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Research Article

Strength of Association Between Generalized/Nonspecific Covid-19 Signs & Symptoms With SARS-COV 2 Specific ORF, N, E Genes Identified Through Real Time PCR

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Abstract

Background: Constant mutation in the SARS-COV2 virus genetic material is resulting in the appearance of new variants frequently hence the overall virulence, treatment resistance, replication modalities, transmissions rates and COVID-19 signs & symptoms are all changing regularly.

Methodology: From 1 January 2021 to 30 August 2022, the clinical lab at Fatima Jinnah General & Chest Hospital Quetta, Balochistan, determined a total of 3375 individuals to be COVID-19 positive because RT-PCR detected ORF, N, and E genes or their various Bi & Tri combinations in their samples. A questionnaire-based interview was conducted with each participant during sample collection. Body temperature more than 370c was recorded as Fever/ Chill. Age, Comorbidities, A-symptomatic individuals & Vaccination status were all neglected during this study. Frequency tables were generated using MS-excel 2016, while Odds ratios were calculated using Chi-square test of association whereby 2x2 contingency tables between Mono, Bi & Tri combinations for ORF, N & E genes were cross associated with various generalized nonspecific COVID -19 signs and symptoms using Epi-info software. Absence of Genetic sequencing was the major limitation.

Results: The study showed that individually the presence of ORF gene was found to be strongly associated " Shortness of Breath/Difficulty in Breathing", Diarrhea, Head ache & Vomitting. While the presence of N-gene was found to be strongly associated with Loss of smell & taste, Head ache,Presistant Chest Pain & Bluish lips/Face. Where as the presence of E-gene was found to be strongly associated with Cough, Shortness of breath/ Difficulty in breathing, Sore throat, Diarrhea, Head ache & Laziness. In addition, the study also found that different Bi & Tri combinations of ORF, N & E genes in a COVID-19 positive patient expressed generalized non-specific COVID-19 signs & symptoms differently.

Discussion & Conclusion: The presence of various SARS-COV2 genetic markers significantly alters the clinical presentation of COVID-19.

Keywords: RT-PCR; SARS-COV2; ORF N & E Genes; Association

Introduction

There were 41,409 people in all documented, from at least 23 different nations, with 26 different clinical presentations. Six symptoms—fever (58.66%), cough (54.52%), dyspnea (30.82%), malaise (29.75%), weariness (28.16%), and sputum/ secretion (25.33%)—had a general prevalence more than or equal to 25%. Other prevalent symptoms included headache (12.17%), chest discomfort (11.49%), diarrhea (9.59%), sneezing (14.71%), sore throat (14.41%), rhinitis (14.29%), goosebumps (13.49%), dermatological signs (20.45%), anorexia (20.26%), myalgia (16.9%), and rhinitis. The manifestations of dermatology were only documented in one study. Hemoptysis was the least common indication or symptom (1.65%). The three most common symptoms in trials involving more than 100 patients were dyspnea (30.64%), cough (54.21%), and fever (57.93%) [1-13].

Certain symptoms, such as dyspnea, fever, cough, and headache, are generally nonspecific for SARS-CoV2. Patients who are asymptomatic may have mild forms of infection, while others may have severe pneumonia that can be fatal [2-3].

The initial symptoms of the illness were the trio of fever, coughing, and shortness of breath. Later, the US Center for

Disease Control and Prevention (CDC) expanded this list to include chills, headache, sore throat, muscle discomfort, and loss of taste or smell (neurological manifestations) [4-15].
Any possible association between generalized nonspecific COVID-19 signs and symptoms with SARS-COV 2 specific ORF, N & E genes would open up a new door to research in future.

Literature Review

It is certain that age manifest the clinical sings & symptoms of COVID-19 differently. According to one study, people over 60 have more bilateral lobe lesions, greater levels of inflammatory markers, and higher blood urea nitrogen levels. Patients who are older than 60 have a higher risk of respiratory failure and prolonged illness courses. The intensity is, however, less severe in people under 60 [5-14].

According to one additional study that claims a total of 72,314 verified cases in China the majority of the patients (87%) are between the ages of 30 and 79. There were no fatalities among those under the age of nine. However, the case-fatality rate (CFR) is 8.0% for those aged 70 to 79 and 14.8% for people 80 years of age and older. The CFR is 10.5, 7.3, 6.3, 6.0, and 5.6%, respectively, for patients with various concomitant diseases, including cancer, chronic respiratory disease, diabetes, cardiovascular disease, and hypertension. According to these findings, COVID-19 patients with comorbid conditions have higher fatality rates than those who don't have underlying diseases [6-15].
Coronaviruses have genomes that range in size from 26 to 32 kilobases and have a variety of open reading frames (ORFs) [7]. Coronaviruses have a varying number of open reading frames in their genomes, which range in size from 26 to 32 kilobases (ORFs). The spike surface glycoprotein (S), small envelope protein (E), matrix protein (M), and nucleocapsid protein (N) are the four major structural proteins and the eight accessory proteins (3a, 3b, p6, 7a, 7b, 8b, 9b, and orf14) are situated in the 3′-terminus of the SARS-CoV-2 genome [8-16,17].

