allows us to affirm that for the total sample, the averages of the four anthropometric indicators (WHI, WWCI, BMI and BFP) are similar, in the two sexes, with the exception of the BFP that is higher in the female sex. Likewise, the averages of the variables and indicators, compared by sex, are not statistically significant with the exception of the BFP ($p<0.001$) (Table 1).

By discriminating these results, by age groups, it is necessary that all the averages, with the exception of the WHI, increase with age (Table 2).

On the other hand, bivariate correlations were obtained between the variables and the indicators, and the results reveal that for the male sex there is a high positive correlation between Weight-Age (0.76); Size-Age (0.83); Weight-Height (0.81); Weight-WWCI(0.74); Weight-BMI (0.84) and moderate negative correlation between WHI-WWCI (-0.61). For the female sex there is a high positive correlation between Weight-Height (0.78); Weight-BMI (0.87); Weight-BFP (0.89); high negative correlation between WHI-WWCI (-0.79); there is also a moderate positive correlation between Age-Weight (0.65); and very high positive correlation between BMI-BFP (0.99). It is observed that for the female sex the Weight-Age and Age-Height correlations are lower; the Weight-BMI and Weight-BFP correlations are greater.

Regressions were obtained between BMI-WWCI, BMI-BFP and BMI-Weight, by sex and all have positive linear regression, with values of the coefficient of determination low in the case of BMI-WWCI; very high in the case of BMI-WWCI male sex, high in BMI-WWCI female; high in the case of BMI-Weight for both sexes (Figure 1).

Table 3 presents the values of the Median BMI and the BFP by age and sex, and it can be observed that they behave in a very similar way, because the values are similar for BMI, except for 18 years which is much higher in the female sex and at 19 years which is much higher in the male sex; in relation to the BFP, it has to behave similarly from 10 to 12 years in the male sex, is less than 13, 14 and 15 years, increases at 16 years, decreases at 17 years and increases from 18 to 19 years; in the female sex it increases from 10 to 16 years, decreases at 17 years, increases at 18 and falls again at 19 years; in addition to all ages, it is higher in the female sex.

The qualitative analysis of the data allows us to affirm that the classification of adolescents, according to each of the indicators, is for BMI according to the two criteria considered, the following:

BMI, according to the WHO/CDC criterion, classifies the total sample of adolescents in the Normal category with a lower percentage than the BMI/CTS criterion (87.8% vs 91.6%), although they turn out to be not significant ($p=0.072$); in addition, it classifies a higher percentage in the Excess category (11.2% vs 7.4%), which turn out to be statistically significant ($p=0.06$). When comparing the percentages by sex, in the WHO/CDC criterion, it classifies a higher percentage of the female sex in Normal (94.1% vs 83.0%); likewise, it classifies a higher percentage in the Excess category (16.2% vs 4.8%), which turn out to be significant ($p=0.001$).

For the BMI/CTS criterion, it must be classified 91.6% in the Normal category, and of them the highest percentage of the female sex (93.5% vs 90.05), which turn out to be non-significant ($p=0.196$); in the Excess category it classifies 7.4% and of them a higher percentage in the male sex (9.5% vs 4.8%) which are statistically not significant ($p=0.067$).

For WHI, WWCI and BFP, indicators, all three have to classify the same percentage of the total sample in the Normal category (80.3%; 79.6% and 79.8%); as well as equal percentage in the Excess category (14.5%; 15.5% and 15.0%). When discriminating by sex, the WHI classifies the same percentage in the two sexes (83.0%), and a similar percentage in the Excess category; the WWCI and the BFP classify a percentage in the Higher Normal category in the female sex (85.5% and 61.8%), and in the male sex they classify 75.1% and 52.7%, respectively; while in the Excess category they classify a higher percentage in the male sex (19.1% and 52.7%) (Table 2).

The comparison of these percentages, in the two categories, turns out to be statistically significant, for Normal with the WWCI ($p=0.008$) and for Excess ($p=0.018$); with the BFP in the Normal category they are significant ($p=0.059$) and in the Excess category they are not statistically significant ($p=0.144$).

When discriminating by age and sex, it is observed that for the WWCI and BMI indicators the average is higher in the male sex and for the WHI and BFP indicators it is higher in the female sex; in addition, for the WHI, WWCI and BMI indicators the median is higher in the female sex (Table 3).

