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Prevalence of Anemia Among Chronic Obstructive Pulmonary Disease Patients and Identification of Diagnostic Markers Associated with the Disease

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ABSTRACT

Background: A co-morbidity of chronic obstructive pulmonary disease (COPD) has been identified as anemia. Chronic obstructive pulmonary disease is now known to have systemic inflammation, making it a potential cause of chronic anemia (ACD). According to preliminary findings, anemia may be more common than predicted in COPD patients, occurring in 10% to 15% of cases. This study aims to investigate the prevalence of anemia in COPD patients and look for biomarkers linked to the disease.

Methods: In a retrospective study conducted at a university hospital in Kwara state, Nigeria, 280 patients (166 men and 114 women) with spirometry-confirmed COPD were assessed for the incidence of anemia. The gene expression profiles of microarray data, including GSE148004, were acquired from the Gene Expression Omnibus (GEO) database to discover potential candidate key genes linked to the onset and prognosis of COPD. DAVID was used to examine the pathway enrichment analysis of the differentially expressed genes (DEGs) from the Kyoto Encyclopedia of Genes and Genomes (KEGG).

Results: Anemia was found in 104 people (88 men and 16 women), providing a frequency of 37.1%. Additionally, 57.1% of the patients developed acute exacerbations. There were 9458 DEGs overall, of which 3772 were up-regulated, and 5686 were down-regulated. In the current study, DEGs revealed that the NEDD9 gene was over-expressed in COPD patients.

Conclusion: Patients with COPD typically experience anemia, which is linked to higher morbidity in hospital admissions and exacerbations. These patients' clinical outcomes may be improved by treating their anemia. A potential biological relationship between anemia and COPD is IL1R1.

Keywords: Anemia, COPD, DEGs, NEDD9, IL1R1and spirometry

1.0 INTRODUCTION

Anemia is a condition marked by a hemoglobin deficiency in the entire blood [1, 2]. According to the World Health Organization [3], the world's population, which makes up around 24.8% of the population, has the highest prevalence of it. Age, sex, lifestyle, and altitudinal ranges are some of the variables that have an impact on Anemia [4]. Nutritional deficiencies are the most frequent cause of anemia and are most frequently seen in underdeveloped nations [4, 5]. Anemia has been discovered to be a co-morbidity in COPD patients. Risk factors for COPD include smoking, age, and co-morbidities. Anemia is a common comorbidity in patients with COPD, with prevalence varying from 7.5 to 33% [6, 7].

Poor lung airflow is a hallmark of the respiratory disorder known as a chronic obstructive pulmonary disease (COPD), ranked as the third greatest cause of death [8]. Numerous factors contribute to its development, including the lungs' partial destruction, excessive mucus production, and inflammation and swelling of the bronchial mucosa. Globally, COPD is responsible for 5.7% of deaths and 13.1% of prevalence [9, 10]. Anemia of chronic illness is the most reported type of anemia in people with COPD (ACD). Even if a diagnosis of anemia is not expensive, having COPD as a concomitant condition result in higher treatment costs [11]. Anemia in COPD is not improved by raising erythropoietin (EPO) levels [12–14]. The problems connected to ACD are characterized by increased cytokine production. Anemia is significantly influenced by cytokines that mediate the immunological or inflammatory response, including tumor necrosis factor, interleukin-1, and interferons. These cytokines are responsible for all of the processes that contribute to the development of ACD, including reduced red blood cell survival, a reduced erythropoietin response to anemia, and reduced erythroid colonies' ability to form in response to erythropoietin, and abnormal mobilization of reticuloendothelial iron stores. Due to high hepcidin in long-lasting inflammation, phagocytes reduce blood iron levels and limit inflammation by consuming iron, which causes iron deficit and iron deficiency anemia in COPD. One of the causes of anemia and EPO resistance in COPD may be due to this [14].

The prevalence of anemia in COPD patients varies across studies, reflecting different study design factors, variations in outpatient and hospitalized patients, variations in stable and COPD patients experiencing an acute exacerbation, regional anemia prevalence, and various anemia definitions used in these studies. Therefore, it is necessary to conduct a study to determine the prevalence of Anemia in COPD and its related diagnosis.

