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Study of Three Clustering

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Highlights

Algorithms for Microarray Data

Comparison of Anthropometric Indicators

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Hydrological Resources in the Southern Andes of Ecuador: Brief Study of the Tabacay River Micro-Basin

By María E. Calle Luzuriaga, María A. Luzuriaga, Karina M. Gonzalez
& José Isidro Yamasqui

Yachay Tech University

Abstract- This research provides a brief review on several topics as a foundation to comprehend the current situation on water and hydrological resources management and monitoring at a local scale, considering the case of the Tabacay river micro-basin the southeast of Ecuador. This study is performed in aims to explore the data collected by representative meteorological and pluviometry stations of the corresponding geographical space within the micro-basin. In this context, a comparative analysis on rainfall for the recent years is provided. It also presents the evaluation of the existing challenges in the water and sanitation management and further recommendations for control and use of the natural resources in subject.

Keywords: water and sanitation, tabacay river, micro-basin, hydrological resources, rainfall.

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Hydrological Resources in the Southern Andes of Ecuador: Brief Study of the Tabacay River Micro-Basin

María E. Calle Luzuriaga ^α, María A. Luzuriaga ^σ, Karina M. Gonzalez ^ρ & José Isidro Yamasqui ^ω

Abstract- This research provides a brief review on several topics as a foundation to comprehend the current situation on water and hydrological resources management and monitoring at a local scale, considering the case of the Tabacay river micro-basin the southeast of Ecuador. This study is performed in aims to explore the data collected by representative meteorological and pluviometry stations of the corresponding geographical space within the micro-basin. In this context, a comparative analysis on rainfall for the recent years is provided. It also presents the evaluation of the existing challenges in the water and sanitation management and further recommendations for control and use of the natural resources in subject.

Keywords: water and sanitation, tabacay river, micro-basin, hydrological resources, rainfall.

1. INTRODUCTION

Water resources and the hydrological cycle are an essential part of healthy ecosystems and life itself. Water is a natural supply but, in many scenarios, it is rather scarce since only the 3% approximately can be drinkable according to the American Geosciences Institute (AGI, 2022). On it depends both origin and continuation of life on Earth. Nowadays water conservation is one of the principal goals of science and activism and therefore, the ecological implications of water treatment, distribution, and consumption. Water and derived resources conservation directly implies making positive contributions to the hydrological cycle, yet there is a constant negative damage to its quality due to anthropogenic impact (León, et al., 2018). Strategies for the protection and sustainable management water resources should be prioritize and given the importance they imply. These strategies must contemplate the natural elements of a hydrological system such as groundwater, rivers, lakes, springs, rainfall, creeks, etc. (Nalbantis et al., 2011).

Although it is stipulated as a human right and one of the sustainable development goals (goal 6), water distribution is unequal across the world. Governments and global institutions such as the United


Nations invest large amounts of money in the development of plans to improve the current situation and enhance water and sanitation supply. The emerge of new technologies has helped in numerous problems derived from the use and consumption of water and hydrological resources. Now there are ecological approaches to minimize wastewater, new sustainable filtration systems and therefore, better environmental management and control of such fragile reserve. Within this framework, several models have been developed to support and monitor environmental management and policy development. (Jerves-Cobo, et al., 2019).

The human factor also must be considered since anthropogenic activities can highly alter ecosystems and their hydrological cycle, especially in a country that highly depends on livestock and agronomy. Agriculture is one of the largest sectors of the productive matrix of Ecuador, representing up to 30% of the total working class in the country, according to a 2019 survey by World Bank. In many areas of developing countries, the population is supplied with water from rivers, streams, lakes, etc. (Arnal-Arnal, et al., 2002).

In aims to tackle and contribute to the global Sustainable Development Goals, the National Institute of Statistics and Censuses of Ecuador (INEC), has been improving and consolidating its monitoring system since 2016, specifically on SDG 6.1 and 6.2 (Water and sanitation) and the SDG indicators were adapted to Ecuadorian framework (Moreno, et al., 2020). From the several surveys and studies done under this institution and others in the field, it is evident that safe water distribution is the main issue in the water sector. Yet, another highly significant concern is the water and hydrological resources management. In this research we consider the Tabacay River micro-basin that provides of drinking water to the city of Azogues and its surroundings, in the southeast of Ecuador.

The leading causes that affect the ecological status of the Tabacay River micro-basin system are mostly administrative problems and territorial management of areas allowed for livestock and agriculture (Olivares-Navarro, 2017). Also, the lack of education and training on topics such as ecology and water use and consumption, which are a crucial factor in the sustainability of both cities and communities. The framework of this research is to provide a general insight

Author ^α: Universidad de Tecnología Experimental Yachay Tech, School of Chemistry and Engineering. e-mail: maria.calle@yachaytech.edu.ec

Author ^σ : Universidad Católica de Cuenca, Cañar campus.

Author ^ρ: EMAPAL: Water and sanitation management municipal company, Azogues, Ecuador.

on the status of the micro-basin and the data collected on the meteorological stations. We aimed to validate an integrated ecological perspective on the Tabacay river micro-basin monitoring and management.

II. WATER MANAGEMENT IN ECUADOR

Ecuador is in the northwestern region of South America. The total population is around 17.8 million people, according to the 2021 census. According to the reported statistics around 70% is urban and 30% rural (INEC, 2022). The country has four main regions: the Andean highlands, the Amazon, the coastal region, and Galapagos islands. Although there are some geographical and administrative differences between these regions all of them are under the same law code and National Constitution and a legal territorial organization.

In terms of hydrological resources, they share the Organic Water Resources Law on Water Uses and Exploitation (2014), most of the activities regarding use and consumption of water and related resources are regulated by the National Secretary of Water (SENAGUA). The Agency for the Water Regulation and Control (ARCA) oversees the water and sanitation services. (Moreno, et al., 2020). In Ecuador, water and sanitation services are considered a public right. These services are mostly in charge of local governments and the management of them is under local public enterprises. Yet, that happens for the urban areas. In the rural communities, which represent a third of the country, water distribution and administration depend upon community organizations called water joints.

Therefore, the quality-of-service provision does not run homogeneously for every city, rather it is diverse. In those terms, the situation demands constant supervision and regulation for the State to guarantee that the Ecuadorian population can access to such basic services with the quality they imply and the international standards for water consumption. Every water provider enterprise must follow certain technical norms with specifications on physical-chemical and microbiological parameters that ensure the distribution of potable water.

In rural Ecuador, 25% of the population still has no access to improved sanitation facilities (INEC, 2022). The restricted financial budget available in developing countries, there is an increasing demand for environmentally and economically sustainable water and sanitation systems. Urban water and wastewater treatment offers many other public health advantages since they fulfill certain quality criteria opening the door to resource recovery as well as water recycling for selected agricultural and industrial uses (Alvarado, et al., 2017).

III. CASE OF STUDY: THE TABACAY RIVER MICRO-BASIN

The Cañar province is in the southeast of Ecuador. The relief is mountainous, and the most significant elevations are at 4,518 and 3,838 meters above sea level. The climate is very specific to the Andes Mountain range, and the climatic floors are mostly humid mesothermal and semi-thermal paramos.



Figure 1: Panoramic views of the Tabacay micro-basin topography

There are seven counties: Azogues, Biblián, Cañar, Deleg, Tambo, Suscal and La Troncal. The political, financial, and administrative headquarters of this province is in the city of Azogues, as the provincial capital. From there, the most relevant decisions are handled, however, each county has its own local administration.

For the Tabacay micro-basin the main tributaries are the Llaucay, Nudpud, Condoryacu, Rosario, Mapayacu and Rubís streams. The micro-basin has an area of 6650 hectares (66.5 km²) and administratively belongs to the Azogues county and is divided into 2 parishes: Guapán and Bayas.



Figure 2: Hydrometeorological network of the Tabacay micro-basin

The Tabacay River micro-basin is part of the Santiago River basin, whose surface is 66.85 km², which provides drinking water to the rural and urban areas of the city of Azogues (Gutierrez, 2017).

Over the recent years the hydrological monitoring and the use of water resources from this micro-basin has improved. However, there are many problems whose repercussion is of great impact on environmental, social, and economic management within this geographical area.

In the Tabacay river micro-basin, many problems were detected that have been mentioned for their damaging impact on environmental and hydrological management within this geographic space. These problems are agriculture in the paramo and expansion of agricultural in unsuitable areas, water pollution and deficient quantity and quality of potable water, erosion provoked by the previous mentioned motives, sediment production, soil degradation caused by poor road infrastructure, irrigation without planning and defective initiatives for the conservation and recovery of native vegetation. Another key concern is the lack of mitigation of environmental impacts: construction of roads, exploitation of mines, Problem due to the use of water. Lastly, there is an obvious deficit on environmental education and technical assistance (Olivares-Navarro, 2017).

The water and sanitation management municipal company of Azogues, Ecuador, also known

as EMAPAL-EP has been working on improving the water and hydrological resources control and management of the Tabacay river micro-basin. The company has several meteorological and pluviometry stations that are indispensable tools that measure and monitor the various parameters that allow to track the micro-basin situation and how it changes through the years. These weather stations use specific sensors for each parameter that collects information on atmospheric behavior covering strategic areas and study the water catchment creeks of the corresponding geographical space.

