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HEALTH SCIENCES

Healthcare workers exposed to COVID-19 patients present an inflammatory status and Th2/Th17/Th22 immune profile: findings from before vaccine application in Brazil

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Abstract: Healthcare workers present an increased risk of contagion for the SARS-CoV-2 virus due to their labor exposure. Here, we describe the clinical, laboratory, and immunological characteristics of healthcare workers, before vaccine application, exposed to SARS-CoV-2-infected patients. We collected sociodemographic, clinical, and laboratory information from 50 professionals who worked during the COVID-19 pandemic at the Clinical Hospital of the Northwest in Brazil. The results showed that most workers are women, over 50 years old, and worked as nursing technicians. Approximately 56% of workers were positive for a previous infection by RT-PCR and/or anti-SARS-CoV-2-immunoglobulin tests. Increased levels of hematocrit, neutrophils, NK lymphocytes, and fibrinogen, were found in positive healthcare workers, suggesting a light inflammatory status. The immunological findings showed an increase in IL-17 production and a Th2/Th17/Th22 profile followed by high serology for anti-SARS-CoV-2 IgM and IgG. Those data reveal the importance of studies with healthcare workers to investigate if the continuous exposition to the virus may result in chronic activation of the immune system and/or pulmonary inflammation in this target group.

Key words: COVID-19, Healthcare workers, Inflammation, SARS-CoV-2, Th2/Th17/Th22.

INTRODUCTION

The coronavirus disease 2019 (COVID-19), which emerged in China, was considered the most serious outbreak of pneumonia in the last 100 years, reaching more than 200 countries around the world (Gorbalenya et al. 2020, Lu et al. 2020, Li et al. 2020a, Melo et al. 2020). The transmissibility of COVID-19 is due to the high viral load in the upper respiratory tract, even among asymptomatic patients, which distinguishes it from other respiratory diseases (Arons et al. 2020, Li et al. 2020b). Virus spread occurs through direct contact with contaminated surfaces, aerosols, and respiratory droplets (Chan et al. 2020). The infected person may not present symptoms (asymptomatic), develops few clinical manifestations such as fever, cough, flu, weakness (mild disease), or progress to pneumonia, dyspnea, hypoxia, lung involvement

on imaging tests, respiratory failure, systemic shock, multi-organ failure (severe disease), and to die (Merad & Martin 2020, Buitrago-Garcia et al. 2020, De Souza Silva et al. 2020, Furukawa et al. 2020).

Worldwide, thousands of healthcare workers have taken leave from professional activities due to COVID-19 infection, which also caused a significant number of deaths. In China, about 29% of healthcare workers were infected with COVID-19 during the first three months of the pandemic (Wang et al. 2020a). Spain and Italy also had 22% and 20% of healthcare workers taking work left, respectively (Suárez-Garcia et al. 2020, Arons et al. 2020). The fundamental role of healthcare workers during the COVID-19 pandemic should be recognized and serve as an example for future generations. However, no study, to date, described the immunobiology of healthcare workers exposed to COVID-19-infected patients and how these professionals physiologically reply to daily contact with SARS-CoV-2. Because of constant SARS-COV-2 exposure, it is possible that health workers might develop unique reactions to infection than non-healthcare workers. Here, we described the clinical, biochemical, and immunological aspects of 50 healthcare workers exposed to the disease (due to their clinical procedures with contaminated patients), during the pandemic outbreak, in a public hospital unit, in a period before vaccination against SARS-CoV-2.

MATERIALS AND METHODS

Characterization of volunteers and collection of blood samples

Volunteers of this study consist of 50 healthcare workers from the Clinical Hospital of the Federal University of Pernambuco in Brazil, who worked throughout the COVID-19 pandemic period. The inclusion criteria were to work with infected patients and/or process biological samples of infected patients during COVID-19 pandemic. The socio-demographic, clinical and epidemiological data of healthcare workers were collected through a questionnaire. Likewise, blood samples to perform laboratorial (hematological, coagulogram, serological, and biochemical) and immunological analyses were collected in different tubes (BD Vacutainer®). All volunteer who participated in this study signed a 'Free and Informed Consent Form'. In addition, all experimental protocols were approved by the Human Ethics Committee from Federal University of Pernambuco (nº 4.206.047/2020; CAAE 30332120.8.3001.5191).

Laboratorial analysis of blood samples

The blood of all voluntaries was collected on the same day. After the collection and processing of blood from the healthcare workers, blood and serum samples were sent to the laboratory analysis. The blood count was performed in a Yumizen H2500® hematological analyzer, followed by a review of the blood smear slides under an optical microscope. The coagulogram was analyzed in an automatic STA Compact Max® analyzer. The sample serum was used to evaluate the serological and biochemical parameters using the Architect i2000SR® fully automated immune panels and the CMD 800i automatic biochemical analyzer (Wiener lab®), respectively.

Immunological tests

Immunological analysis from sera samples

The sera collected, according to the 2.1 item, were used for cytokine dosage assays and for the qualitative detection of anti-SARS-CoV-2 immunoglobulins. The Th1/Th2/Th17 human cytokines were measured through of Cytometric Bead Array (CBA) kit (BD Bioscience®) with all data

acquired by FACSVerse platform and processed using the BD FACSuite® software. Exclusively to this assay, we provided seven voluntary samples (which no are healthcare professionals) to compare with our healthcare workers' values. The qualitative detection of IgG and IgM was performed twice, with two months of interval, and using the SARS-CoV-2 Antibody Test Kit by the colloidal gold immunochromatography technique (Lepu Technology®).