The SARS-CoV-2 was found to be more related to two SARS-like bat CoVs from Zhoushan in eastern China, bat-SL-CoVZC45 and bat-SL-CoVZXC21, than to the SARS-CoV and the MERS-CoV, according to analysis of the genome from the samples of nine patients. Laboratory specific detection Next-generation sequencing or real-time reverse transcriptase-polymerase chain reaction (RT-PCR) techniques for the SARS-CoV-2 virus were developed as a result of the isolation of the causative agent and determination of its partial genomic sequence [9-20].

The Open Reading Frame (ORF) segments in the SARS COV 2 virus genome encode structural and non-structural proteins [8]. The ORF1a/b gene in SARS COV 2 nucleic acid is used for diagnostics by RT PCR. It produces non-structural proteins (nsp1–16), which are necessary for the viral genome's maintenance and replication apparatus. Adaptive mutations in ORF1a/b may boost viral replication or increase treatment resistance, hence increasing virulence [10]. The "N" genome encodes the "N" structural protein which participates in a number of viral genome-related functions, such as viral genome signaling, viral replication, and host cell immunity to viral infections [11]. Similarly, the "E" genome encodes for the "E" structural protein Through interactions with the host cell membrane protein, the E protein participates in the viral growth and maturation stages [12-25].

From the very first case the SARS-COV 2 infection, a continuous mutation is reported in the virus as a result of which new variants popup frequently hence the overall virulence, treatment resistance, replication modalities & transmissions rates are all changing regularly hence in these circumstances it is of great importance to frequently monitor the symptomology of COVID-19. Currently no published literature has assessed the relationship of COVID-19 symptomology (i.e., COUGH, SHORTNESSS OF BREATH/DIFFICULTY IN BREATHING, Fever/Chills, NEW MUSCLE/BODY ACHE, SORE THROAT, LOSS OF SMELL & TASTE, DIARRHEA, HEAD ACHE, NAUSEA, VOMITTING, RUNNY NOSE, PERSISTANT CHEST PAIN, LAZINESS, BLUISH LIPS/FACE) with the ORF, N & E genes which are few of the diagnostic markers assessed for SARS COV-2 infection assessed during real-time PCR test.

Methodology

From 1 January 2021 to 30 August 2022, the clinical lab at Fatima Jinnah General & Chest Hospital Quetta, Balochistan, determined a total of 3375 individuals to be COVID-19 positive because RT-PCR detected ORF, N, and E genes or their various Bi & Tri combinations in their samples. A questionnaire-based interview was conducted with each participant during sample collection. Body temperature more than 370c was recorded as Fever/Chill. Age, Comorbidities, A-symptomatic individuals & Vaccination status were all neglected during this study. Frequency tables were generated using MS-excel 2016, while Odds ratios were calculated using Chi-square test of association whereby 2x2 contingency tables between Mono, Bi & Tri combinations for ORF, N & E genes were cross associated with various generalized nonspecific COVID -19 signs and symptoms using Epi-info software. Absence of Genetic sequencing was the major limitation.

Results

From 01 January 2021 till 30th August 2022 a total of 3375 local residents across Balochistan were declared to be COVID-19 positive by the RT-PCR. 48% (n=1620) individuals out of 3375 study participants were found to be positive for all the three major diagnostic markers of SARS-COV 2 (i.e. ORF + N + E). Similarly, the RT-PCR reports of 12% (n=405) study participants were positive for only N & E (N + E) genes together. Moreover 13% (n= 438) study participants were positive only for ORF & N (ORF + N) genes together. While 10% (n=337) study participants were positive only for ORF & E (ORF+E) genes combination. It was also seen that 08% (n=270) study participants were positive only for ORF gene, 06% (n=203) study participants expressed only "N" gene while only 03% (n=102) of the study participants were positive only for "E" gene only as shown below:

Viral Load of SARS-COV2 Among Study Participants:
If a sample gets positive and show presence of ORF, N & E genes in any combination on lesser RT-PCR cycles i.e., below 21 cycles is generally believed to possess high viral load while if a sample becomes positive at 21 or beyond RT-PCR cycles it is beloved to have low vial load. In this Study out of 3375 study participants 57%(n=1915) were positive with high viral load while rest of the 42% (n=1460) were found to be positive with low viral load as shown in the following chart:

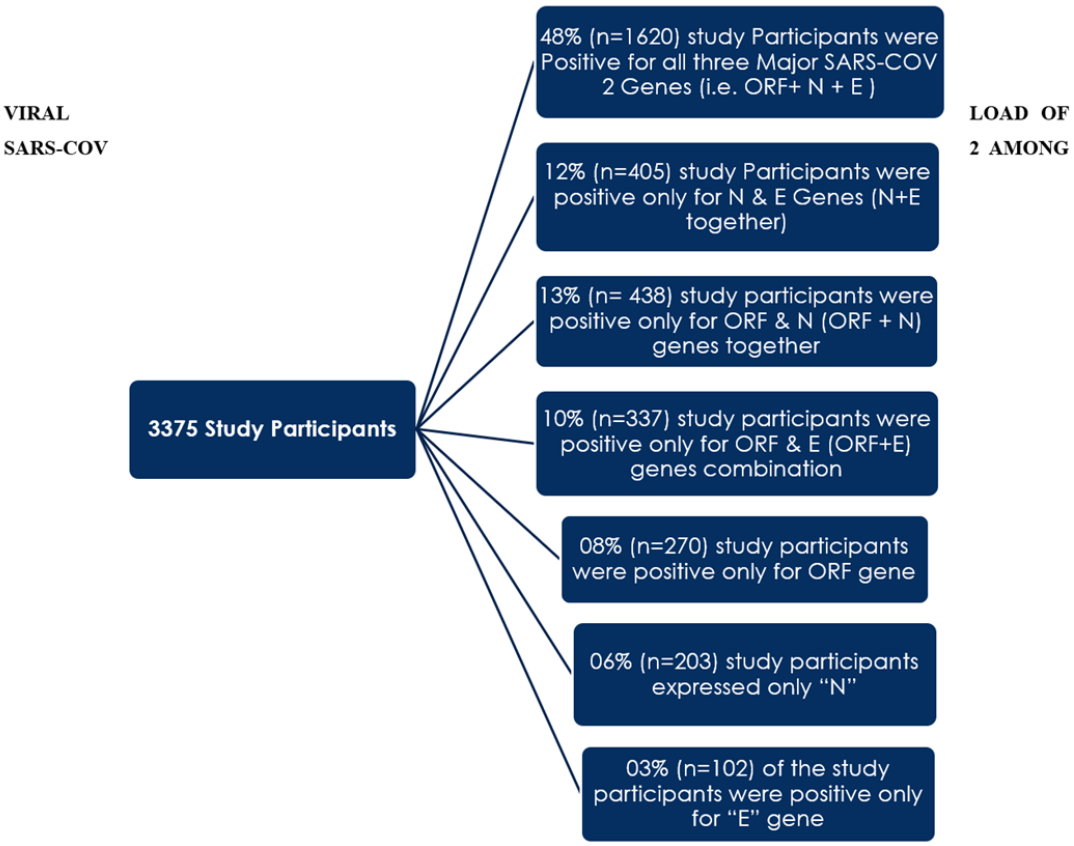

Figure 1: Sub classification of the study participants based on results of RT-PCR reports.

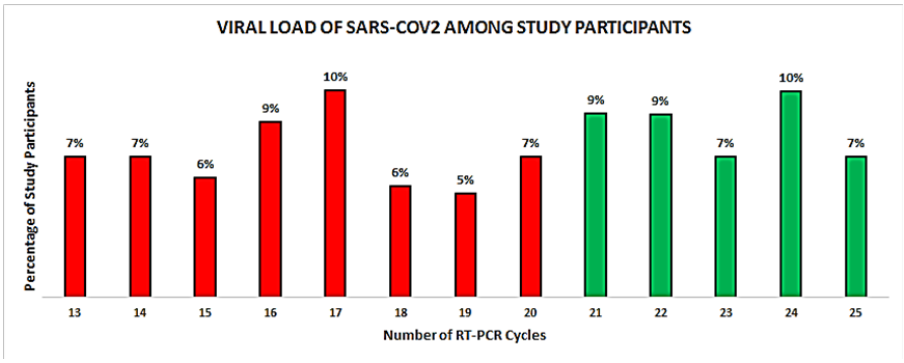

Chart 1: Viral load of study participants.

The following table further summarizes the above chart and is showing cycle wise positivity rate:

Table 1: Positivity rate against RT-PCR Cycles.

S.No.	N.O. of RT-PCR Cycles	N.O of Positive study participants
1	13	236
2	14	250
3	15	201
4	16	294
5	17	347
6	18	186
7	19	174
8	20	227
9	21	309
10	22	307
11	23	247
12	24	345
13	25	252
GRAND TOTAL		**3375**

Frequency & Percentages of the Generalized/Non Specific Sign and Symptoms Among the Study Participants:
The following table summarizes the overall findings:

Table 2: Overall summary of the major findings.

Sub classification of study participants on the bases of RT-PCR Report	SARS_COV2 (ORF) Gene	SARS_COV2 (N) Gene	SARS_COV2 (E) Gene	Total Number of Positive Samples	Cough		Shortness of Breath/ Difficulty in Breathing		Fever/ Chill		New Mucle/ Body aches		Sore Throat		Loss of Smell & taste		Diarrhoea		Head Ache		Nausea		Vomitting		Runny Nose		Persistant chest pain or pressure		Laziness		Blush lips or face	
					Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
All 03 Major Genes	+	+	+	1620 (48%)	513	1107	604	1016	1203	417	1310	310	519	1101	357	1263	284	1336	41	1579	842	778	1002	618	1338	282	1139	481	228	1392	695	925
Different Combination of 02 Major Genes	-	+	+	405 (12%)	37	368	49	356	258	147	175	230	154	251	351	54	136	269	283	122	97	308	310	95	91	314	375	30	145	260	209	196
	+	+	-	438 (13%)	158	280	109	329	223	215	146	292	16	422	269	169	377	61	80	358	323	115	102	336	199	239	118	320	340	98	294	144
	+	-	+	337 (10%)	the	94	we	78	161	176	307	30	219	118	283	54	120	217	190	147	237	100	13	324	198	139	165	172	39	298	261	76
Only single Major gene	+	-	-	270 (08%)	50	220	217	53	148	122	144	126	106	164	9	261	203	67	160	110	35	235	209	61	102	168	136	134	96	174	21	249
	-	+	-	203 (06%)	57	146	71	132	50	153	160	43	52	151	109	94	18	185	114	89	73	130	135	68	132	71	138	43	154	49	120	83
	-	-	+	102 (03%)	80	22	95	7	73	29	40	62	94	8	11	91	95	7	70	32	35	67	49	53	28	74	24	100	56	46	36	66
GRAND TOTAL→				3375	1138	2237	1404	1971	2116	1259	2282	1093	1160	2215	1389	1986	1233	2142	938	2437	1642	1733	1820	1555	2088	1287	2095	1280	1058	2317	1636	1739
PERCENTAGES →					34%	66%	42%	58%	63%	37%	68%	32%	34%	66%	41%	59%	37%	63%	28%	72%	49%	51%	54%	46%	62%	38%	62%	38%	31%	69%	48%	52%