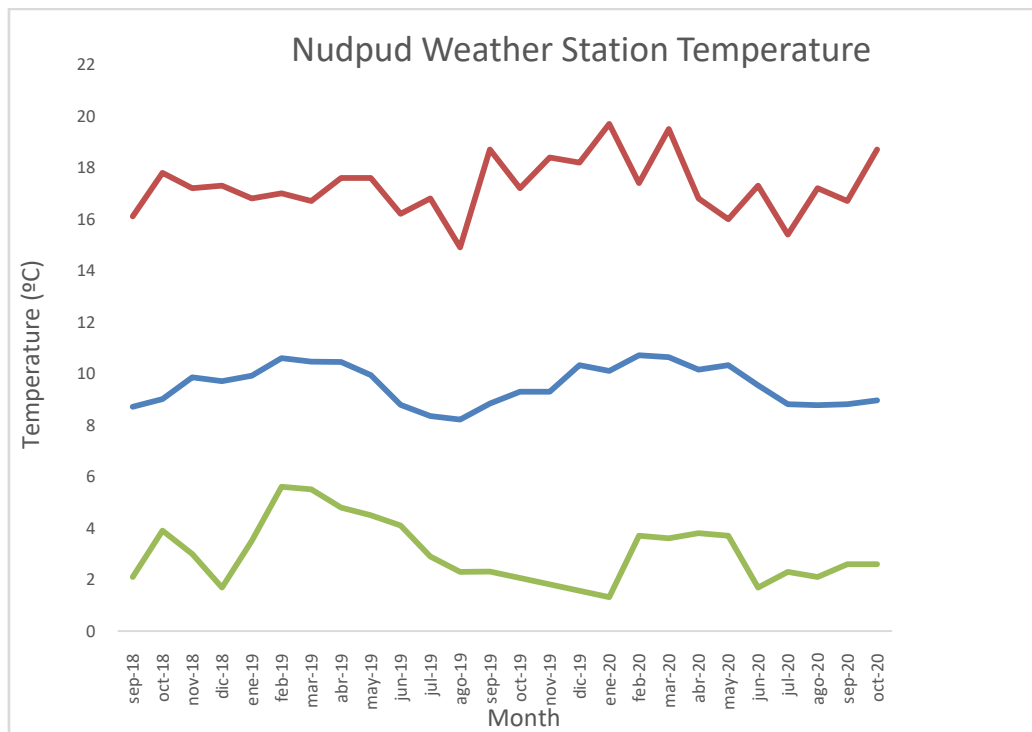
IV. WATER CATCHMENT CREEKS, RAIN GAUGES AND METEOROLOGICAL STATIONS

Meteorological and pluviometry stations are located through the micro-basin based on several criteria that allows to collect representative data that can provide worth statistics. These criteria considerate having such tools near water catchment creeks since it is of interest knowing how much it rains in the area that provides water to a city. Also, the rain gauges are in easy access and geographically strategic locations.

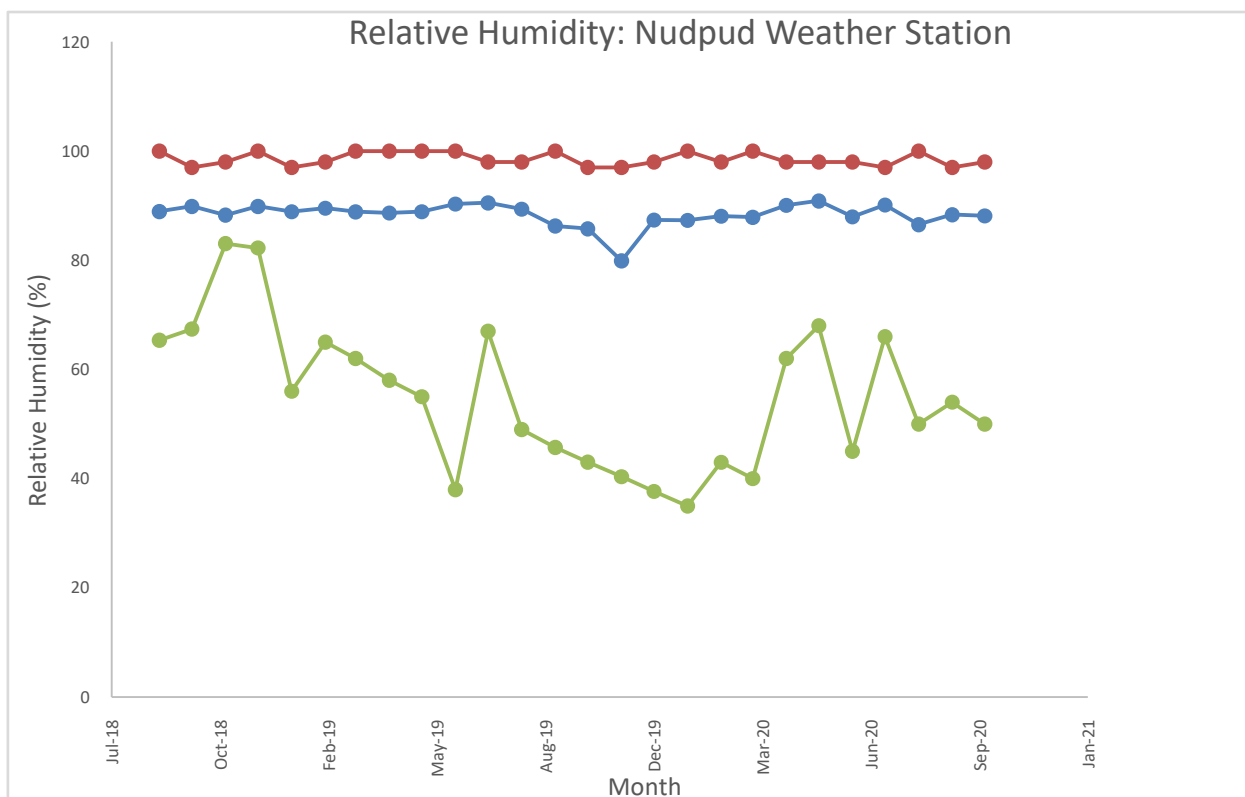
There are 7 rain gauges in the Tabacay micro-basin, in this brief study we considered the most relevant stations: Nudpud, Guapán, Aguilán, Molobog and Pugiola. The most efficient monitoring system is by the Nudpud station; thus, the most illustrative data is provided by the mentioned station.

V. NUDPUD WEATHER STATION

The area where the station is located is at an altitude of 3230masl, so it is observed that temperatures have a variable behavior over time.

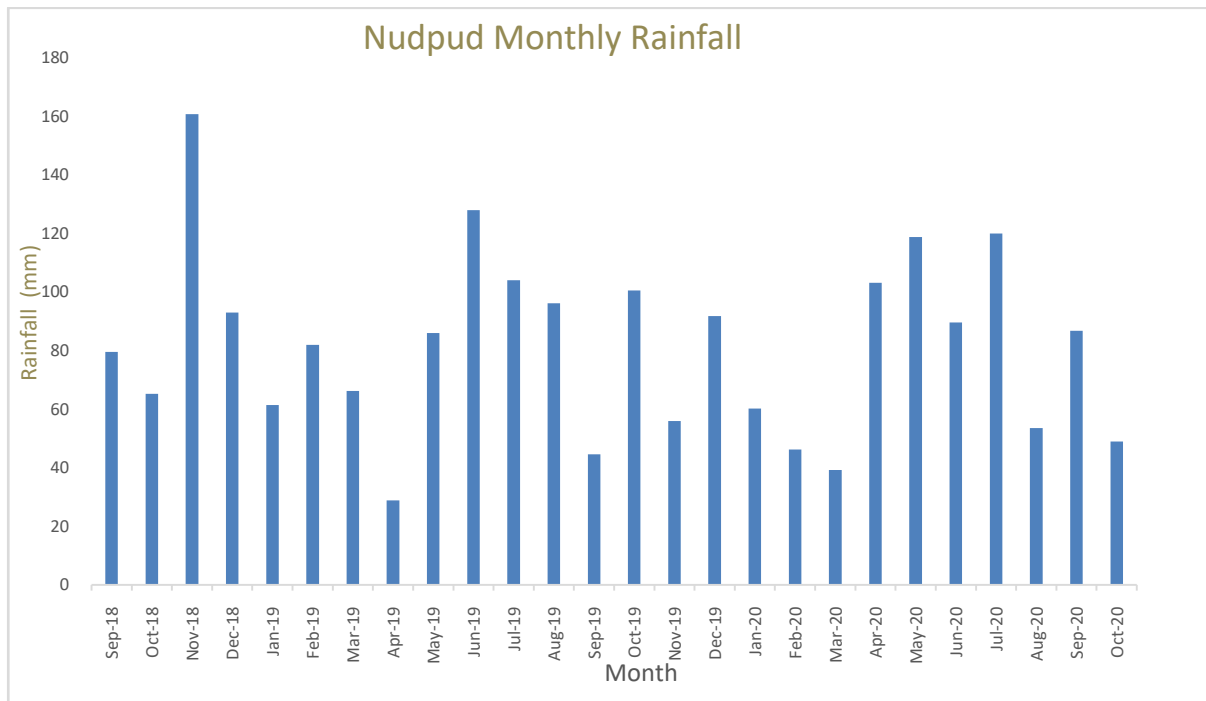


Relative humidity indicates the amount of water in the environment and a condition for whether there is precipitation or fog. Relative humidity remains within normal ranges in the area.