IV. Discussion

For the discussion, the concern of the United Nations was taken into account, regarding No communicable Diseases, particularly overweight and obesity, since in May 2013 it convened a meeting with the heads of state, in order to treat the prevalence, morbidity and mortality of non-communicable diseases, which was held on September 19 and 20, 2011, and concluded that these diseases are a threat to the economies of member countries, as well as recognize the importance of assisting countries with fewer resources and intensifying measures taken; they also considered the "European Charter against Obesity,

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approved in November 2006; the Aruba Convention for Action Against Obesity, June 2011." [23].

In addition to this, there is the fact that the WHO has published figures related to overweight and obesity in children and adolescents,

In such a way that in line with this proposal, the results of this work are obtained, which show that the averages of age, weight and height are higher in the male sex; for the WHI and BFP indicators they are higher in the female sex and for the WWCI and BMI indicators they are higher in the male sex. These results differ from those obtained by Ojeda Nahuelcura et al [24], who report similar averages for males and females, in age and weight, and slightly higher weight in males. When classifying them by age group, it is observed that in the group of 10 to 12 years the averages of age, weight, height, BMI, WWCI and BFP are higher in the female sex, and for the WHI they are equal in the two sexes; in the group of 13 to 15 years the average age is equal, weight, height, WHI and WWCI with higher averages in the male sex and for the BMI and BFP higher in the female sex; for the group 16 to 19 years, equal mean age and BMI, higher average in weight, height, WHI in the male sex, and higher in BFP for the female sex.

Regarding the classification of BMI, according to the WHO/CDC criterion, 87.8% of adolescents are classified in the Normal category, and with the CTS criterion 91.6% are classified in the Normal category; both percentages higher than those reported by Duin-Balza et al [25]; on the other hand, these results are higher than those reported by Ojeda Nahuelcura et al [24] in a study of adolescents, with similar average age, who report 38.3% in Normal. In relation to overweight and obesity, the WHO/CDC criterion classifies 11.2% in Excess and the CTS criterion 7.5%, while the reference study classifies 48.9% in Excess. Likewise, the average BMI value is 20.8±3.4 kg/^{m2}, similar to that obtained by Guerrero et al [26], in a study of 150 adolescents from the central region of Venezuela. When discriminating by sex, the averages are similar and not significant (21.0±4.4 and 20.5±3.4), slightly higher than those reported by Bauce [19] in a sample of 304 schoolchildren, and higher than those obtained by Bauce [27] in a study that includes 484 students from Caracas (18.7±3.7 and 18.6±3.2). On the other hand, these two criteria classify in Excess 11.2% and 7.5%, respectively, percentages that are statistically significant, in addition to those reported by Bauce [28]." When discriminated by age group, it has to be that for the group of 10 to 12 years the average is 19.0±3.3 kg/^{m2}; for group 13 to 15 years 21.3±4.1 kg/^{m2} and for group 16 to 19 years 23.3±3.3 kg/^{m2}; values slightly higher than the averages reported by Bauce [29] 18.3±3.4 kg/^{m2}; 20.14±2.7 kg/^{m2} and 22.3±3.1 kg/^{m2} for the groups 9 to 11 years, 13 to 15 years and 16 or more years, respectively.

Given these results about BMI, in different groups, which have a similar behavior, both for the total and for each sex, in addition to being non-significant differences, it can be said that this indicator remains reliable to evaluate overweight and obesity.

In relation to the WHI it can be observed that the average of the group is 0.45±0.1, higher in the female sex, and the statistically significant difference; On the other hand, the WHI classifies 82.9% in Normal and 17.1% in Risk, and when discriminating by sex, it has to classify equal percentages of each sex in Normal, a value that differs from that reported by Matos-Imbert et al [30], who report much lower percentage; in Risk, the WHI classifies similar percentages, although slightly higher in the male sex, which turn out to be lower than those reported by these authors.

It should be borne in mind that according to Sánchez et al [31], the WHI is an indicator that "eliminates the need to compare with a pattern of percentiles, because they remain stable during growth, it does not vary between 6 and 14 years".