2.0 METHODOLOGY

2.1 Survey Information and Analysis

We performed a retrospective analysis of COPD cases previously identified at the UITH Ilorin Pulmonology Clinic, utilizing clinical and spirometry findings between 2003 and the present. Fisher's formula was used to calculate the sample size. It was determined that the standard normal deviation was 1.96, equivalent to a 95% confidence level. 23.1% was the predicted prevalence from a prior study [15]. The sample size was determined to be at least 272. To guarantee that the study is appropriately powered even when attrition occurs, 10% of the minimal sample size [16] was added. 300 patients were therefore required for the investigation.

The University of Ilorin Teaching Hospital provided the diagnosis and treatment for a total of 280 COPD cases that fit our criteria; 166 of the patients were men, and 114 were women. From their records, we gleaned the following information: Sex, Age, Status (Stable or Exacerbated cases). Also laboratory blood tests (Haemoglobin level, Packed cell volume (PCV) and Red blood cell count) was recorded.

The clinical condition and treatment of the COPD cases were used to categorize them. The cases that were handled at the clinic as outpatients and those that had acutely worsened cases that required hospital admission were classified together.

We classified the patients as anaemic using the following criteria:

Haemoglobin level <13.0 g/dl in males, <12.0 g/dl in females.

PCV <40% in males, <37% in females.

RBC count $<4.5 \times 10^{12}$ /l in males, $<3.8 \times 10^{12}$ /l in females [17].

2.2 Ethical Consideration

Ethical approval was obtained from the Ethics and Research Committee of the University of Ilorin Teaching Hospital, Ilorin, Kwara State with approval number: ERC PAN/2022/08/0307.

2.3 Statistical Methods

Statistical analysis (frequency distribution and chi square) was done by using SPSS 15.0 "p" value of <0.05 was taken as significant.

2.4 Microarray Data and Data Pre-processing

In 2022 [18], the microarray data (GSE148004) was downloaded from the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/). The platform used was the GPL13497Agilent-026652 Whole Human Genome Microarray 4x44K v2. A total of 16 samples, including 7 COPD samples and 9 control samples, were available. The data from the expression profile chip was pre-processed using the Affy package in Bioconductor (http://www.bioconductor.org/packages/release/bioc/html/affy.html) [19] and Affymetrix annotation files from Brain Array Lab (Affymetrix, Santa Clara, CA, USA; http://www.affymetrix.com/analysis/). Background correction, quartile data normalization, and probe summarization were performed using the robust multiarray average technique (http://www.bioconductor.org). Use [20] to obtain a gene expression matrix.

2.5 Identification of DEGs

The expression values for the normalized data were calculated using R's limma package [21]. DEGs were determined using the student's t test. The raw P value was transformed into the false discovery rate (FDR) using the Benjamini& Hochberg method [22]. The threshold was set at FDR 1.

2.6 Enrichment analysis for DEGs

The DEGs were functionally enriched in the biological process, molecular function, and cellular component categories according to the GO database (http://geneontology.org/) [23].

3.0 RESULTS

For this investigation, a total of 280 COPD cases were used, 166 of whom were men and 114 of whom were women (Figure 1). According to Figure 2, patients between the ages of 61 and 70 have the largest prevalence (42.9%), those under the age of 40 have the lowest prevalence (7.1%), and those over the age of 80 have the highest prevalence (8.6%).

In the COPD population that was studied using Table 1, the prevalence was higher in patients over the age of 50; there were 256 patients with COPD overall, 97 of whom were male and 159 of whom were female; 12 patients—7 males and 5 females—were under the age of 40; and 8 patients were between the ages of 41 and 50. (2 males and 6 females).

It was determined that most of the patients were between the ages of 51 and 80. 160 (57.14%) of the 280 COPD patients who participated in the study were unstable, and 120 (42.86%) were stable (Figure 3).