The month with the highest rainfall recorded in the Nudpud area is November 2018, while the other months the precipitation has been moderate to high.

A comparison between the monthly precipitation of some of the areas belonging to the Tabacay micro-basin was performed:



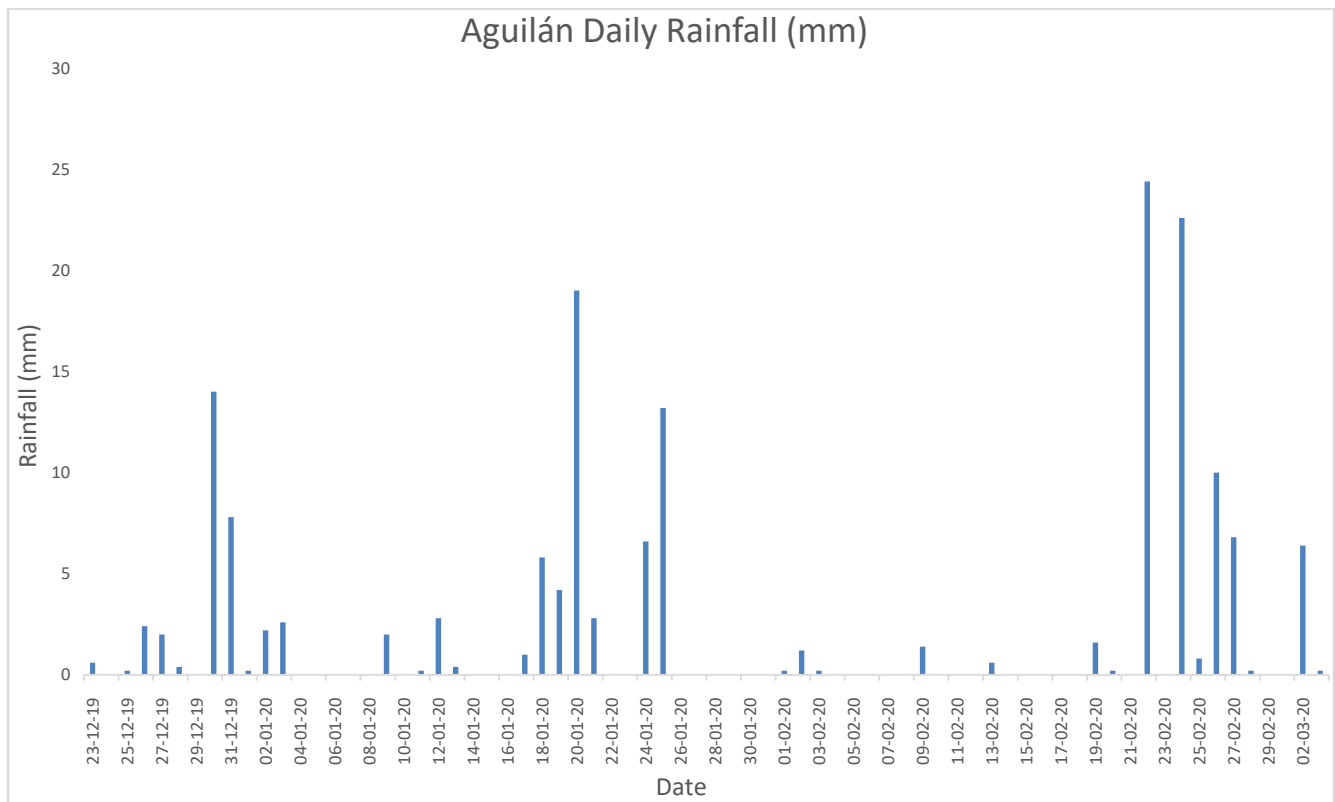
Comparison of data recorded by years

Date	Temperature (°C)					RelativeHumidity (%)	Rainfall (mm)
	Med	Max	Min	High	Low		
nov-18	8.72	16.1	2.1	9.3	8.21	88.91	79.6
nov-19	8.84	18.7	2.3	9.31	8.49	86.25	44.6
nov-20	8.81	16.7	2.6	9.22	8.41	88.32	86.8

When analyzing the average, maximum and minimum temperatures, it is observed that the years of comparison have a similar behavior with temperature ranges according to the altitude of the area, as well as the relative humidity are in ranges of 86% - 88%.

VI. AGÜILÁN RAIN GAUGE

The months of greatest rainfall recorded in the Aguilán area is in the month of February of the year 2020, while in the other months there is moderate to high rainfall in the area.



It is determined that there is a different behavior between the compared years, having a greater amount of rain in 2020.

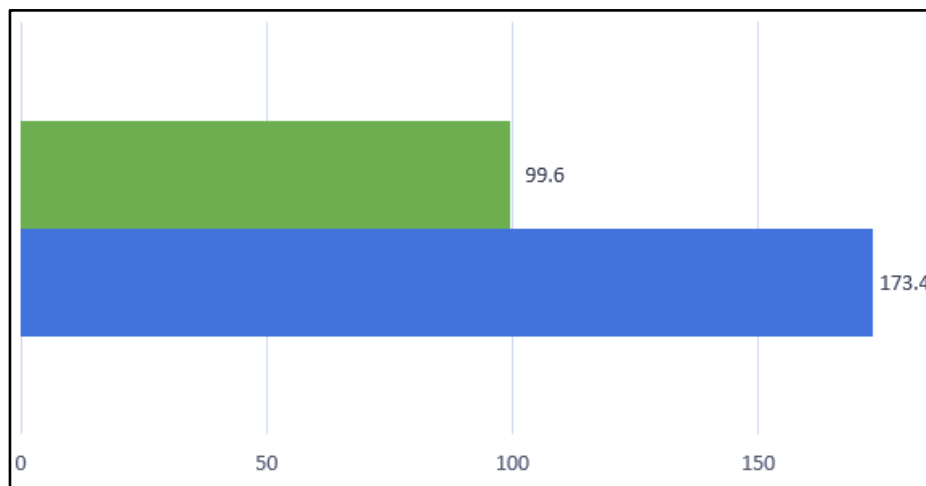
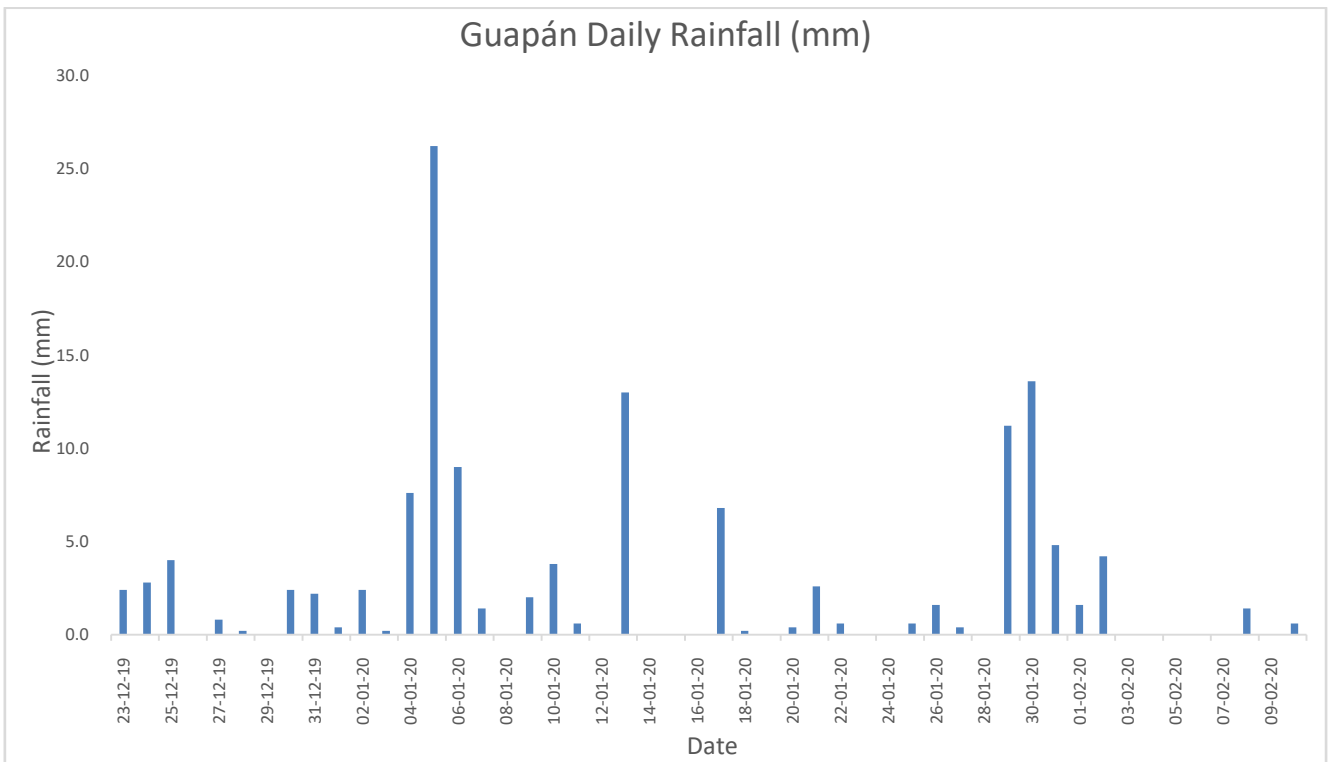


Figure 3: Monthly comparison of rainfall (mm) in Aguilán Rain Gauge. In green the month of November 2021, in blue the month of November 2020

VII. GUAPÁN RAIN GAUGE

The months of greatest rainfall recorded in the Guapán area is in the month of January of the year 2020, while in the other months there is moderate to high rainfall in the area.