Regarding the BFP, the average is higher in the female sex, and the difference is statistically significant ($p<0.001$), which coincides with what Bauce [32] obtained in a group under 20 years old, which turn out to be statistically significant. When discriminating by age group, it has to be that for 10 to 12 years, the averages by sex are not significant, while for the groups 13 to 15 years and 16 to 19 years, the difference of the averages by sex if it is statistically significant ($p<001$); this result differs from that obtained in a study conducted by Herrera-Cuenca et al [33], which reveals that there is no significant difference between the percentages of obesity prevalence in children and adolescents, obtained by BMI and BFP. Additionally, it has to be that Bauce [7], in a study that included different age groups, it obtained a high correlation between the BFP, obtained by Deurenberg's formula, and the BMI for the group under 20 years and averages with significant difference between sexes. On the other hand, a study carried out by Padilla [34], reveals a correlation of 0.75 between BMI and BFP, while in this study a correlation of 0.99 and a coefficient of determination of 0.9916 have been obtained, which indicates that the variability of BMI is explained by 99.16% by the variability of BFP; this result coincides with that obtained by Bauce in a study that includes 361 children aged 6 to 11 years, and the correlation between BMI and BFP is 0.99 for the male sex and for the female sex (35). In addition, the regressions between the BMI with the BFP and the WWCI, reflect the linear behavior and show the regression coefficient, for each of the two sexes, and behave in a much more homogeneous way than those reported by this same author, which means that these two indicators can be used with confidence to evaluate overweight and obesity in children and adolescents.

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In another study conducted by Medina-Bustos et al [36], in which they incorporate the median by age and sex, it was observed that this is very similar in the two sexes, for the WHI, behavior that is the same in this study, with the exception of the age of 15 years, in which the median is higher in the female sex; for the IPCC the median is greater than 10 at 13 years and 15 years, as well as for BMI which is greater at 11, 15 and 18 years and the BFP at 10 and 13 to 19 years, higher in the female sex.

The relationship between BMI and BFP turns out to be stronger in this study, than that obtained by Di Gianfilippo et al [37], since the sex determination coefficient is close to value 1, so for the male sex it is 0.9908 and for the female sex it is 0.9802, which indicates that the variability of BMI is explained by 99.08% and 98.02% by the variability of the PGC, for the male and female sex, respectively.

V. Conclusions

It is necessary to emphasize that for the evaluation of anthropometric measurements, the elaboration of indicators is indispensable, the which are combinations of the measurements made in each individual [38], according to this four indicators were identified: BMI, WHI, WWCI and BFP, to evaluate this group of adolescents, and taking into account the results and the discussion, it can be concluded that:

BMI remains the most commonly used indicator to assess overweight and obesity.

The WHI, despite being an indicator that eliminates the need to compare with a pattern of percentiles, seems to over evaluate when classifying adolescents at Risk or Excess, both male and female; however, the averages by sex are not significant.

The WWCI, like the WHI, seems over-evaluated when classifying adolescents at Risk or Excess, although the averages by sex are not significant; however, it correlates moderately with BMI, 0.61 and 0.62, for males and females respectively.

The PGC seems to be the indicator that behaves best, since it classifies with percentages similar to those of BMI/CTS, in Excess, both male and female adolescents; In addition, it correlates highly with BMI, 0.99 and 0.89 for male and female respectively, and the averages by sex are statistically significant, as are the percentages, by sex in the Normal and Excess categories. In view of this, the BMI indicators, WWCI and BFP, can be combined, to form a Scale and evaluate groups of adolescents, in a more efficient and reliable way, with overweight and obesity.

To complement, Dr Bull said: "WHO encourages countries to strive to change environmental factors that increase the risk of obesity in our children. More specifically, it is necessary to reduce the consumption of very elaborate foods that are cheap, with high caloric content and low nutritional value. In addition, it is advisable that children devote less leisure time to sedentary activities and that include the use of screens. To this end, it is necessary to promote physical activity through sport and active recreation' [39].

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Table 1: Average and deviation of anthropometric variables, by sex and age group