Table 3: Table adopted from the above table.

S.NO.	Generalized/Non Specific COVID-19 Signs & Symptoms	Number of study Participant giving YES Responces	Percentages of study Participant giving YES Responces	Number of study Participant giving NO Responces	Percentage of study Participant giving NO Responces
1	COUGH	1138	34%	2237	66%
2	SHORTNESSS OF BREATH/ DIFFICULTY IN BREATHING	1404	42%	1971	58%
3	Fever/Chills	2116	63%	1259	37%
4	NEW MUSCLE/BODY ACHE	2282	68%	1093	32%
5	SORE THROAT	1160	34%	2215	66%
6	LOSS OF SMELL & TASTE	1389	41%	1986	59%
7	DIARRHEA	1233	37%	2142	63%
8	HEAD ACHE	938	28%	2437	72%
9	NAUSEA	1642	49%	1733	51%
10	VOMITTING	1820	54%	1555	46%
11	RUNNY NOSE	2088	62%	1287	38%
12	PERSISTANT CHEST PAIN	2095	62%	1280	38%
13	LAZINESS	1058	31%	2317	69%
14	BLUISH LIPS/FACE	1636	48%	1739	52%

34% of the participants has cough while 66% had no cough, similarly 42% of the study participants reported to have S.O.B while 58% did not have any S.O.B. Moreover 63% had fever/ chill while 37% did not had. New muscular or body ach was reported by 68% individuals while 32% had no new muscular/ body ache. Similarly, sore throat was reported by 34% of the study participants while 66% of the study participants did not report any sore throat symptom.

Loss of smell and taste was reported by 41% of the study participants while 59% did not report this symptom. Diarrhea was reported by 37% of the study participants while 63% did not report any diarrhea. 28% of the study participants reported headache while 72% did not report any such symptom. Nausea was reported by 49% of the study participants while 51% did not report this symptom. Similarly, 54% of the study participants reported vomiting while 46% did not report this symptom. Runny nose was reported by 62% of the study participants while 38% did not report any such symptom. 68% of the study participants reported persistent chest pain while 38% did not report any chest pain. Laziness was reported by 31% of the study participants and lastly bluish lips/Face was reported by 48% of the study participants while 52% did not report any symptom.

Strength of association between generalized/non- specific covid-19 signs and symptoms reported by all study participants whose RT-PCR test was positive for all three SARS-COV2 genes (i.e., Orf + n + e) compare to others:
The following two tables summarizes the overall findings as shown below:

The presence of all three SARS-COV 2 genetic markers (i.e. ORF + N + E) was strongly associated with certain generalized/ nonspecific COVID-19 signs & symptoms like Fever/ Chills, New Muscular/Bodily Ache, Nausea, Vomiting & Persistent chest pain.

Strength of association between generalized/non- specific covid-19 signs and symptoms reported by all study participants whose RT-PCR test was positive for different combinations of two SARS-COV2 genes (i.e., Orf, n, e) identified during rt-pcr process compare to others:
As previously mentioned during the RT-PCR analysis process among all the 3375 study participants only THREE different (ORF, N, E) paired combinations were identified as summarized below:

Identified during RT-PCR:
Over all 3375 study participants were sub divided into three different classes the nasopharyngeal and throat samples of these study participant only yields a pair of 2 genes out of all the three major genes that is ORF, N, & E gene. One class of study participants were identified with only N & E genes. Similarly, one class of study participants were positive only with ORF & N gene while some of the study participants only

Table 4: Contingency 2x2 table between generalized/ non-specific COVID-19 Signs and symptoms reported by all the study participants whose RT-PCR report was positive for all the three SARS-COV 2 Genes (i.e., ORF + N + E) Compare to others.