It is determined that there is a different behavior between the compared years, the year with the least

precipitation is 2020, while 2019 is the greatest amount of precipitation

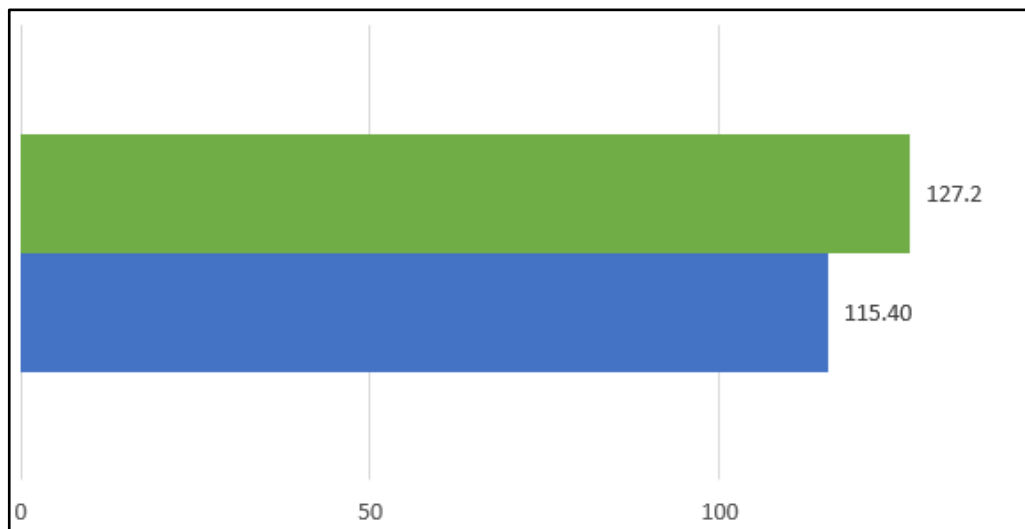
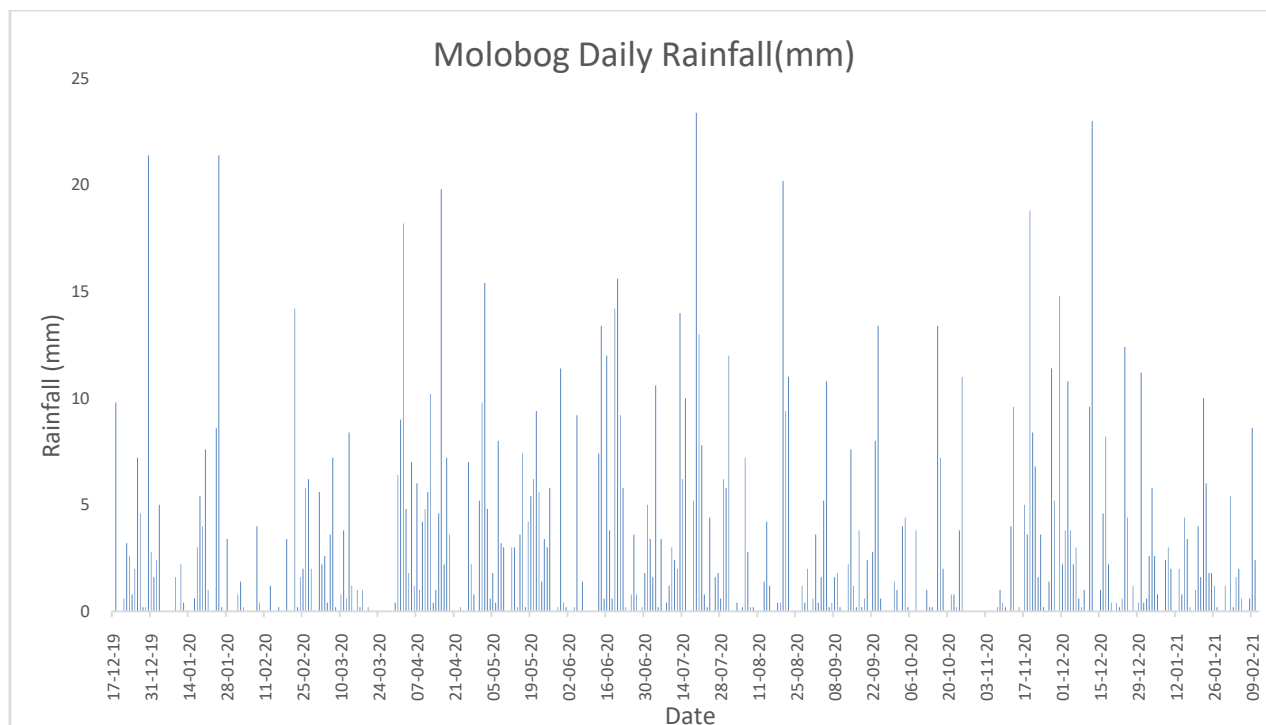


Figure 4: Monthly comparison of rainfall (mm) in Guapán Rain Gauge. In green the month of November 2021, in blue the month of November 2020.

VIII. MOLOBOG RAIN GAUGE

The months of greatest rainfall recorded in the Molobog area are in the months of January, July, and December of the year 2020, while in the other months there is moderate to high rainfall in the area.



It is determined that there is a different behavior, the rainfall of 2021 is greater than that of 2020.

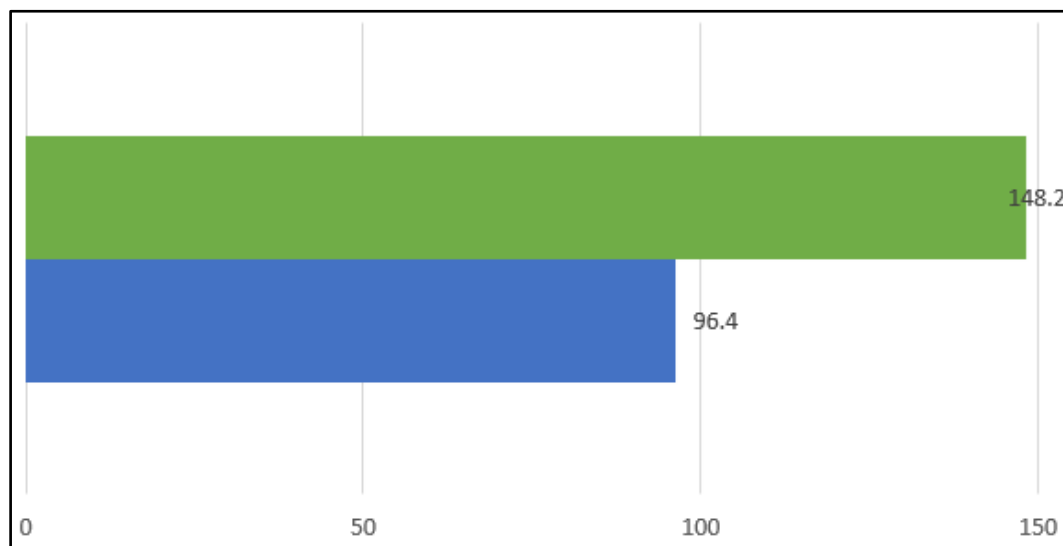
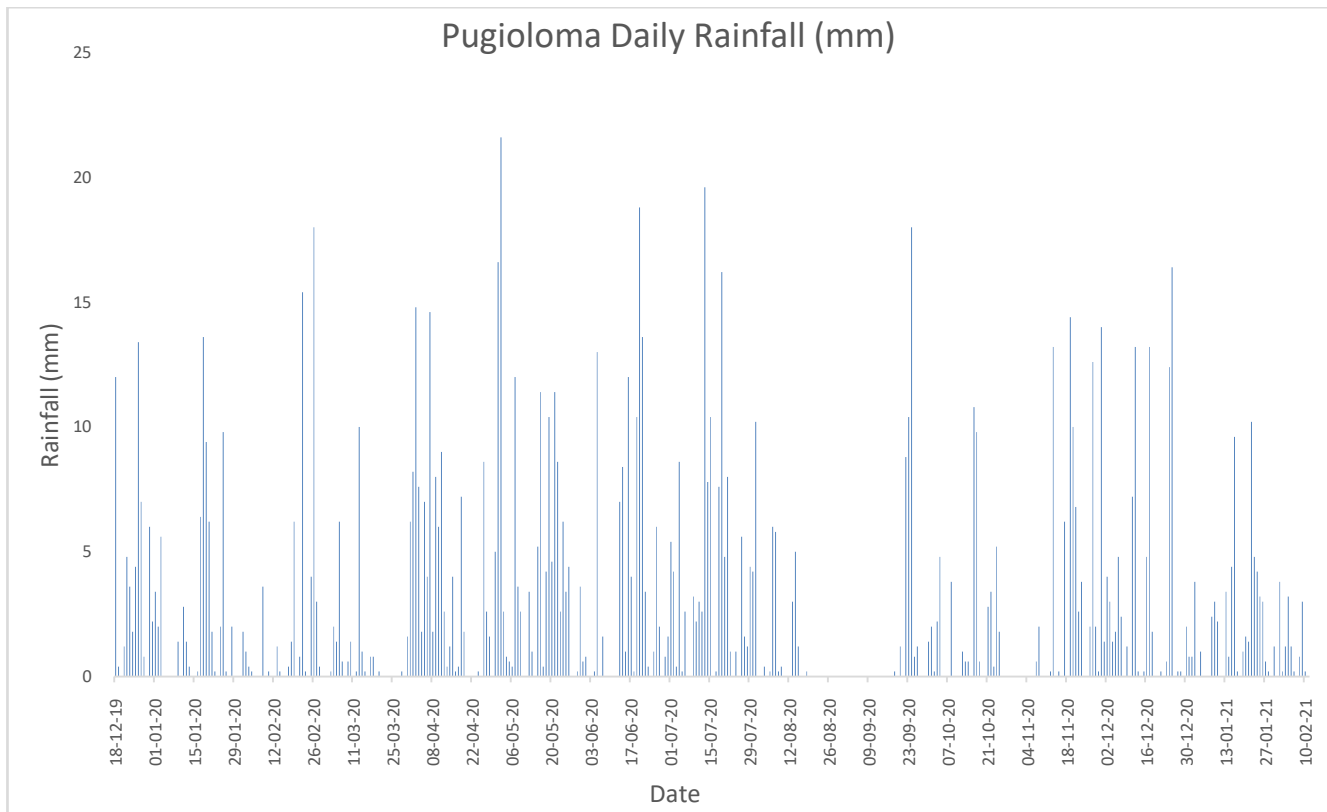