Sex	Age	Weight	Height	BMI	WHI	WWCI	BFP
Muestra total							
Male (n=241)	13,5±2,7	51,5±17,0	154,5±15,5	21,0±4,4	0,45±0,1	0,75±0,3	12,2±5,5
Female (n=187)	11,0±2,2	38,3±11,7	149,9±10,7	20,5±3,4	0,44±0,1	0,76±0,3	22,3±4,4
Total (n=428)	13,4±2,8	49,4±15,3	152,5±15,6	20,8±3,4	0,45±0,1	0,76±0,3	16,6±7,3
p	0,013	0,0001	0,0001	0,150	0,465	0,745	0,0001
Group from 10 to 12 years							
Sex	**Age**	**Weight**	**Height**	**BMI**	**WHI**	**WWCI**	**BFP**
Male (n=93)	10,9±0,9	37,4±09,8	140,0±09,2	18,9±3,5	0,48±0,05	0,57±0,14	19,8±4,3
Female (n=89)	11,0±0,9	40,0±10,1	143,4±10,3	20,2±3,7	0,48±0,04	0,61±0,14	20,2±3,7
Total (n=182)	10,9±0,9	38,7±09,9	141,7±09,9	19,0±3,3	0,48±0,04	0,59±0,14	14,5±6,9
Group from 13 a 15 years							
Male (n=93)	13,8±0,7	54,7±13,1	158,3±9,4	21,8±4,7	0,45±0,04	0,78±0,16	13,1±5,7
Female (n=55)	13,7±0,8	49,5±08,1	154,7±7,4	22,5±3,3	0,44±0,04	0,73±0,10	22,5±3,3
Total (n=148)	13,7±0,8	52,7±11,7	157,0±8,8	21,3±4,1	0,44±0,04	0,76±0,15	16,6±6,7
Group from 16 a 19 years							
Mañe (n=55)	17,5±1,2	69,5±11,6	172,2±07,2	23,3±3,3	0,40±0,08	1,08±0,48	15,8±4,1
Female (n=42)	17,7±1,4	57,1±08,1	157,3±06,0	23,0±3,4	0,38±0,11	1,11±0,59	26,3±4,2
Total (n=97)	17,6±1,2	64,4±12,3	165,9±10,0	23,3±3,3	0,39±0,10	1,10±0,50	20,4±6,6

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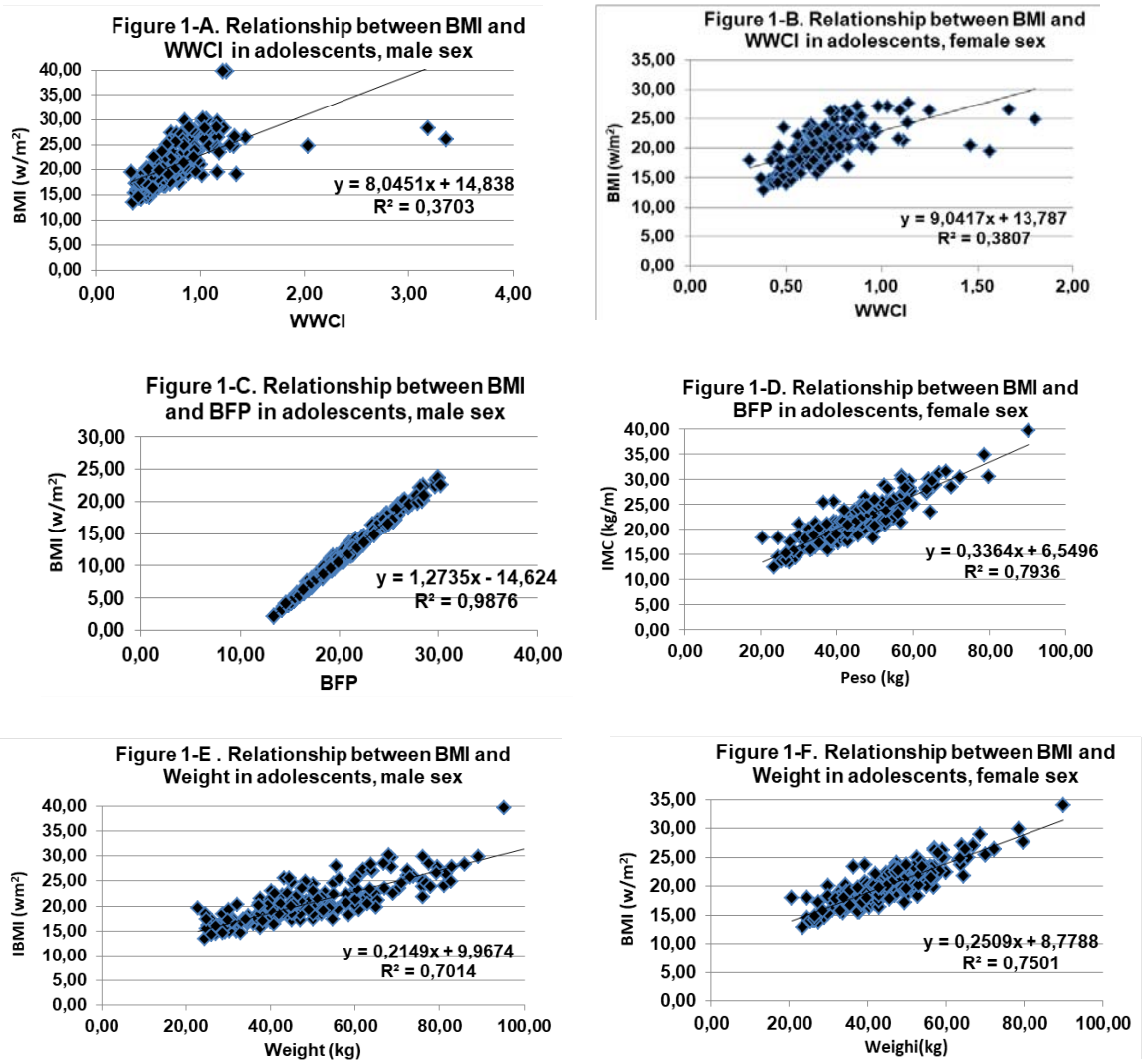