		COUGH		SHORTNESSS OF BREATH/DIFFICULTY IN BREATHING		Fever/Chills		NEW MUSCLE/ BODY ACHE		SORE THROAT		LOSS OF SMALL & TASTE		DIARRHEA	
		yes	No	yes	No	yes	No	yes	No	yes	No	yes	No	yes	No
SARS-COV-2 (ORF+N+E) GENES	+	513	1107	604	1016	1203	417	1310	310	519	1101	357	1263	284	1336
	-	625	1130	800	955	913	842	972	783	695	1060	1032	723	949	806

		HEAD ACHE		NAUSEA		VOMITTING		RUNNY NOSE		PERSISTANT CHEST PAIN		LAZINESS		BLUISH LIPS/ FACE	
		yes	No	yes	No	yes	No	yes	No	yes	No	yes	No	yes	No
SARS-COV-2 (ORF+N+E) GENES	+	41	1579	842	778	1002	618	1338	282	1139	481	228	1392	695	925
	-	897	858	800	955	818	937	750	1005	956	799	830	925	941	814

Table 5: Odds Values, P-Values & C.I for table no 04.

	Major SARS-COV2 Symtoms	V/S	Samples With Positive ORF+N+E Genes			Results
1	COUGH	0.83	0.015	0.72	0.96	Weak Association
2	SHORTNESSS OF BREATH/DIFFICULTY IN BREATHING	0.7	0.00	0.61	0.81	Weak Association
3	Fever/Chills	2.66	0.00	2.3	3.07	**Strong Association**
4	NEW MUSCLE/ BODY ACHE	3.4	0.00	2.91	3.97	**Strong Association**
5	SORE THROAT	0.8	0.00	0.73	0.88	Weak Association
6	LOSS OF SMELL & TASTE	0.198	0.00	0.33	0.41	Weak Association
7	DIARRHEA	0.18	0.00	0.15	0.21	Weak Association
8	HEAD ACHE	0.024	0.00	0.02	0.034	Weak Association
9	NAUSEA	1.29	0.00	1.12	1.48	**Strong Association**
10	VOMITTING	1.85	0.00	1.61	2.13	**Strong Association**
11	RUNNY NOSE	6.35	0.00	5.42	7.45	**Strong Association**
12	PERSISTANT CHEST PAIN	1.97	0.00	1.71	2.28	**Strong Association**
13	LAZINESS	0.18	0.00	0.15	0.216	Weak Association
14	BLUISH LIPS/FACE	0.64	0.00	0.56	0.74	Weak Association
		O.R	P-Value	LCI	UCI	

Table 6: Subclasses of Study participants with paired ORF, N, E genes Combinations.

ORF Gene	N Gene	E Gene	Total Number of Study Participants identified with this Combination	Percentage among all 3375 Study Participants
-	+	+	405	12%
+	+	-	438	13%
+	-	+	337	10%

Table 7: Contingency 2x2 table between generalized/ non-specific COVID-19 Signs and symptoms reported by all the study participants whose RT-PCR report was positive for different combinations of two SARS-COV 2 Genes (i.e. ORF, N, E) Compare to others.

Different Combinations of 2 major Sars-Cov-2 Genes as Identified				Cough		O.R	C.I	P-value
ORF	N	E		Yes	No			
-	+	+	yes	37	368	0.09	0.06 - 0.13	0.00
			No	401	374			
+	+	-	Yes	158	280	0.93	0.72 - 1.18	0.56
			No	280	462			
+	-	+	Yes	243	94	8.5	6.44 - 11.44	0.00
			No	195	648			

"Cough" was found to be strongly associated with the Genetic combination of (ORF + E) genes.

Different Combinations of 2 major Sars-Cov-2 Genes as Identified				Shortness of Breath /Difficulty in Breathing		O.R	C.I	P-value
ORF	N	E		Yes	No			
-	+	+	yes	49	356	0.15	0.10 - 0.21	0.00
			No	368	407			
+	+	-	Yes	109	329	0.46	0.35 - 0.60	0.00
			No	308	434			
+	-	+	Yes	259	78	14.3	10.59 - 19.56	0.00
			No	158	685			

"Shortness of Breath/Difficulty in Breathing" was found to be strongly associated with the genetic combination of (ORF + E) genes.

Different Combinations of 2 major Sars-Cov-2 Genes as Identified				Fever/Chill		O.R	C.I	P-value
ORF	N	E		Yes	No			
-	+	+	yes	258	147	1.7	1.3 - 2.28	0.00
			No	384	391			
+	+	-	Yes	223	215	0.79	0.63 - 1.01	0.06
			No	419	323			
+	-	+	Yes	161	176	0.68	0.53 - 0.88	0.003
			No	481	362			

"Fever/Chill" was found to be strongly associated with the Genetic combination of (N + E) genes.

Different Combinations of 2 major Sars-Cov-2 Genes as Identified				New Muscle/Body Ache		O.R	C.I	P-value
ORF	N	E		Yes	No			
-	+	+	yes	175	230	0.54	0.42 - 0.68	0.00
			No	453	322			
+	+	-	Yes	146	292	0.26	0.21 - 0.34	0.00
			No	482	260			
+	-	+	Yes	307	30	16.64	11.15 - 24.82	0.00
			No	321	522			

"New Muscles/Body Ache" were found to be strongly associated with the Genetic combination of (ORF+ E) genes.

Different Combinations of 2 major Sars-Cov-2 Genes as Identified				Loss of smell & Taste		O.R	C.I	P-value
ORF	N	E		Yes	No			
-	+	+	yes	351	54	2.62	1.89 - 3.63	0.00
			No	552	223			
+	+	-	Yes	269	169	0.27	0.20 - 0.35	0.00
			No	634	108			
+	-	+	Yes	283	54	1.88	1.35 - 2.61	0.00
			No	620	223			

"Loss of Smell & Taste" was found to be strongly associated with the Genetic combination of (N + E) genes.