Figure 5: Monthly comparison of rainfall (mm) in Molobog Rain Gauge. In green the month of November 2021, in blue the month of November 2020

IX. PUGIOLOMA RAIN GAUGE

The months of greatest rainfall recorded in the Pugioloima area are in the months of April, June, July, and December of the year 2020, while in the other months there is moderate to high rainfall in the area.



It is determined that there is a different behavior, the amount of rainfall of 2021 is less than 2020.

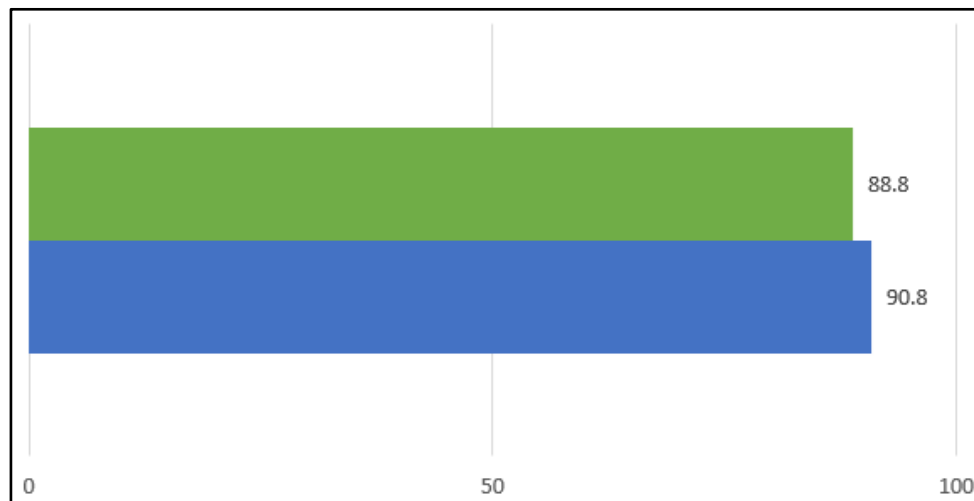


Figure 6: Monthly comparison of rainfall (mm) in Pugiola Rain Gauge. In green the month of November 2021, in blue the month of November 2020

X. CONCLUSION

Access to potable water and sanitation services remains as one of the biggest challenges in the water sector in Ecuador. The distribution is dynamic across the country and the management of the hydrological resources highly depends on the local governments. In the province of Cañar, the Tabacay river micro-basin constitutes the water supply for the city of Azogues and its tributaries. The principal findings of this brief study

are, first, that the status of the micro-basin remains in Grosso modo, constant through the last years in terms of rainfall. The precipitation data collected show that there are very similar volumes of rain catchment in the different months of the last three years. The Nudpud station, which represents of the main stations within the micro-basin, provided with information about the temperature in the area for the last three years, that evidences a rather low to not considerable variation in weather. The other rain gauges considered show that

there is a steady flow of rain between the periods measured.

Second, the water sector has without a doubt a door open to improve its performance and that relies on national agencies and government since they have the budget to support municipal companies in the implementation of quality monitoring and water potabilization services. Also, to support the strengthening of hydrological resources control, regulation, and policies. Third, the anthropogenic factor is unavoidable since the agricultural sector is one the major productive industries in Ecuador. Yet there is the need to create spaces to educate and promote the awareness in environmental and sustainable management of basins, micro-basins and water derived services in general. In this context, there is a considerable potential of improving the conservation of resources.

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Corresponding Orcid numbers:

Maria E. Calle Luzuriaga: 0000-0003-0827-7664

Maria A. Luzuriaga: 0000-0002-5122-9177

Karina M. Gonzalez: 0000-0001-9377-3894

José I. Yamasquí: 0000-0002-7372-8831

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Comparative Study of three Clustering Algorithms for Microarray Data

By Noveenaa Pious & Dicky John Davis G

Sri Ramachandra Institute of Higher Education & Research

Abstract- High throughput genomic data analysis is becoming an increasingly integral part of biomedical research. The information derived from gene expression analysis helps in diagnosing the treatment modality given to the patient. However, the amount of data is humongous and becomes complex to examine manually. Unsupervised machine learning algorithms perform complex tasks on an unlabelled data by clustering to comprehend the underlying structure and behaviour of the pattern. Clustering microarray data, examines the differential expressed genes found by grouping the genes based on the similarity of the expression values. In this study, we propose to elucidate the best clustering algorithm for gene expression data on various clinical conditions. The proposed study was carried on three gene expression datasets of Severe acute respiratory syndrome, Amyotrophic lateral sclerosis and Parkinson's disease. Differentially expressed genes were found at three p-values 0.01, 0.05, 0.001 and the most significant number of genes were retrieved at p-value 0.05. We experimented the differential expressed genes on three clustering algorithms, namely Hierarchical clustering, k-means clustering and fuzzy clustering of the three diseases. The performance of the three clustering algorithms was evaluated using the internal validity index, wherein Hierarchical clustering was found to be best for gene expression data.

Keywords: *hierarchical clustering; k-means clustering; fuzzy clustering; differentially expressed genes; microarray data.*

GJSFR-G Classification: DDC Code: 005.1 LCC Code: QA76.6



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Comparative Study of three Clustering Algorithms for Microarray Data

Noveenaa Pious ^α & Dicky John Davis G ^σ

Abstract- High throughput genomic data analysis is becoming an increasingly integral part of biomedical research. The information derived from gene expression analysis helps in diagnosing the treatment modality given to the patient. However, the amount of data is humongous and becomes complex to examine manually. Unsupervised machine learning algorithms perform complex tasks on an unlabelled data by clustering to comprehend the underlying structure and behaviour of the pattern. Clustering microarray data, examines the differential expressed genes found by grouping the genes based on the similarity of the expression values. In this study, we propose to elucidate the best clustering algorithm for gene expression data on various clinical conditions. The proposed study was carried on three gene expression datasets of Severe acute respiratory syndrome, Amyotrophic lateral sclerosis and Parkinson's disease. Differentially expressed genes were found at three p-values 0.01, 0.05, 0.001 and the most significant number of genes were retrieved at p-value 0.05. We experimented the differential expressed genes on three clustering algorithms, namely Hierarchical clustering, k-means clustering and fuzzy clustering of the three diseases. The performance of the three clustering algorithms was evaluated using the internal validity index, wherein Hierarchical clustering was found to be best for gene expression data.

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I. INTRODUCTION

Microarray analysis refers in finding different patterns in the genes expressed under specific situation or in a specific cell and to look for associations between regulation of gene expression levels and phenotypic variations (Tarca AL, Romero R 2006). The advancement of microarrays was utilized to examine large number of genes within a sample in two distinct cell states such as in normal state or diseased state. Microarray used to unravel and observe the genes that change accordingly due to trigger of environmental factors by producing stimulating responses. The expression patterns in microarray are pivotal as it intends to comprehend over the two states i.e., whether a gene is under or over-expressed in a diseased region (Rodriguez-Esteban and Jiang 2017). The differential expressed genes track the patterns or evolution of novel genes in different diseases namely cancer, nervous

disorders, and so on. In this study we focus on clustering techniques to capture the behaviour that have the similar patterns and functions by determining if the genes are correlated or dissimilar, or affiliated to the characteristics of the disease (Thalamuthu et al. 2006). We identified the differential expressed genes by using t-test in the control and treated samples of Severe acute respiratory syndrome (SARS), Amyotrophic lateral sclerosis (ALS), and Parkinson's disease (PD). We experimented the differential expressed genes with the three clustering techniques based on connectivity clustering model and centroid clustering such as Hierarchical clustering, k-means clustering and fuzzy clustering. The performance of clustering models was evaluated to comprehend the well-separation using Silhouette coefficient (Rousseeuw 1987). The proposed study approaches to annotate the suitable clustering technique for the different diseases.