Figure 1: Relationship between BMI and indicators WWCI, BFP and Weight, in adolescent by sex

Table 2: Percentage comparison of the classification of anthropometric indicators, according to percentiles, by sex, according to category

	Deficit				Normal				Excess			
	Male		Female		Male		Female		Male		Female	
IMC/Age CDC/OMS	n	%	n	%	n	%	n	%	n	%	n	%
	2	0,8	2	1,1	200	83,0	175	94,1	39	16,2	9	4,8
IMC/Age CCSS	n	%	n	%	n	%	n	%	n	%	n	%
	1	0,4	3	1,6	217	90,0	174	93,5	23	9,5	9	4,8
WHI	n	%	n	%	n	%	n	%	n	%	n	%
	0	0,0	0	0,0	200	83,0	156	83,9	41	17,0	30	16,1
WWCI	n	%	n	%	n	%	n	%	n	%	n	%
	14	5,8	7	3,8	181	75,1	159	85,5	46	19,1	20	10,8
BFP	n	%	n	%	n	%	n	%	n	%	n	%
	92	38,2	61	32,8	127	52,7	115	61,8	22	9,1	10	5,4

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Table 3: Median and standard deviation of BMI and PGC, by age and sex

	Male			Female		
Age	n	BMI (kg/m2) Median ± SD	BFP (%) Mediana ± SD	n	BMI (kg/m2) Median ± SD	BFP (%) Mediana ± SD
10	37	17,23±2,75	17,58±3,29	32	17,90±3,25	18,38±3,90
11	25	17,53±4,37	18,17±5,24	21	18,84±2,64	19,74±3,17
12	31	19,98±3,17	21,34± 3,80	36	20,07±2,73	21,45±3,28
13	39	20,53±4,09	11,43±4,91	30	20,15±2,75	21,77±3,30
14	37	20,80±2,86	11,98±3,44	13	19,91±2,44	21,71±2,93
15	16	19,93±8,37	11,16±10,05	13	21,18±2,82	23,47±3,37
16	16	22,53±3,44	14,52±4,13	10	23,04±2,21	25,93±2,65
17	16	21,12±3,57	13,06±4,28	6	21,44±2,87	24,24±3,45
18	5	21,73±2,51	14,02±3,01	7	26,40±2,87	30,42±4,67
19	19	25,84±2,85	19,18±3,42	18	22,95±3,84	26,51±4,60

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Acknowledgments

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The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.

Manuscript Style Instruction (Optional)

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11'", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

Structure and Format of Manuscript

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

a) A title which should be relevant to the theme of the paper.
b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
d) An introduction, giving fundamental background objectives.
e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
f) Results which should be presented concisely by well-designed tables and figures.
g) Suitable statistical data should also be given.
h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
j) There should be brief acknowledgments.
k) There ought to be references in the conventional format. Global Journals recommends APA format.

Authors should carefully consider the preparation of papers to ensure that they communicate effectively. Papers are much more likely to be accepted if they are carefully designed and laid out, contain few or no errors, are summarizing, and follow instructions. They will also be published with much fewer delays than those that require much technical and editorial correction.

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The title page must carry an informative title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) where the work was carried out.

Author details

The full postal address of any related author(s) must be specified.

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The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Keywords

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, “What words would a source have to include to be truly valuable in a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

Numerical Methods

Numerical methods used should be transparent and, where appropriate, supported by references.

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Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

Formulas and equations

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

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Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.

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Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

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Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

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Techniques for writing a good quality Science Frontier Research paper:

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

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14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

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23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

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This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

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- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

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Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

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An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

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Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.



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Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

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- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
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- Do not present similar data more than once.
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Approach:

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Put figures and tables, appropriately numbered, in order at the end of the report.

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- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

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Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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