Different Combinations of 2 major Sars-Cov-2 Genes as Identified				Sore Throat		O.R	C.I	P-value
ORF	N	E		Yes	No			
-	+	+	yes	154	251	1.4	1.09 - 1.81	0.007
			No	235	540			
+	+	-	Yes	16	422	0.03	0.02 - 0.06	0.00
			No	373	369			
+	-	+	Yes	219	118	7.34	5.55 - 9.72	0.00
			No	170	673			

"Sore Throat" was found to be strongly associated with the Genetic combination of (ORF + E) genes.

Different Combinations of 2 major Sars-Cov-2 Genes as Identified				Diarrhoea		O.R	C.I	P-value
ORF	N	E		Yes	No			
-	+	+	yes	136	269	0.28	0.21 - 0.36	0.00
			No	497	278			
+	+	-	Yes	377	61	11.73	8.60 - 15.99	0.00
			No	256	486			
+	-	+	Yes	120	217	0.35	0.27 - 0.46	0.00
			No	513	330			

"Diarrhea" was found to be strongly associated with the Genetic combination of (ORF + N) genes.

Different Combinations of 2 major Sars-Cov-2 Genes as Identified				Head Ache		O.R	C.I	P-value
ORF	N	E		Yes	No			
-	+	+	yes	283	122	4.33	3.34 - 5.61	0.00
			No	270	505			
+	+	-	Yes	80	358	0.12	0.09 - 0.16	0.00
			No	473	269			
+	-	+	Yes	190	147	1.70	1.32 - 2.20	0.00
			No	363	480			

"Head ache" was found to be strongly associated with the Genetic combination of (N+E) genes.

Different Combinations of 2 major Sars-Cov-2 Genes as Identified				Nausea		O.R	C.I	P-value
ORF	N	E		Yes	No			
-	+	+	yes	97	308	0.12	0.09 - 0.15	0.00
			No	560	215			
+	+	-	Yes	323	115	3.43	2.65 - 4.45	0.00
			No	334	408			
+	-	+	Yes	237	100	2.38	1.82 - 3.12	0.00
			No	420	423			

"Nausea" was found to be strongly associated with Genetic combination of (ORF + N) genes.

Different Combinations of 2 major Sars-Cov-2 Genes as Identified				Vomitting		O.R	C.I	P-value
ORF	N	E		Yes	No			
-	+	+	yes	310	95	18.72	13.82 - 25.36	0.00
			No	115	660			
+	+	-	Yes	102	336	0.39	0.30 - 0.51	0.00
			No	323	419			
+	-	+	Yes	13	324	0.04	0.02 - 0.07	0.00
			No	412	431			

"Vomiting" was found to be strongly associated with Genetic combination of (N + E) genes.

Different Combinations of 2 major Sars-Cov-2 Genes as Identified				Runny Nose		O.R	C.I	P-value
ORF	N	E		Yes	No			
-	+	+	yes	91	314	0.27	0.21 - 0.36	0.00
			No	397	378			
+	+	-	Yes	199	239	1.30	1.02 - 1.65	0.02
			No	289	453			
+	-	+	Yes	198	139	2.71	2.09 - 3.52	0.00
			No	290	553			

"Runny Nose" was found to be strongly associated with Genetic combination of (ORF + E) genes.

Different Combinations of 2 major Sars-Cov-2 Genes as Identified				Persistant Chest Pain Or Pressure		O.R	C.I	P-value
ORF	N	E		Yes	No			
-	+	+	yes	375	30	21.73	14.5 - 32.40	0.00
			No	283	492			
+	+	-	Yes	118	320	0.13	0.10 - 0.18	0.00
			No	540	202			
+	-	+	Yes	165	172	0.68	0.52 - 0.87	0.00
			No	493	350			

"Persistent Chest pain/Pressure" was found to be strongly associated with Genetic combination of (N + E) genes.

Different Combinations of 2 major Sars-Cov-2 Genes as Identified				Laziness		O.R	C.I	P-value
ORF	N	E		Yes	No			
-	+	+	yes	145	260	0.58	0.45 - 0.74	0.00
			No	379	396			
+	+	-	Yes	340	98	10.52	7.95 - 13.91	0.00
			No	184	558			
+	-	+	Yes	39	298	0.09	0.06 - 0.13	0.00
			No	485	358			

"Laziness" was found to be strongly associated with Genetic combination of (ORF + N) genes.

Different Combinations of 2 major Sars-Cov-2 Genes as Identified				Bluish Lips/Face		O.R	C.I	P-value
ORF	N	E		Yes	No			
-	+	+	yes	209	196	0.42	0.32 - 0.54	0.00
			No	555	220			
+	+	-	Yes	294	144	1.18	0.92 - 1.51	0.18
			No	470	272			
+	-	+	Yes	261	76	2.32	1.73 - 3.10	0.00
			No	503	340			

"Bluish lips/Face" was found to be strongly associated with Genetic combination of (ORF + E) genes.

