Study of Carboxyhemoglobin Levels of Patients of Respiratory Viral Infections: An important clinical indicator of severity

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Abstract
Respiratory viral infections are the common and frequent infections affecting humans throughout the year. As these viral pathogens in majority of cases of persistent infections reach the lungs, their intracellular replication in pulmonary cells can lead to interfere the composition of haemoglobin molecule by means of liberating carbon monoxide as metabolic and catabolic products of virus replication. The blood samples of patients admitted in the Sharda Hospital, U.P. with respiratory distress like condition were taken for the study. The serum was separated and the carboxyhemoglobin levels were analysed employing ELISA technique as per the manufacture protocol.

Present study reports carboxyhemoglobin level in 59 patients in terms of ng/ml of blood. Since these levels have been observed in case of COPD (Chronic Obstructive Pulmonary Distress) indoor patients, the reported values could serve as cut off levels of carboxyhemoglobin in severe patients of COPD.

Keywords: Carboxyhemoglobin, COPD, Carbon Monoxide, Respiratory viral infections.

Introduction

Acute respiratory infections rank first in terms of disease burden leading to severe morbidity and mortality[1]. Some respiratory viruses like Rhinovirus, Coronavirus, Adenovirus infect the upper region, others like Respiratory Syncytial Virus, Metapneumovirus, Influenza, Parainfluenza and Adenovirus infect the lower tract. The recent addition to this group is the Severe Acute Respiratory Syndrome (SARS) Coronavirus -2 that appeared in late 2020[11]. Prevalence studies of these respiratory viruses show that throughout the year depending upon the environmental conditions and host availability, these respiratory viruses appear and cause varied type of illness[8]. Respiratory viruses may appear as asymptomatic forms to mild and sometimes as chronic lung infections or chronic obstructive pulmonary disease (COPD)[3-6,14].

COPD in turn can lead to number of secondary complications and as the illness advances, it affects the normal functioning of the respiratory system, inflammation of the bronchi, blocking of the airways, damage to lungs and respiratory epithelium and asthma[13]. Biochemically, COPD may also lead to oxidative stress, inflammation of WBC cells reduced cytokine and related factors[17]. When the various metabolic activities are going on in our body, carbon Monoxide (CO) is released, specifically during the synthesis of Heme-by-Heme oxygenase (HO). The Heme which is a major component of Haemoglobin in the RBC has a good binding affinity with the carbon monoxide (240 times) compared to Oxygen which is carried by the Haemoglobin[2,9,10,16]. Viruses can also activate expression of HO and thus can participate in CO accumulation[7].

We hypothesize that when the respiratory viruses infect the cells of different body organs, they may release CO which may impact the oxygen carrying capacity of the Haemoglobin leading to increased number of carboxyhemoglobin (COHb) formation in the cell. This may indirectly lead to less oxygen binding and thus creating a hypoxemia like situation. During the time of COVID-19, an increase in the level of carboxyhemoglobin was observed[15]. In the present study, we report the carboxyhemoglobin levels detected in various patients with respiratory distress like conditions and attempt to create a baseline to estimate the seriousness of the infection.

Material and Methods

The blood samples of patients admitted in the Sharda Hospital with respiratory distress like condition were taken. The blood samples were also collected from the outdoor patients. The consent for the study was taken from all the patients prior to the study and patient Information sheet was also filled. Serum was separated from the blood in the laboratory The samples were then brought to the laboratory and stored at -80°C. The clinical presentation of the patients was divided into two parts, one based on their current symptoms and second based on any existing comorbidity. Carboxyhemoglobin levels were analysed employing ELISA technique as per the manufacturer's protocol and optical density was noted down. The standard of carboxyhemoglobin (Human HbCO standard) was prepared as 8 serial dilutions and then tested along with patients' sample.

Results

The OD (Optical density) values for the standard run were measured and are displayed in table 1. A graph was also plotted and a linear rise w.r.t. quantity was obtained (Fig. 1). A total of 59 blood samples were analysed for the presence of carboxyhemoglobin levels, of which 42 were of male and 17 were of female. These individuals were also divided into

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4 age groups; group 1 (18-25 years), group II (26-50 years), group III (51-69 years) and group IV (above 70 years) individuals. In group I, there were 6 males and 2 females, group II had 12 males and 5 females, group III had maximum individuals i.e. 19 males and 8 females while group IV had 5 males and 2 females (Table 2 and table 3). Since respiratory distress and carboxyhemoglobin levels alter in smokers/non-smokers, hence this parameter was also checked. Out of the total individuals, 17 males (28.81%) and 3 females (5.08%) were active smokers while 9 males and 2 females were ex-smokers i.e. they had quit smoking.