II. METHODS

a) Experimental design

Each disease is affected by genetic or environmental factors. neuro degenerative disorders namely PD and ALS and respiratory syndrome namely SARS. The three diseases were studied on, by collecting datasets from Gene Expression Omnibus (GEO) present in National Centre for Biotechnology Information (NCBI). The dataset comprises of two sections, namely the GPL platform and GSE series. We used GPL 201 Affymetrix Human HG-Focus Target Array for all the three diseases consisting of raw gene information. The GSE series provides the sample information for SARS (GSE53394), ALS (GSE41444) and PD (GSE20333). We selected the three gene expression series with primary focus on control and treated samples to interpret the differential expressed genes.

Firstly, the GSE53394 series was to investigate the peripheral blood mononuclear cells from the gene expression samples of SARS patients in comparison with the healthy controls (Reghunathan et al. 2005). Here, the differential expressed genes from the SARS patients were not merely caused by the immune response against viral infection but were related to the presence of inherently inflammatory reactions. There was no majority of up-regulated genes leading to the conclusion that the SARS-CoV virus followed unfamiliar approach to avoid the host immune system. Secondly, the GSE20333 series were retrieved from the profiling of

Author ^α ^σ: Department of Bioinformatics, Faculty of Engineering & Technology, Sri Ramachandra Institute of Higher Education & Research, Porur, Chennai, Tamil Nadu – 600116, India.
e-mail: dicky@sriramachandra.edu.in

gene expression performed on human substantia nigra pars compacta (SNpc) of PD patients, where the reduced expression of SKP1 gene resulted in the non-functioning of protein regulatory in PD patients (Grünblatt et al. 2004). The third gene expression series GSE41444 was based on ALS patients, the affected patients increased the activity of muscle atrophy due to correlated genes present in the gene network and lead to the development of muscle homeostasis (Bernardini et al. 2013).

b) Dataset

The three diseases were studied on, by collecting datasets from Gene Expression Omnibus (GEO) present in National Centre for Biotechnology Information (NCBI) to perform the gene expression analysis. The flowchart of gene expression analysis is portrayed in Figure 1. The GPL201 was used, which primarily consists of features such as ID, Gene symbols, Gene title, Gen Bank accession etc. But only two features i.e. ID and Gene symbols was selected for the pre-processing phase, since these attributes provide the prior gene information. The data from the GPL201 probe is merged with the GSE samples provides the converged dataset. In order to stabilize the variation of the gene expression levels, the process of normalization is required. Here, the normalization is carried out using \log_2 function for the pre-processed data. The normalization is visualized using histograms and box plot to apprehend the variation of intensities of variation before and after normalization (Bengtsson and Hössjer 2006). Normalization is necessary since the measurements hold different scales from different hybridizations (Steinhoff and Vingron 2006). Furthermore, the conversion of multiplicative errors into additive ones is one of the advantages of logarithmic normalization (Cui, Kerr, and Churchill 2003). Following that, the unpaired student t-test is performed on the normalized data to predict the differential expressed genes under the p-values. The unpaired student t-test was applied on the control and treated samples of the three gene expression datasets. The difference between the measurements indicates if a gene expressed is up or down-regulated in a particular disease. Furthermore, the unpaired t-test produces two values t-statistics and p-value in which p-value representing the probability of the observed data under the assumption indicating the null hypothesis is true. During the process, we experimented different levels of p-values at 0.01, 0.05 and 0.001 to observe the differential expressed genes. Additionally, the \log_2 fold change was carried out to check and apprehend the up and down regulated genes in the three diseases. In most of the cases, the fold change produces incorrect results due to the inability to capture the differentially expressed genes. The \log_2 was illustrated using volcano plots of the up and down regulated genes.

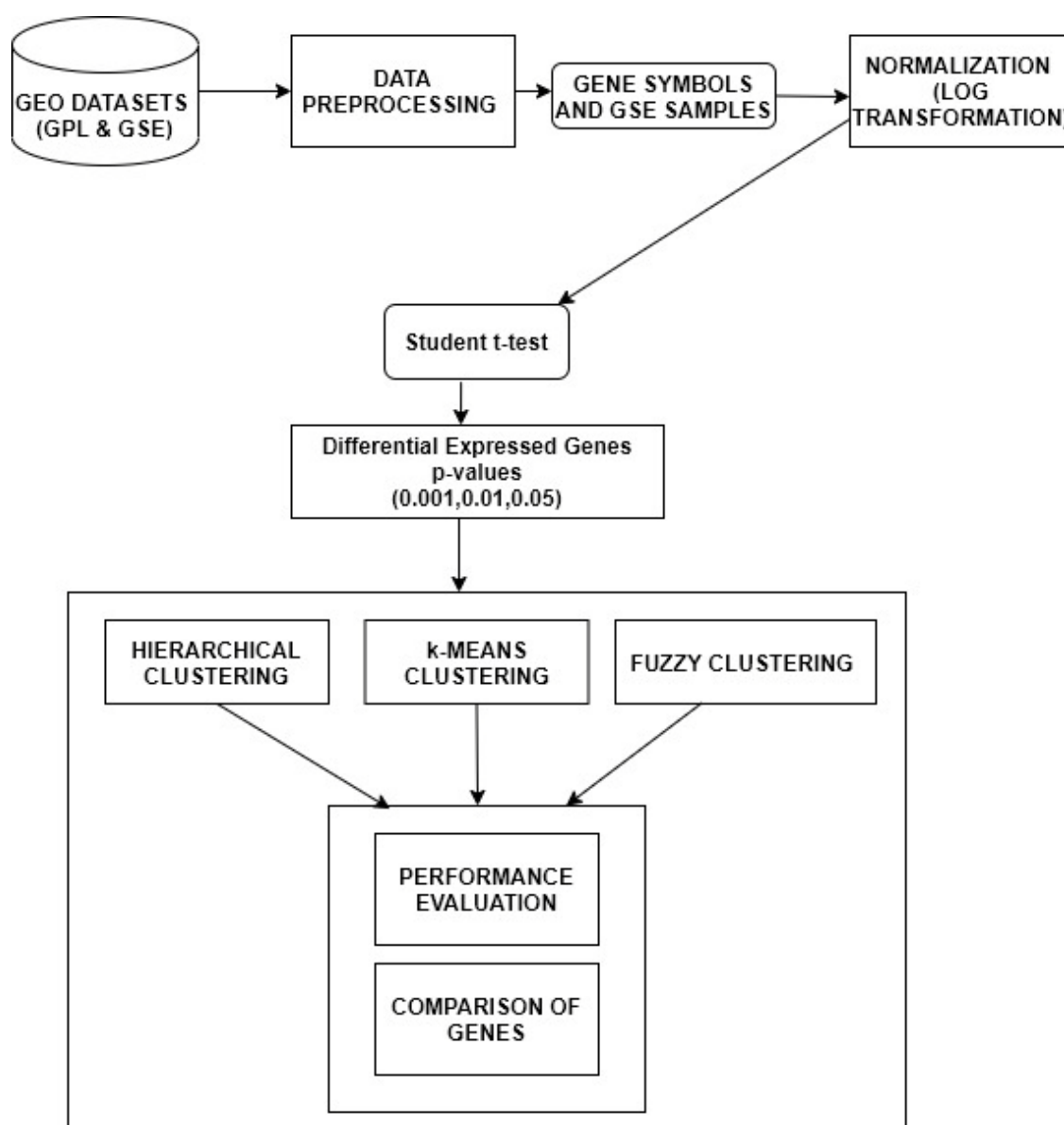


Figure 1: Flowchart for gene expression analysis

The three clustering algorithms namely Hierarchical Clustering, K-means Clustering, Fuzzy Clustering are applied on differential expressed genes retrieved from p-values and compared using the internal validity index. The comparison is retrieved by taking genes formed under different perspective clusters in three different algorithms. Here, the best clustering algorithm is proposed based on biologically significant genes. Relatively, the three clustering techniques were applied on the differential expressed genes under the three p-values. For instance, the agglomerative strategy pairs the nearby samples first and adds them to the mean of samples and constructs them into clusters in a hierarchy. It merges the closest pair of clusters in order to obtain a single cluster, as it defines the cluster proximity. Similar genes with their respective computed values are ordered and clustered together provide

higher expression level (Molla et al. 2004). Hierarchical clustering is visualized using heat maps by displaying the gene symbols providing the variation of intensities of the gene expression samples. The Euclidean distance is the mostly commonly distance measure in the clusters and the linkage criteria such as ward, complete and single was experimented, and complete linkage was chosen to perform well on the three diseases which associates the furthest neighbours. The distance between the two clusters is based on the most distant points in the different clusters V_i and V_j respectively. The Euclidean distance can be calculated as,

$$\|a - b\|_2 = \sqrt{\sum_i (a - b)^2} \dots \dots \dots (1)$$

In agglomerative hierarchical clustering, the inter-cluster distance is being premediated in order to

determine the distance between two separate clusters. The complete linkage method given by,

$$d(V_i, V_j) = \max \{d(x, y) \mid x \in V_i, y \in V_j\} \dots\dots\dots(2)$$

Each dendrogram represents the distance on which the cluster was formed. The dendograms cuts were observed and obtained at $k=3$ based on the performance of the clusters. The k-means clustering algorithm works by predefining the number of clusters. Firstly, it will choose a random centroid for each data point. The algorithm groups repeatedly until no data point is left alone. Euclidean distance method is the distance used for the clustering the data point and the centroids. The significant genes are assigned to each cluster, and it is repeated until clusters assignments are stable. Here, the algorithm follows the assignment strategy. It defines the cluster centre and assigns the data point to the nearest cluster. The cluster centres are placed as far as possible for the best accuracy. The cluster centres are based on the dataset used. In these datasets, it assigns the genes according to three cluster centres. The iterations take place simultaneously when no more centroids could not move further between the data points. Finally, the squared error function is deployed for minimizing the objective function using the formula,

$$S(V) = \sum \sum (\|x_i - y_j\|) 2c_{ij} = 1 \quad c_{ij} = 1 \dots\dots\dots(3)$$