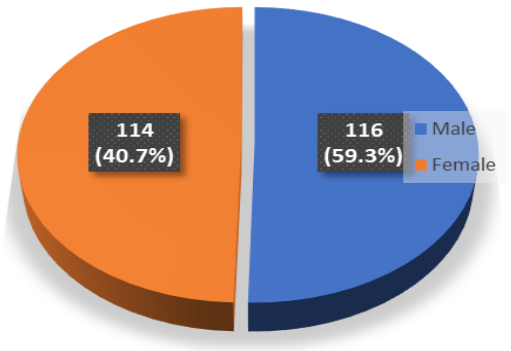

Figure 1. Prevalence of COPD based on Gender

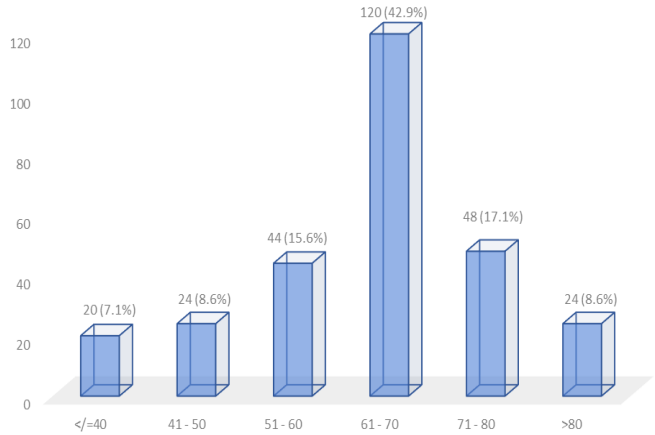

Figure 2. Prevalence of COPD Based on Age Distribution

Table 1. Age and Gender Distribution of COPD Patients

Variable	Male	Female	Total	χ^2	p value
Age group					
≤40	7 (58.3)	5 (41.7)	12	13.235	0.039
41 – 50	2 (25.0)	6 (75.0)	8		
51 – 60	3 (12.5)	21 (87.5)	24		
61 – 70	21 (47.7)	23 (52.3)	44		
71 – 80	49 (40.8)	71 (59.2)	120		
>80	15 (31.3)	33 (68.8)	48		

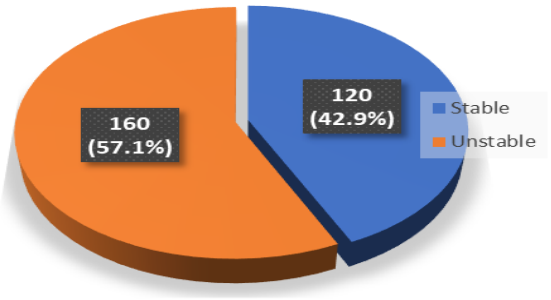

Figure 3. Stability Status of patients with COPD

Table 2. Anemia in Studied Population using PCV

Variable	Frequency	Percent
PCV		
Anemia	104	37.1
No Anemia	176	62.9

PCV is <40% in males and <37% in females

Table 3. Gender of anaemic patients in studied population

Variable	Male	Female	Total	χ^2	p value
PCV					
Anemia	88 (44.0)	16 (20.0)	104	14.098	<0.001
No Anemia	112 (56.0)	64 (80.0)	176		

Table 4. Anemia in Studied Population using Haemoglobin Level

Variable	Frequency	Percent
Haemoglobin level		
Anemia	88	31.4
No Anemia	192	68.6

104 individuals overall (37.1%) were anaemic, while 176 patients (62.9%) were not (Table 2). Based on gender, Table 3 shows that 64 female patients (80%) are not anaemic, while 16 female patients (20%) out of 200 female patients were confirmed anaemic. Overall, 88 (44%) out of 200 male patients were anaemic, while 112 male patients (56%) are not anaemic (Table 4).

3.1 Data Preprocessing and DEG Screening

A GEO dataset (GSE148004) was pre-processed to provide a dataset of 7 COPD and 9 control samples (Figure 4). The limma package in R found 9458 DEGs in total, comprising 3772 up-regulated genes and 5686 down-regulated genes. The top statistically significant up- and down-regulated genes are listed in Table 5. In order to show the distribution of DEGs, a volcano plot was utilized (Figure 5).