It was seen that in group I, there were 2 male active smokers. In group II, there were 4 males (and 3 who had quit smoking/ex-smokers) and 1 female. In group III there were 9 males (4 had quit smoking) and 1 female (and 2 had quit smoking) and in group IV, there were 2 males (and 2 had quit smoking) and 1 female active smokers. The symptoms presented by patients were breathlessness, fever, cough and sputum, while co-morbidities seen were hypertension, diabetes and tuberculosis. Symptoms and co-morbidities other than these were also placed in figs. 2, 3 and 4.

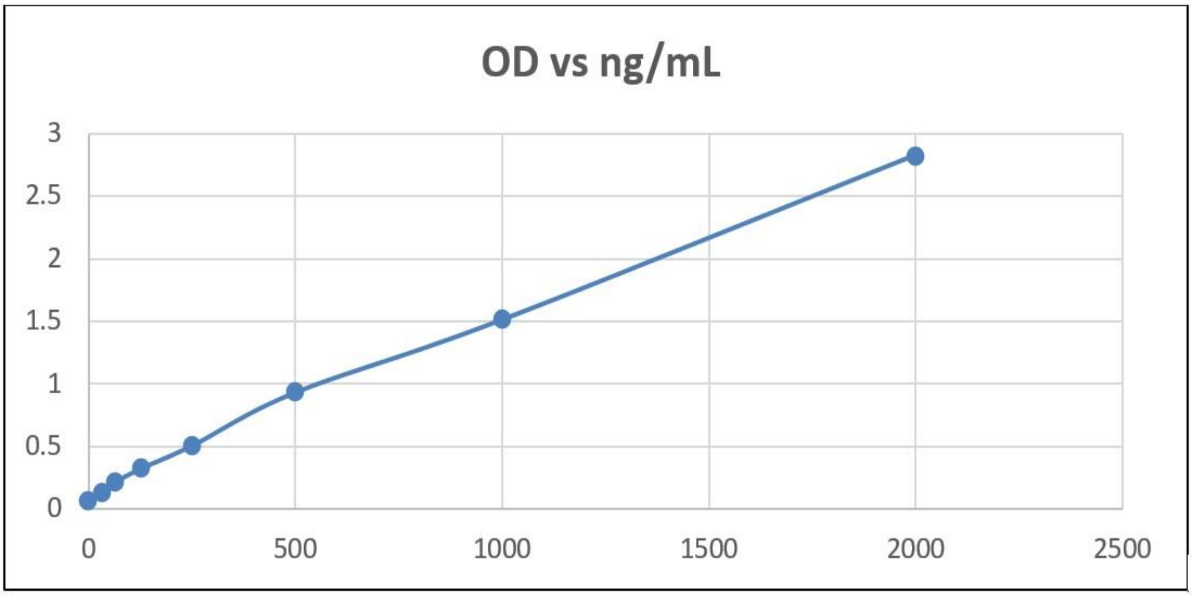

Fig. 1: Graph plotted for Optical Density v/s concentration of Carboxyhemoglobin in ng/ml for the individual standards