The optimal k clusters were determined using the three methods such as elbow technique of total within sum of square, gap-statistics and silhouette score. One of the most popularly known for soft clustering of assigning data points to the cluster centre and influential methods is Fuzzy Clustering algorithm (Bezdek, 1981). The algorithm predicts precariousness in the expression levels of the genes and targets the areas of tumour formation (Scaria et al. 2016). The important characteristics of FCM, it calculates based on the weighting components of the data points. It oscillates between 0 and 1 for each cluster formation. Furthermore, when it reaches infinity, the outcomes become the centre. FCM algorithm aids to recognize the patterns clearly, provides a precise interpretation of the genomics data. The FCM algorithm is calculated using the formula,

$$D(U, V) = \sum \sum (\mu_{ij}) m \|x_i - v_j\| 2c_j = 1 \quad n_i = 1 \dots\dots\dots(4)$$

III. RESULTS

The proposed technique has been implemented in the working platform of "R" (version 3.4). The GEO datasets for SARS (GSE53394), PD (GSE20333), ALS (GSE41444) are downloaded in this platform. With respects to the genomic datasets, Hierarchical Clustering, K-means Clustering and Fuzzy Clustering techniques are compared. The up-regulated and down regulated genes identified using \log_2 fold change for the

three diseases are depicted in Figure 2. The comparisons between the three clustering algorithms were made using silhouette coefficient. The number of overlaps found in the genes were also represented using Bioinformatics & Evolutionary Genomics Venn diagram tools. Table 1 portrays the number of samples used for each disease using GEO datasets and the number of samples used for each disease taken for experimentation from GEO datasets. It provides the details of number of genes expressed under the p-values 0.05, 0.01, 0.001 with the respective details of the disease. The performance of clustering for the different diseases were described based on silhouette coefficient for the optimal clusters (Table 2). For the PD, the p-value at 0.001 was not included for the clustering analysis, since there were a smaller number of genes found at this p-value.

Table 1: Observation of genes

Dataset			p-values		
GEO Samples	Diseases	No of samples	0.001	0.01	0.05
GSE53394	SARS	14	426	1182	2293
GSE20333	PD	14	4	813	148
GSE41444	ALS	14	231	928	2469

Table 2: Performance of Clustering

GEO samples	Optimal k clusters	Clustering Algorithms	Silhouette score (p-values)		
			0.001	0.01	0.05
GSE53394	3	Hierarchical	0.68	0.89	0.90
	3	k-means	0.527	0.691	0.76
	3	Fuzzy	0.341	0.361	0.427
GSE41444	3	Hierarchical	0.439	0.482	0.481
	3	k-means	0.452	0.47	0.49
	3	Fuzzy	0.411	0.452	0.478
GSE20333	3	Hierarchical	-	0.81	0.93
	3	k-means	-	0.81	0.87
	3	Fuzzy	-	0.535	0.526

The optimal clusters were experimented on different values at $k=2$ and $k=3$ and was chosen based on the elbow technique using the measures of gap statistics, within the sum of squares and performance of clustering. Figure 3 represents the optimal results of hierarchical clustering and provides the details of average silhouette width of each cluster obtained from the three different diseases.

From the results of the three clustering algorithms based on the validation of internal clustering indices, the hierarchical clustering performs well on the optimal cluster k -value than k -means and fuzzy clustering on a gene expression data on all the three different diseases. The fuzzy clustering executed poorly

in all the three datasets, and the silhouette score remained relatively low around 0.5, that inherently indicates that soft clustering was not suitable for the gene expression data. With hierarchical clustering achieved the best silhouette score over the range of 0.9 at the p -value of 0.05 were most of the significant genes were retrieved from the two diseases such as SARS and PD and k -means obtained around 0.7- 0.8. For the ALS disease, the silhouette coefficient scored less in all the three clustering algorithms. From this study, it conquers that the connectivity-based clustering models provides the hierarchical analysis of genes on a gene expression data.

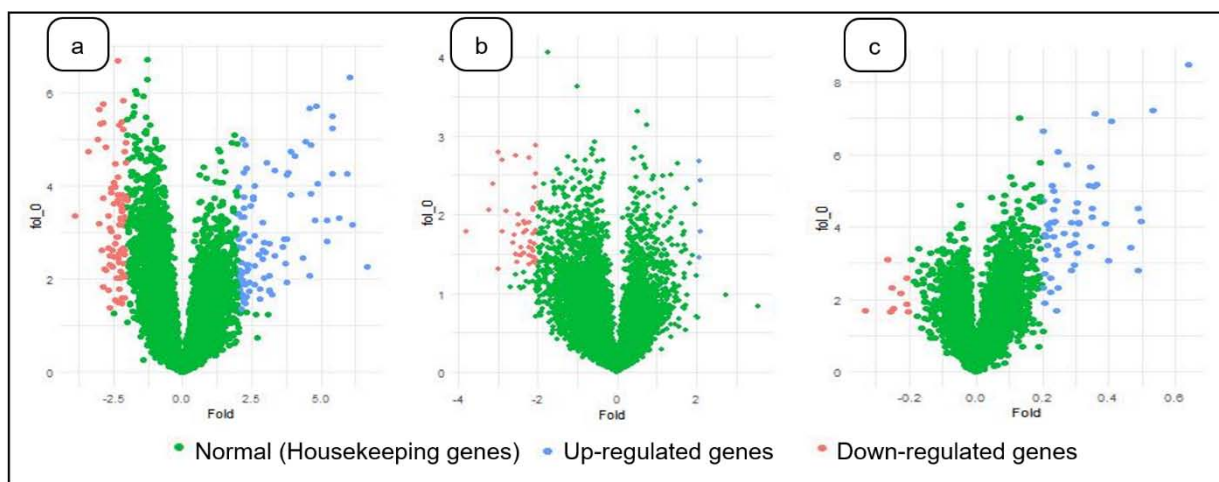


Figure 2: Log₂ fold change of a) Severe acute respiratory syndrome, b) Parkinson's Disease and c) Amyotrophic lateral sclerosis

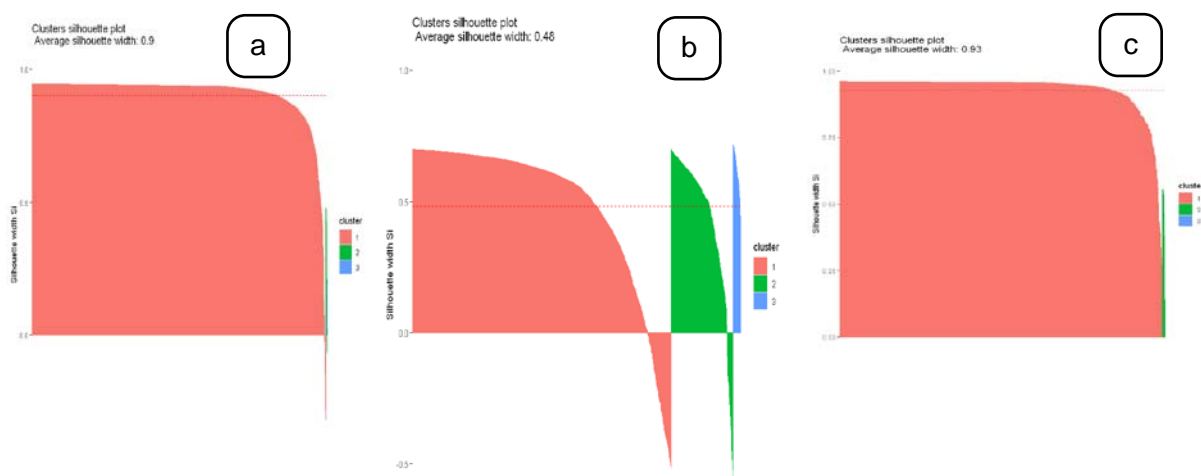


Figure 3: Silhouette scores of hierarchical clustering in a) Severe acute respiratory syndrome, b) Amyotrophic lateral sclerosis and c) Parkinson's Disease

IV. DISCUSSION

From the study of the three diseases, it's found that the significant number of differentially expressed genes was found to be from the p -value 0.05. Comparatively, the hierarchical clustering provides better clustering results using internal validity index. In order to comprehend the hierarchy of differentially expressed genes, whether they fall in the same cluster provides a definite clarity of similar biological functions and aids for the development of drug design. Renji and Manikandan found the genes such as LCN2, LTF and S100A9 were mostly up-regulated genes in the SARS patients (Reghunathan et al. 2005). While analysing the hierarchy of the three up-regulated genes at p -value 0.05 from the clusters, it's remarkable that the genes LCN2 and LTF are evident from cluster 1 and fall in the same branch of the hierarchy indicating similarity whereas S100A9 gene was found to be in cluster 2 revealing 11 genes follow the similar biological pattern from cluster 2.