Strength of association between generalized/non- specific covid-19 signs and symptoms reported by all study participants whose RT-PCR test was positive for a single SARS-COV2 genes (i.e., Orf or n or e) compare to others:

Table 8: Contingency 2x2 table between generalized/ non-specific COVID-19 Signs and symptoms reported by all the study participants whose RT-PCR report was positive for a single SARS-COV 2 Genes (i.e. ORF/ N/ E) Compare to others. The following two tables summarizes the overall findings as shown below:

		COUGH		SHORTNESSS OF BREATH/DIFFICULTY IN BREATHING		Fever/Chills		NEW MUSCLE/ BODY ACHE		SORE THROAT		LOSS OF SMALL & TASTE		DIARRHEA	
		yes	No	yes	No	yes	No	yes	No	yes	No	yes	No	yes	No
ORF	+	50	220	217	53	148	122	144	126	106	164	9	261	203	67
	-	1088	2017	1187	1918	1968	1137	2138	967	1054	2051	1380	1725	1030	2075
		HEAD ACHE		NAUSEA		VOMITTING		RUNNY NOSE		PERSISTANT CHEST PAIN		LAZINESS		BLUISH LIPS/ FACE	
		yes	No	yes	No	yes	No	yes	No	yes	No	yes	No	yes	No
ORF	+	160	110	35	235	209	61	102	168	136	134	96	174	21	249
	-	778	2327	1607	1498	1611	1494	1986	1119	1959	1146	962	2143	1615	1490
		COUGH		SHORTNESSS OF BREATH/DIFFICULTY IN BREATHING		Fever/Chills		NEW MUSCLE/ BODY ACHE		SORE THROAT		LOSS OF SMALL & TASTE		DIARRHEA	
		yes	No	yes	No	yes	No	yes	No	yes	No	yes	No	yes	No
N	+	57	146	71	132	50	153	160	43	52	151	109	94	18	185
	-	1081	2091	1333	1839	2066	1106	2122	1050	1108	2064	1280	1892	1215	1957
		HEAD ACHE		NAUSEA		VOMITTING		RUNNY NOSE		PERSISTANT CHEST PAIN		LAZINESS		BLUISH LIPS/ FACE	
		yes	No	yes	No	yes	No	yes	No	yes	No	yes	No	yes	No
N	+	114	89	73	130	135	68	132	71	160	43	154	49	120	83
	-	824	2348	1569	1603	1685	1487	1956	1216	1935	1237	904	2268	1516	1656
		COUGH		SHORTNESSS OF BREATH/DIFFICULTY IN BREATHING		Fever/Chills		NEW MUSCLE/ BODY ACHE		SORE THROAT		LOSS OF SMALL & TASTE		DIARRHEA	
		yes	No	yes	No	yes	No	yes	No	yes	No	yes	No	yes	No
E	+	80	22	95	7	73	29	40	62	94	8	11	91	95	7
	-	1058	2215	1309	1962	2043	1230	2242	1031	1066	2207	1378	1895	1138	2135
		HEAD ACHE		NAUSEA		VOMITTING		RUNNY NOSE		PERSISTANT CHEST PAIN		LAZINESS		BLUISH LIPS/ FACE	
		yes	No	yes	No	yes	No	yes	No	yes	No	yes	No	yes	No
E	+	70	32	35	67	49	53	28	74	24	100	56	46	36	66
	-	868	2405	1607	1666	1771	1502	2060	1213	2071	1180	1002	2271	1600	1673

express ORF & E genes on to their RT-PCR report. The details of each sub class along with their numbers and percentages are shown in the above table.

The presence of ORF gene was found to be strongly associated " Shortness of Breath/Difficulty in Breathing", Diarrhea, Head ache & Vomitting while Laiziness was found to have no association with the presence of ORF gene. Similarly the presence of N-gene was found to be strongly associated with Loss of smell & taste, Head ache,Presistant Chest Pain & Bluish lips/ Face, N-gene was found to have no association with cough, New Muscular/Body ache & Runny Nose.

The presence of E-gene was strongly associated with Cough, Shortness of breath/ Difficulty in breathing, Sore throat, Diarrhea, Head ache & Laziness while the E-gene was found to have no association with Fever/Chill & Vomiting.

Discussion & Conclusion:

Previously it has been well established from at least 23 different nations, with 26 different clinical presentations that Six symptoms—fever (58.66%), cough (54.52%), dyspnea (30.82%), malaise (29.75%), weariness (28.16%), and sputum/ secretion (25.33%)—had a general prevalence more than or equal to 25%. Other prevalent symptoms included headache

Table 9: Odds Values, P-Values & C.I for table no 08.