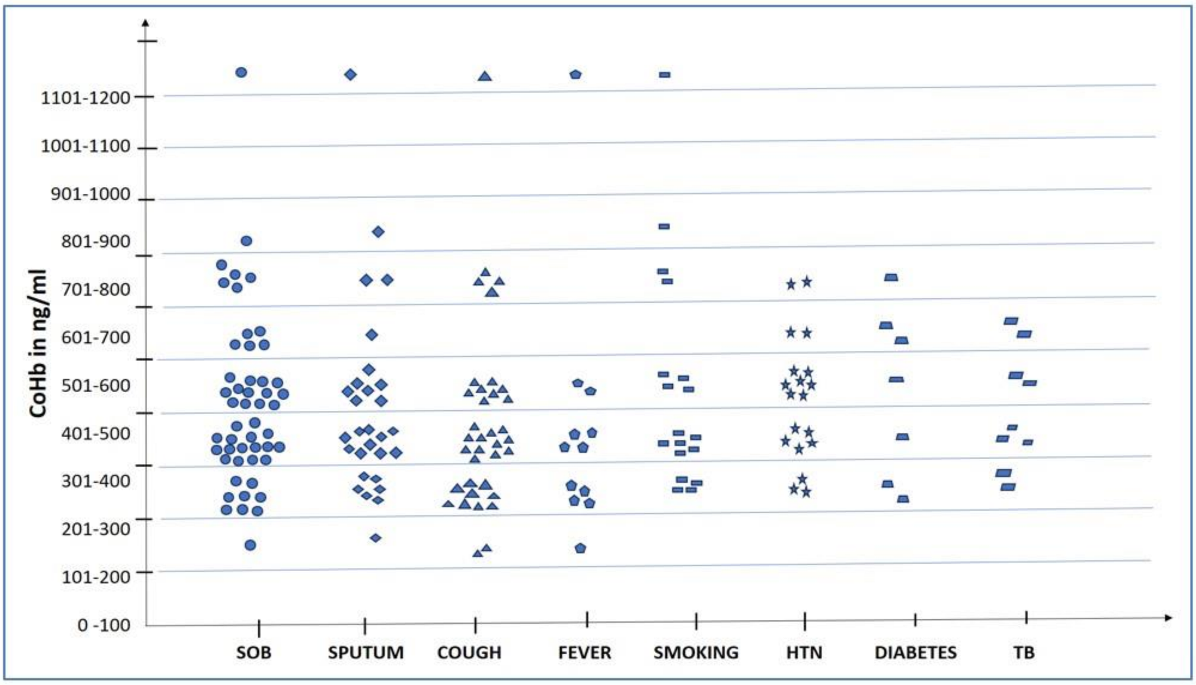

Fig. 2: Pictorial representation of the Carboxyhemoglobin levels w.r.t the clinical presentation in individual cases. (SOB: Shortness of Breath, HTN: Hypertension; TB: Tuberculosis; CoHB: Carboxyhemoglobin)

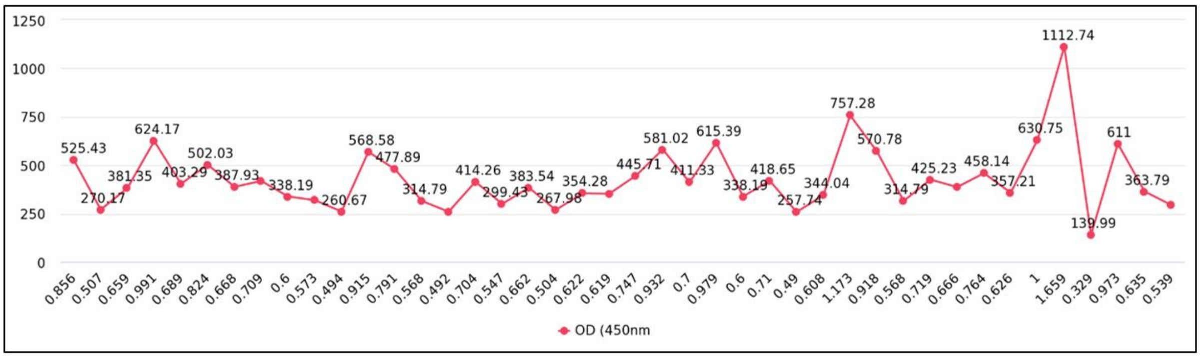

Fig. 3: Graph plotted for Optical Density v/s concentration of Carboxyhemoglobin in ng/ml for the study individuals (male patients).