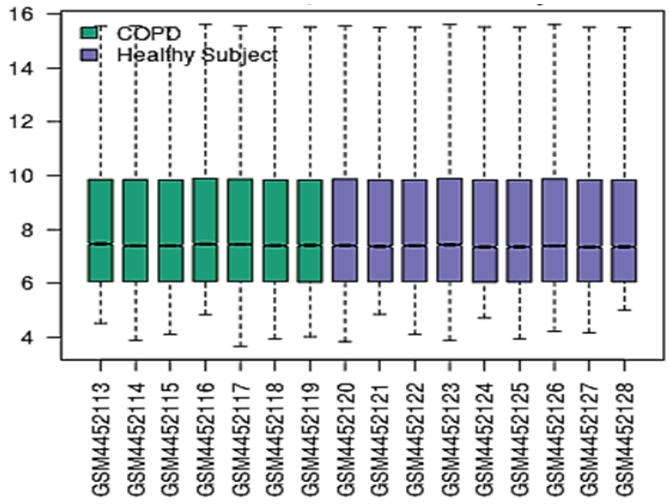

Figure 4. Classification of Samples Adopted for the Study

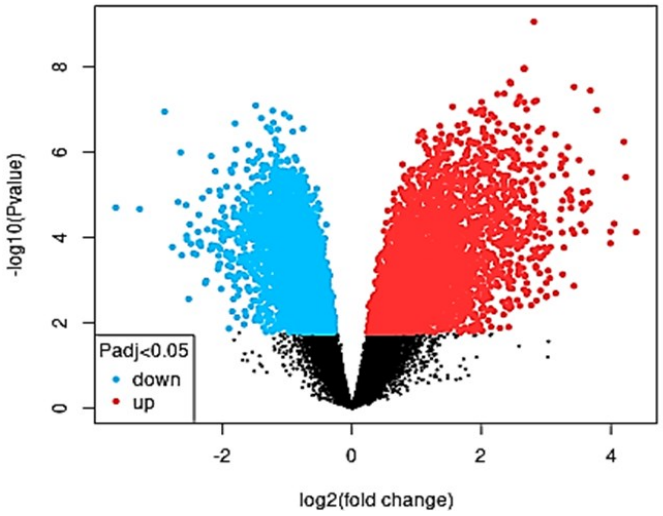

Figure 5. Up regulated and downregulated genes between the healthy and COPD patients

3.2 Gene Ontology (GO) Analysis

The research on biological pathways and functions was done using the R program cluster Profiler [24]. The GO categories "molecular function," "cellular component," and "biological process" were all enhanced. The majority of the up-regulated and down-regulated DEGs were associated with metabolic processes, such as the metabolism of organonitrogen compounds (BP, GO:1901564),

Table 5. Differentially Expressed Genes between the Healthy and COPD Patients

Gene symbol	Gene title	log_2(fold change)	p-Value
	Up-regulated genes		
NEDD9	neural precursor cell expressed, developmentally down-regulated 9	2.81	9.053
CYFIP2	cytoplasmic FMR1 interacting protein 2	2.663	7.962
IL18R1	interleukin 18 receptor 1	2.657	7.942
JARID2	Jumonji and AT-rich interaction domain containing 2	2.439	7.641
NEDD9	neural precursor cell expressed, developmentally down-regulated 9	2.453	7.605
LIPN	lipase family member N	3.43	7.523
IL1R1	interleukin 1 receptor type 1	3.683	7.438
CXorf65	chromosome X open reading frame	2.351	7.365
ATG2A	autophagy related 2A	2.59	7.289
	Down-regulated genes		
RDH12	retinol dehydrogenase 12	-1.488	7.088
SRSF7	serine and arginine rich splicing factor 7	-1.226	6.965
PNPLA3	patatin like phospholipase domain containing 3	-2.897	6.944
IL7	interleukin 7	-1.049	6.89
SLC7A6OS	solute carrier family 7 member 6 opposite strand	-0.968	6.814
LYPLAL1	lysophospholipase like 1	-1.403	6.789
EPHX1	epoxide hydrolase 1	-1.807	6.668
GSTZ1	glutathione S-transferase zeta 1	-1.264	6.546