	Major SARS-COV2 Symtoms	V/S	Samples With ONLY ORF gene Positive			Results
1	COUGH	0.42	0.000	0.38	0.57	Weak Assiciation
2	SHORTNESSS OF BREATH/DIFFICULTY IN BREATHING	6.61	0.00	4.85	9.01	Strong Association
3	Fever/Chills	0.7	0.00	0.54	0.90	Weak Association
4	NEW MUSCLE/ BODY ACHE	0.51	0.00	0.4	0.66	Weak Association
5	SORE THROAT	1.25	0.08	0.97	1.62	Statistically NO Significat Association
6	LOSS OF SMELL & TASTE	0.043	0.00	0.02	0.08	Weak Association
7	DIARRHEA	6.1	0.00	4.58	8.12	Strong Association
8	HEAD ACHE	4.35	0.00	3.36	5.61	Strong Association
9	NAUSEA	0.13	0.000	0.096	0.19	Weak Association
10	VOMITTING	3.17	0.00	2.36	4.26	Strong Association
11	RUNNY NOSE	0.34	0.00	0.26	0.44	Weak Association
12	PERSISTANT CHEST PAIN	0.59	0.00	0.46	0.76	Weak Association
13	LAZINESS	1.22	0.00	0.947	1.35	Statistically NO Significat Association
14	BLUISH LIPS/FACE	0.077	0.00	0.04	0.122	Weak Association
		O.R	P-Value	LCI	UCI	

Table 10: Odds Values, P-Values & C.I for table no 08.

	Major SARS-COV2 Symtoms	V/S	Samples With ONLY N gene Positive			Results
1	COUGH	0.75	0.079	0.55	1.034	No Association
2	SHORTNESSS OF BREATH/DIFFICULTY IN BREATHING	0.74	0.04	0.55	0.99	Weak Association
3	Fever/Chills	0.17	0.00	0.12	0.24	Weak Association
4	NEW MUSCLE/ BODY ACHE	1.84	0.00	1.30	2.59	No Association
5	SORE THROAT	0.64	0.00	0.46	0.88	Weak Association
6	LOSS OF SMELL & TASTE	1.71	0.00	1.28	2.27	Strong Association
7	DIARRHEA	0.156	0.00	0.096	0.25	Weak Association
8	HEAD ACHE	4.61	0.00	3.49	6.07	Strong Association
9	NAUSEA	0.57	0.000	0.427	0.77	Weak Association
10	VOMITTING	1.75	0.00	1.29	2.36	Strong Association
11	RUNNY NOSE	1.15	0.33	0.85	1.55	No Association
12	PERSISTANT CHEST PAIN	2.37	0.00	1.68	3.35	Strong Association
13	LAZINESS	7.88	0.00	5.66	10.97	Strong Association
14	BLUISH LIPS/FACE	1.57	0.00	1.18	2.10	Strong Association
		O.R	P-Value	LCI	UCI	

(12.17%), chest discomfort (11.49%), diarrhea (9.59%), sneezing (14.71%), sore throat (14.41%), rhinitis (14.29%), goosebumps (13.49%), dermatological signs (20.45%), anorexia (20.26%), myalgia (16.9%), and rhinitis. The manifestations of dermatology were only documented in one study. Hemoptysis was the least common indication or symptom (1.65%). The three most common symptoms in trials involving more than 100 patients were dyspnea (30.64%), cough (54.21%), and fever (57.93%) [1-13]. The study participants of our study also reported similar generalized/ nonspecific COVID-19 signs & symptoms.

From the very first case the SARS-COV 2 infection, a continuous mutation is reported in the virus as a result of which new variants popup frequently hence the overall virulence, treatment resistance, replication modalities & transmissions rates are all changing regularly hence in these circumstances it is of great importance to frequently monitor the symptomology of COVID-19. Currently no published literature has assessed the relationship of COVID-19 symptomology (i.e., COUGH, SHORTNESSS OF BREATH/DIFFICULTY IN BREATHING, Fever/Chills, NEW MUSCLE/BODY ACHE, SORE THROAT, LOSS OF SMELL & TASTE, DIARRHEA, HEAD ACHE, NAUSEA, VOMITTING, RUNNY NOSE, PERSISTANT CHEST PAIN, LAZINESS, BLUISH LIPS/FACE) with the ORF, N & E genes which are few of the diagnostic markers assessed for SARS COV-2 infection assessed during real-time PCR test.

The study findings showed that the presence of all three SARS-COV 2 genetic markers (i.e. ORF + N + E) was strongly associated with certain generalized/ nonspecific COVID-19 signs & symptoms like Fever/Chills, New Muscular/Bodily Ache, Nausea, Vomiting & Persistent chest pain.

Similarly, pair combination of different SARS-COV 2 virus genes identified showed that (ORF + E) gene pair presence was strongly associated Cough, Shortness of breath/Difficulty in breathing, New Muscular/Body pain, Sore throat, Runny nose and Bluish lips/face. Moreover, the (N + E) pair was found to be strongly associated with Loss of smell & Taste, Head ache, Vomiting & Persistent chest pain/pressure. Furthermore, the (ORF + N) pairing was found to be strongly associated with Diarrhea, Nausea & Laziness.

Our study also showed that individually the presence of ORF gene was found to be strongly associated " Shortness of Breath/Difficulty in Breathing", Diarrhea, Head ache & Vomitting while Laiziness was found to have no association with the presence of ORF gene. Similarly the presence of N-gene was found to be strongly associated with Loss of smell & taste, Head ache,Presistant Chest Pain & Bluish lips/Face, N-gene was found to have no association with cough, New Muscular/ Body ache & Runny Nose. While the presence of E-gene was strongly associated with Cough, Shortness of breath/ Difficulty in breathing, Sore throat, Diarrhea, Head ache & Laziness while the E-gene was found to have no association with Fever/ Chill & Vomiting.

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