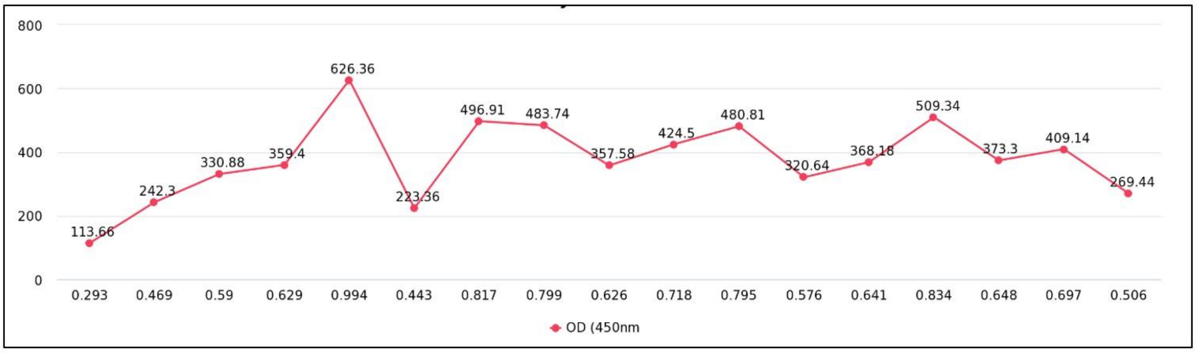

Fig. 4: Graph plotted for Optical Density v/s concentration of Carboxyhemoglobin in ng/ml for the study individuals (female patients).

Table 1
Quantitative estimation of the Standards of Carboxyhemoglobin

Standard prepared (as Serial dilution)	Concentration in ng/mL	OD (Optical Density) obtained at 450nm
Standard 1	2000	2.828
Standard 2	1000	1.514
Standard 3	500	0.933
Standard 4	250	0.508
Standard 5	125	0.323
Standard 6	62.5	0.213
Standard 7	31.2	0.139
Standard 8	0	0.069

Breathlessness was observed in majority of the study subjects (84.74%) followed by cough (67.79%), sputum (49.15%) and fever (20.33%). Of the co-morbidities, hypertension was observed in many individuals (35.59%), followed by tuberculosis (18.64%) and diabetes (15.25%). Other symptoms included skin allergy, chest pain, joint pain, vomiting, HCV positivity etc. When the levels of carboxyhemoglobin were observed, it was seen that the OD values ranged from 0.275 to 1.659. The values of carboxyhemoglobin in study patients were observed in the range of 120ng/ml to 1100ng/ml (Table 2,3; Figs. 3, 4).

Of these individuals, 2 had lowest carboxyhemoglobin level within the range 100-200ng/ml; 10 were in the range of 201-300ng/ml; 20 were in the range of 301-400ng/ml; 14 were in the range of 401-500ng/ml; 6 were in the range of 501-600ng/ml; 5 were in the range of 601-700ng/ml, one subject was in the range of 701-800ng/ml and 1001-1100ng/ml respectively. No patients showed values in the range of 801-900ng/ml and 901-1000ng/ml. When the symptoms were correlated with the levels of carboxyhemoglobin, it was observed that maximum number of individuals were those that fell in the category of 301 to 400 and 401 to 500ng/ml. Most of the individuals in this group showed fever, cough, breathlessness and also had co-morbidities (Figs.3, 4).

Discussion

Respiratory viral infections are one of the leading causes of morbidity and mortality throughput the year in all parts of the world. Due to accumulation of frequent mutations in these viruses, it becomes difficult to find precise vaccine or treatment for their cure. As the viral infections make their way through the respiratory system, they by and large lead to biochemical and cellular changes in the system. Chronic Obstructive Pulmonary Distress (COPD) is one such event which leads to 5% of the mortality worldwide. In India also in the recent past, there have been millions of cases of such types[12]. Since the respiration system is involved, the breathing cycle is likely to be affected. However, it is very difficult to understand what effect did the COPD had on various types of individual condition.

Conclusion

Present study deals with association of carboxyhemoglobin between clinical conditions of patients with corresponding levels of carboxyhemoglobin. The clinical severity as caused by respiratory viruses may be contributed by increased carboxyhemoglobin along with a reduction in the oxygen level of the blood.

The study indicated important observation that in individuals with various case presentations such as shortness of breath, hypertension, blood pressure etc., the corresponding levels of carboxyhemoglobin were observed in range of highest correlation appear between 400-500ng/ml. After further confirmation by studying more number of cases, this level of carboxyhemoglobin could be developed as new clinical indicator of severity of viral infections

Table 2
Details of Symptoms, Comorbidities and Carboxyhemoglobin in Male study subjects.