Table 6. Biological Process with GO Terms and Enrichment

Term name	Term ID	Term size	Adjusted p_value
organonitrogen compound metabolic process	GO:1901564	6522	3.82E-64
cellular macromolecule metabolic process	GO:0044260	3234	2.05E-49
organonitrogen compound biosynthetic process	GO:1901566	1777	1.01E-46
organic substance biosynthetic process	GO:1901576	5854	3.62E-44
biosynthetic process	GO:0009058	5941	1.18E-42
cellular biosynthetic process	GO:0044249	5782	4.45E-41
protein metabolic process	GO:0019538	5462	2.90E-40
cellular nitrogen compound biosynthetic process	GO:0044271	4853	4.22E-31
cellular amide metabolic process	GO:0043603	1201	9.55E-31
cellular macromolecule biosynthetic process	GO:0034645	1193	2.92E-30

Table 7. Molecular Function with GO Terms and Enrichment

Term name	Term ID	Term size	Adjusted p_value
protein binding	GO:0005515	14832	5.48E-58
catalytic activity	GO:0003824	5682	7.68E-38
catalytic activity, acting on a nucleic acid	GO:0140640	609	6.29E-20
catalytic activity, acting on RNA	GO:0140098	394	3.68E-19
catalytic activity, acting on a tRNA	GO:0140101	137	3.24E-14
nucleoside phosphate binding	GO:1901265	2177	1.06E-12
nucleotide binding	GO:0000166	2176	1.35E-12
ligase activity	GO:0016874	178	4.57E-11
transferase activity	GO:0016740	2329	6.32E-11
identical protein binding	GO:0042802	2121	4.89E-09

Table 8. Cellular Component with GO Terms and Enrichment

Term name	Term ID	Term size	Adjusted p_value
cytoplasm	GO:0005737	12270	6.75E-145
Cytosol	GO:0005829	5419	1.15E-81
Mitochondrion	GO:0005739	1684	1.23E-76
Nucleoplasm	GO:0005654	4215	3.29E-69
organelle membrane	GO:0031090	3685	6.64E-57
Envelope	GO:0031975	1270	4.32E-53
organelle envelope	GO:0031967	1270	4.32E-53
mitochondrial envelope	GO:0005740	807	8.42E-45
mitochondrial membrane	GO:0031966	756	1.25E-43
membrane-enclosed lumen	GO:0031974	6637	3.29E-43

the metabolism of cellular macromolecules (BP, GO:0044260), and the metabolism of cellular amides (BP, GO:0005515), with the majority of the metabolic processes consisting of biosynthetic processes (Table 6).

Catalytic processes, such as catalytic activity acting on a nucleic acid (MF, GO:0140640), catalytic activity acting on RNA (MF, GO:0140098), and catalytic activity acting on a tRNA, are among the molecular processes that make up the components of cells (MF, GO:0140101). Ligase activity (MF, GO:0016874), transferase activity (MF, GO:0016740), and nucleoside phosphate binding are other molecular functions (MF, GO:1901265) (Table 7). The cellular junction, cytoplasm, mitochondrion, plasma membrane, and nucleoplasm make up the final part of the cell (Table 8).

4.0 DISCUSSION

This Anemia is a serious global public health problem affecting young children and pregnant women. The World Health Organization estimates that 42% of children under 5 years of age and 40% of pregnant women worldwide are anaemic [25]. The prevalence of Anemia among children aged 6–59 months in Nigeria was 68.1% [26].

At the Pulmonology clinic, UITH Ilorin, a total of 280 previous cases of COPD (166 men and 114 women) with clinical and spirometry results were assessed for the frequency of anemia. Anemia in 104 different cases was found.

Our study's findings indicate that, when utilizing the PCV (40%) and Hb level (13.0 g/dl) as the decisive factors, respectively, there are 37.1% and 31.4% anaemic COPD patients. According to recent studies [27], the prevalence of Anaemia with COPD may be higher than expected, ranging from 7.5 to 33%. This study demonstrated that older people frequently get COPD (age 61-70yr). COPD is a more common chronic condition in the aging population.