Grunblatt and Mandel in their study of gene expression analysis of PD patients established the genes responsible for degradation and dopamine oxidation (Grunblatt et al. 2004). The main significant genes involved in the process of dopaminergic toxicity were observed under the p -value 0.05 retrieved from the hierarchy at cluster $k=3$ was EGLN1 accountable for stress, the reduction of dopamine was related to genes such as ALDH1A1, ARPP-21, and genes that caused the impairment of proteasome subunits namely PSMD8 and PSMA2 occurred on the same cluster i.e cluster 1. From this hierarchy of genes, it's quite evident the above genes are similar and contribute to the neuro degenerative disorder. By experimenting the differentially expressed genes in ALS disease (Bernardini et al. 2013) at cluster $k=3$, the correlated genes actively taking part in the mitochondrial metabolism obtained from the

former network with the ACTN3 gene by speeding the disease in humans, in addition, PRKARIA, FOXO1 and FBP2 fall under the cluster 2, indicating similarity function of the progression of the ALS disease. From the latter gene network, were CHRNA1 and TRIM32 are clustered together specify the critical activity of mitochondrial network. Hence, the differential expressed genes from all the three diseases provide homogenous biological activity and this similarity pattern is highly indicative and structurally evident in gene expression data using hierarchical clustering as compared to centroid clustering algorithms.

V. CONCLUSION

In the proposed study, our aim was to identify the differential expressed genes, obtained at different levels in a sample. Moreover, the experimental results, indicate that the differential expressed genes were enormous at p -value 0.05. All the three clustering algorithms were able to identify differentially expressed genes at p -value (0.01, 0.05 and 0.001). Each clustering algorithm provide specific challenges while grouping the gene expression data. Hierarchical clustering was found to be the suitable clustering algorithm on identifying the biologically significant genes as well as indicating the similar biological functions based on hierarchy of clusters in comparison with K-means and Fuzzy clustering. Thus, Hierarchical Clustering have paved the way for effective retrieval of significant genes in the microarray gene expression data.

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Declarations

Ethics approval– Not applicable

Availability of data and materials: The datasets used and/or analysed during the current study are available in the NCBI GEO database.

Conflict of interest: The authors declare that they have no conflict of interest.

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

By Gerardo Jose Bauce

Central University of Venezuela

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

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.

GJSFR-G Classification: DDC Code: 616.15 LCC Code: RB145



COMPARISON OF ANTHROPOMETRIC INDICATORS OF OVERWEIGHT AND OBESITY IN ADOLESCENTS FROM THREE REGIONS OF VENEZUELA

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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.

1. 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

Author: School of Nutrition and Dietetics. Faculty of Medicine. Central University of Venezuela. Caracas, Venezuela.
e-mail: gbauce@hotmail.com

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 (BFP ≥ 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.

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 Non-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,

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 $20.8 \pm 3.4 \text{ kg/m}^2$, 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⁾.¹¹ When discriminated by age group, it has to be that for the group of 10 to 12 years the average is $19.0 \pm 3.3 \text{ kg/m}^2$; for group 13 to 15 years $21.3 \pm 4.1 \text{ kg/m}^2$ and for group 16 to 19 years $23.3 \pm 3.3 \text{ kg/m}^2$; values slightly higher than the averages reported by Bauce ⁽²⁹⁾ $18.3 \pm 3.4 \text{ kg/m}^2$; $20.14 \pm 2.7 \text{ kg/m}^2$ and $22.3 \pm 3.1 \text{ kg/m}^2$ 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 ($p < 0.001$); 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.

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

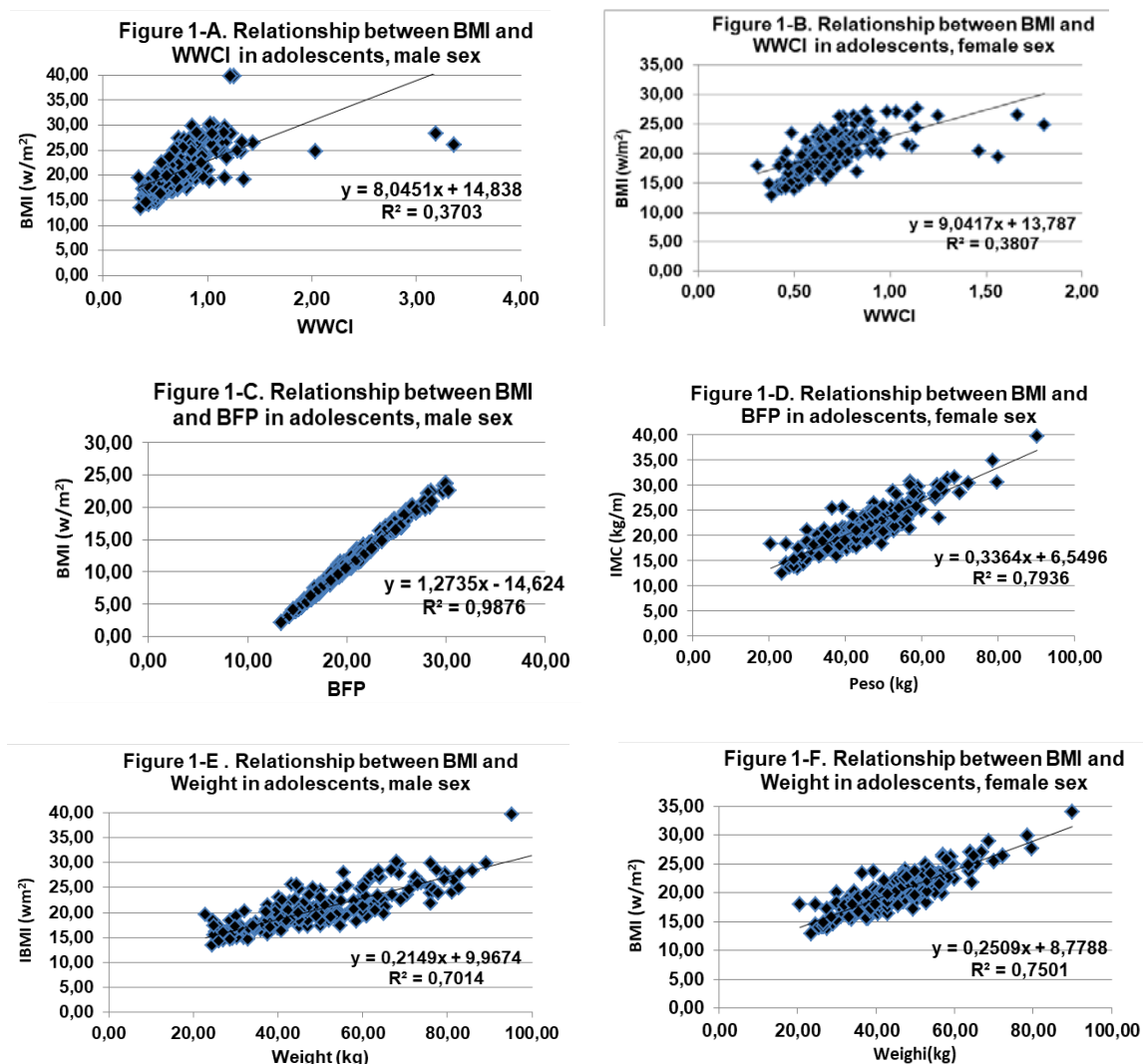


Figure 1: Relationship between BMI and indicators WWCI, BFP and Weight, in adolescent by sex

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

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

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

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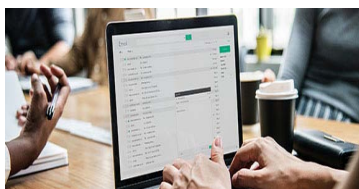
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Acknowledgments

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PREPARING YOUR MANUSCRIPT

Authors can submit papers and articles in an acceptable file format: MS Word (doc, docx), LaTeX (.tex, .zip or .rar including all of your files), Adobe PDF (.pdf), rich text format (.rtf), simple text document (.txt), Open Document Text (.odt), and Apple Pages (.pages). Our professional layout editors will format the entire paper according to our official guidelines. This is one of the highlights of publishing with Global Journals—authors should not be concerned about the formatting of their paper. Global Journals accepts articles and manuscripts in every major language, be it Spanish, Chinese, Japanese, Portuguese, Russian, French, German, Dutch, Italian, Greek, or any other national language, but the title, subtitle, and abstract should be in English. This will facilitate indexing and the pre-peer review process.

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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Author details

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

Abstract

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.

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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.

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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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TIPS FOR WRITING A GOOD QUALITY SCIENCE FRONTIER RESEARCH PAPER

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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.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

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7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

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12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

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 through 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:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

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.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear: Adhere to recommended page limits.



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- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- 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.

Title page:

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.

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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.

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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:

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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.

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Materials:

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.



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.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

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

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If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

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Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

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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:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

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Topics	Grades		
	A-B	C-D	E-F
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
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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