S.N.	Study groups (GI, GII, GIII, GIV)	Patient Code	Age (YRS)/Male	Smoker/non-Smoker	Day of Observation	Clinical Symptoms				Comorbidities				Elisa for Carboxyhemoglobin	
						Breathlessness	Fever	Cough	Sputum	Hypertension	Diabetes	Tuberculosis	Other	OD (450nm	ng/mL
1.	G I	SCS/RD/2022/41	17	×	10TH	×	×	×	×	×	×	√	Chest Pain	0.856	525.43
2.	G I	SCS/RD/2023/125	18	√	1ST	×	√	√	×	×	×	×	Chest Pain, Bodyache	0.507	270.17
3.	G I	SCS/RD/2023/126	18	×	1ST	×	√	×	×	×	×	×	Bodyache	0.659	381.35
4.	G I	SCS/RD/2022/12	19	×	7TH	√	×	√	×	×	×	×	NO	0.991	624.17
5.	G I	SCS/RD/2023/123	21	√	1ST	√	×	×	×	×	×	×	NO	0.689	403.29
6.	G I	SCS/RD/2022/89	24	×	9TH	√	×	√	√	×	×	√	NO	0.824	502.03
7.	G II	SCS/RD/2022/16	27	×	1ST	√	×	√	×	×	×	×	NO	0.668	387.93
8.	G II	SCS/RD/2022/08	27	×	1ST	√	×	×	×	√	×	√	NO	0.709	417.91
9.	G II	SCS/RD/2023/132	32	×	6TH	√	×	√	√	×	×	√	NO	0.6	338.19
10.	G II	SCS/RD/2023/99	34	×	1ST	×	√	√	√	×	×	×	NO	0.573	318.45
11.	G II	SCS/RD/2022/72	35	√	3RD	√	×	√	√	√	×	×	Bronchial Asthma	0.494	260.67
12.	G II	SCS/RD/2022/66	36	× (EX)	2ND	√	×	√	×	√	√	×	Back Pain	0.915	568.58
13.	G II	SCS/RD/2023/140	46	×	7TH	√	×	√	√	√	×	×	Sepsis	0.791	477.89
14.	G II	SCS/RD/2022/65	48	√	11TH	√	×	√	√	×	×	×	Skin Allergy	0.568	314.79
15.	G II	SCS/RD/2022/84	48	√	2ND	√	×	√	×	×	√	×	Thyroid	0.492	259.2

16.	G II	SCS/RD/2022/117	49	√	3RD	√	×	√	√	√	×	×	Vomiting Daily in Morning	0.704	414.26
17.	G II	SCS/RD/2022/79	49	× (EX)	2ND	√	×	√	√	×	×	×	NO	0.547	299.43
18.	G II	SCS/RD/2022/73	50	× (EX)	9TH	√	×	√	√	×	×	×	NO	0.662	383.54
19.	G III	SCS/RD/2023/128	55	×	1ST	×	√	√	×	×	×	×	NO	0.504	267.98
20.	G III	SCS/RD/2022/06	56	×	1ST	√	×	×	×	×	×	×	NO	0.622	354.28
21.	G III	ABG/RD/2023/26	56	×	4TH	√	×	×	×	×	×	√	NO	0.619	352.09
22.	G III	SCS/RD/2022/05	56	√	1ST	√	√	√	√	×	×	×	NO	0.747	445.71
23.	G III	SCS/RD/2022/34	56	×	11TH	√	×	×	×	√	√	×	NO	0.932	581.02
24.	G III	SCS/RD/2022/105	59	×	3RD	√	×	√	×	×	×	×	HCV Positive, Chest Pain	0.7	411.33
25.	G III	SCS/RD/2022/54	60	√	3RD	√	×	×	×	×	×	×	Joint Pain	0.979	615.39
26.	G III	SCS/RD/2023/133	60	√	2ND	√	×	√	×	×	×	×	Chest pain	0.6	338.19
27.	G III	SCS/RD/2022/78	62	×	4TH	√	×	√	√	×	×	×	Sepsis	0.71	418.65
28.	G III	SCS/RD/2022/87	63	√	7TH	√	×	×	√	√	√	×	NO	0.49	257.74
29.	G III	SCS/RD/2022/67	64	√	3RD	√	×	√	√	√	×	×	NO	0.608	344.04
30.	G III	SCS/RD/2023/96	64	√	13TH	√	×	√	√	×	×	×	Chest Pain	1.173	757.28
31.	G III	SCS/RD/2022/11	65	× (EX)	1ST	√	×	√	×	×	×	×	NO	0.918	570.78
32.	G III	SCS/RD/2022/97	68	√	3RD	√	×	√	√	×	×	×	NO	0.568	314.79
33.	G III	SCS/RD/2022/45	68	× (EX)	2ND	√	×	√	√	×	×	×	NO	0.719	425.23
34.	G III	SCS/RD/2022/82	68	× (EX)	8TH	√	×	×	×	×	×	√	Pneumothorax	0.666	386.47
35.	G III	SCS/RD/2022/32	68	√	8TH	√	×	√	√	×	×	√	NO	0.764	458.14
36.	G III	SCS/RD/2023/134	69	× (EX)	15TH	√	×	×	×	√	×	×	NO	0.626	357.21
37.	G III	SCS/RD/2022/95	69	√	4TH	√	×	√	√	√	√	×	Back Pain	1	630.75
38.	G IV	SCS/RD/2023/139	70	√	2ND	√	√	√	√	×	×	×	NO	1.659	1112.74
39.	G IV	SCS/RD/2023/122	70	× (EX)	3RD	√	×	√	√	×	×	×	HCV Positive	0.329	139.99
40.	G IV	SCS/RD/2022/14	75	× (EX)	1ST	√	×	√	√	√	×	×	NO	0.973	611
41.	G IV	SCS/RD/2022/60	77	√	2ND	√	×	×	×	×	×	×	Skin Allergy	0.635	363.79
42.	G IV	SCS/RD/2022/80	78	×	5TH	√	×	√	√	×	×	√	NO	0.539	293.58