Anemia is more common as people age in the general population. In men and women 65 years of age and older, the prevalence of anaemia was 11% and 10.2%, respectively, in the National Health and Nutrition Examination Survey (NHANES-III) [28]. Males were found to have a higher prevalence of anaemia than females. This result contrasts other research [29], whose results indicated no discernible gender difference in the frequency of anemia. Most patients, or 57.1%, had acute exacerbations (160). The total number of anaemic patients was 104 (37.1%), most of whom presented to the hospital with an acute exacerbation. It is necessary to mention that older persons may become iron deficient because of inadequate intake or absorption of iron. Without blood loss, anemia takes several years to develop. The serum ferritin level is the most effective way to diagnose iron deficiency anemia [30]. The causes of anemia in the elderly are divided into three broad groups: nutritional deficiency, anemia of chronic disease (ACD), and unexplained anemia (UA) [31]. Anemia is a common comorbidity in COPD patients associated with reduced functional capacity, impaired quality of life, greater likelihood of hospitalization, and early mortality [32].

The NEDD9 gene was shown to be overexpressed in COPD patients in the current study, according to DEGs. Using subtractive cloning technology, the gene known as Neural Precursor Cell Expressed, Developmentally Downregulated 9 (NEDD9), which is only expressed in the brain during embryonic stages but not in the brains of mature mice, was originally discovered in 1992 [33]. The first study to assign NEDD9 a biological purpose was conducted by Law et al. in 1996 [34]. In a prior study, structural alterations, the ability of the pulmonary epithelium to repair itself, the quantity of E-cadherin protein, and the immunoreactivity of neural precursor cell expressed developmentally down-regulated protein 9 (NEDD9) were assessed in emphysematous (n=7) and non-emphysematous (n=6) areas of lung samples taken from patients with chronic obstructive pulmonary disease.

For Alveoli that are bigger, the epithelium and walls of the alveoli are damaged, type 2 pneumocytes and NEDD9 immunoreactivity are elevated, and E-cadherin proteins are decreased in emphysematous areas [34]. As a result of NEDD9's degradation of E-cadherin in emphysematous areas, the same study demonstrates that E-cadherin levels are lowered there. Reduced levels of E-cadherin also result in the absence of intercellular connections or weak intercellular connections, which contribute to the breakdown of the pulmonary epithelium. Due to the decreased E-cadherin, type 2 pneumocytes could not develop into type 1 pneumocytes, preventing the completion of the pulmonary epithelial restoration. The pathogenesis of pulmonary emphysema is aided by decreased E-cadherin levels, which result in emphysematous changes in human lungs [34]. Additionally, it was

discovered that COPD significantly overexpressed IL1R1 (interleukin 1 receptor type 1) (p=0.05). This was in line with earlier research that demonstrated that the problems connected to Anaemia of chronic disease (ACD) are characterized by increased cytokine production [35]. Anemia of chronic illness is the most reported type of anaemia in people with COPD (ACD). Even if diagnosing anaemia is not expensive, having COPD as a concomitant condition result in higher treatment expenses [36]. Anaemia is significantly influenced by cytokines that mediate the immunological or inflammatory response, including tumor necrosis factor, interleukin 1, and interferons. These cytokines are responsible for all the processes contributing to the development of ACD, such as decreased red cell survival, a blunted erythropoietin response to Anemia, and reduced erythroid colony formation ability in response to erythropoietin, and abnormal mobilization of reticuloendothelial iron stores [37].

Finally, Anaemia frequently occurs in COPD patients and is associated with an increase in morbidity, such as an increase in the frequency of exacerbations and hospital admissions. By addressing their Anaemia, these patients' clinical results might be enhanced. The postulated molecular link between Anaemia and COPD is IL1R1.

Conflicts of Interest

The authors declare that there is no conflict of interests.

Authors' Contributions

OAA conceived and designed the study, performed data collection, contributed to data analysis tools, analysis of data and manuscript writing. M**OB, MAJ, IMW** contributed to writing of manuscript. All authors approved the final copy of the manuscript.

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