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Table 3
Details of Symptoms, Comorbidities and Carboxyhemoglobin in Female study subjects.

S.N.	Study groups (GI, GII, GIII, GIV)	Patient code	Age (Yrs)/Male	Smoker/non-smoker	Day of observation	Clinical symptoms				Comorbidities				Elisa for Carboxyhemoglobin	
						Breathlessness	Fever	Cough	Sputum	Hypertension	Diabetes	Tuberculosis	OTHER	OD (450nm	ng/mL
1.	G I	SCS/RD/ 2023/129	20	×	1ST	×	√	√	×	×	×	×	Severe Headache	0.293	113.66
2.	G I	SCS/RD/ 2022/75	22	×	4TH	√	√	√	×	√	×	√	NO	0.469	242.3
3.	G II	SCS/RD/ 2023/127	29	√	1ST	×	√	√	√	×	×	×	Neck Pain	0.59	330.88
4.	G II	SCS/RD/ 2023/130	30	×	3RD	×	√	×	×	×	×	×	Boduache	0.629	359.4
5.	G II	SCS/RD/ 2023/138	40	×	2ND	√	×	√	×	×	×	×	NO	0.994	626.36
6.	G II	SCS/RD/ 2022/131	48	×	1ST	√	√	√	√	×	×	√	NO	0.443	223.36
7.	G II	SCS/RD/ 2022/101	50	×	3RD	√	×	×	×	√	×	×	HVBS positive, Acidity, Abdomen Pain	0.817	496.91
8.	G III	SCS/RD/ 2022/108	54	×	12TH	×	√	×	×	×	√	×	Kidney Infection	0.799	483.74
9.	G III	SCS/RD/ 2022/09	55	× (EX)	1ST	√	×	×	×	√	√	×	Suffocation	0.6265	357.58
10.	G III	SCS/RD/ 2023/135	55	× (EX)	3RD	√	×	×	×	√	√	×	Liver enlarge	0.718	424.5
11.	G III	SCS/RD/ 2022/38	55	NO	2ND	√	×	√	√	√	×	×	only in rainy season	0.795	480.81
12.	G III	SCS/RD/ 2022/110	55	√	3RD	√	×	√	√	√	×	√	NO	0.576	320.64
13.	G III	SCS/RD/ 2022/47	60	×	3RD	√	×	√	√	√	×	×	NO	0.641	368.18
14.	G III	SCS/RD/ 2022/102	66	×	2ND	√	×	×	×	√	√	×	Hypotherio diism, Body pain	0.834	509.34
15.	G III	SCS/RD/ 2022/49	67	√	4TH	√	×	√	√	√	×	×	Back pain	0.648	373.3
16.	G IV	SCS/RD/ 2022/15	70	×	24TH	√	×	×	×	√	×	×	Constipatio n, Pain in neck	0.697	409.14
17.	G IV	SCS/RD/ 2022/71	73	√	8TH	√	×	√	√	×	×	×	Joint Pain	0.506	269.44

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