Immunological analysis with blood-isolated cell samples

The isolation of peripheral blood mononuclear cells (PBMC), previously collected in the 2.1 item, was performed through blood centrifugation (400 x g, 30 min) under a Ficoll separation gradient (1.077 g/mL; GE Healthcare Life Sciences®), cells were washed twice with PBS (400 x g, 10 min) and stained with anti-CD3-FITC, anti-CD4-PercyPCy5, anti-CD8-PE, anti-CD56-PECy7, anti-CD19-FITC (BD Biosciences®). The anti-IL-10-PE and anti-IL-17-PE intracytoplasmic cytokines were also investigated. The acquisition of the cells was made in 10,000 events in a FACSVerse flow cytometer (BD Biosciences®) and data analysis was performed in the BD FACSuite® software.

Gene expression assay

RNA extraction and cDNA synthesis

Total RNA extraction was performed. PBMC ($5x10^6$) was homogenized in 1 mL of liquid Trizol (Invitrogen). Purification of isolated total RNA was performed through RNeasy® Mini Kit (QIAGEN) following the manufacturer's instructions. The RNA's quality was assured by a NanoDrop 2000 Spectrophotometer (Thermo Scientific Wilmington, USA) and electrophoresis (1% agarose gel). Purified RNA (1 µg) of adequate quality (an OD260/280 from 1.8 to 2.1 and intact rRNA subunits - 28S and 18S) was used to synthesize cDNA by means of Maxima First Strand cDNA Synthesis Kit for RT-qPCR with dsDNase (Thermo Scientific®). For each sample, a negative control RT reaction (without Reverse Transcriptase enzyme) was used.

Primer design and efficiency estimation for qPCR

The primers used to detect STAT4, JAK2, STAT6, STAT3, and FOXO4 genes were designed based on previous studies (Park et al. 2019a, b, Su et al. 2014, Usui et al. 2003, Zhao et al. 2018). The reference genes, ACTB and GAPDH, were used for the relative quantification. Those genes were previously validated in PBMC samples (Eyerich et al. 2009, Lewkowicz et al. 2011, Mousset et al. 2019). The ID GenBank for the primers used are STAT4 (NM_001243835.2), JAK2 (NM_001322195.2), STAT6 (NM_001178081.2), STAT3 (NM_001384992.1), FOXO4 (NM_001170931.2), ACTB (NM_001101.5) and GAPDH (NM_001357943.2). The cDNA from positive PBMC samples was used to exemplify a real test condition.

Real-time qPCR

The RT-qPCR reaction (10 µL) was performed using the Fast SYBR® Green Master Mix kit (Applied Biosystems®). For reading, the AriaMx Real-Time PCR System (Agilent Technologies) equipment was used according to the following parameters: 95°C in 20 sec for polymerase activation, 40 cycles in 95°C for 3 sec for denaturation, and 60°C in 30 sec for annealing and extension. Thus, geometrical media of the reference genes (ACTB and GAPDH) was used to calculate the relative expression of all targets (Livak & Schmittgen 2001).

Polymorphism Assay

The genomic DNA was extracted from peripheral blood leukocytes by the phenol–chloroform method for molecular analysis. Investigation

of polymorphisms -174 G/C IL-6 (rs 1800795), -197 G/A IL-17A (rs1974226), -511 C/T IL-1β (rs 16944), and -308 G/A TNF-α (rs1800629) was performed by PCR-RFLP. Primer sequence pairs for the amplifications were used as established by Cabrera et al. (1995), Unfried et al. (2001), Arman et al. (2006) and Wu et al. (2010a). For -1082 A/G polymorphism of the IL-10 gene (rs1800896) was made using the PCR-ASA – Allele-Specific method. Primer sequence pairs for the amplifications were used as established by Cavet et al. (1999) and Borroni et al. (2004).

Statistical analysis

The D'Agostino test was applied to test the normality of the hypothesis and the statistical differences between groups were analyzed by one-way analysis of variance (ANOVA) with Unpaired *t* test with Welch's correction confirmation. Results about genes expression it was used Mann-Whitey test. All results were expressed as the mean of the values of the groups ± standard deviation and analyzed considering the value of $p<0.05$ as statistically significant. The graphs were processed by GraphPad Prism 9 Software.

RESULTS

The results on the profile of this cohort of healthcare workers showed that 80% were women, 40% were over 51 years old, 56% had a technical level of education and 46% were nursing technicians (see Table I). In addition, 64% worked during the day, 86% with a 12-hour shift, and 62% have two or more jobs in other health services. About 80% of the workers did not have relevant comorbidities (which are risk factors for the worsening of COVID-19).

Bloods of the volunteers (all of them) were collected on the same day, and they were not symptomatic in this phase. Based on any anti-SARS-CoV-2 positive test, rt-PCR, and/or Immunoglobulin, (performed in a period from six months before blood collection) the healthcare workers of this study were divided into two groups, the first group (28 / 56%) was composed of workers who tested positive for infection by SARS-CoV-2. The second group (22 / 44%) was composed of workers who were negative on all tests. Furthermore, about one month before

Table I. Socio-demographic, clinical and epidemiological profile of investigated healthcare workers exposed to SARS-CoV-2-infected patients in the Clinical Hospital.

		N	%
Gender	Women	40	80
	Men	10	20
Age range (years)	20-30 years	2	4
	31-40 years	15	30
	41-50 years	13	26
	Above 51 years	20	40
Schoolarity	Technical level	28	56
	University level	22	44
Occupation	Nursing Technician	23	46
	Nurse	12	24
	Laboratory technician	5	10
	Pharmaceutical	3	6
	Physicians, including residents	3	6
	Physiotherapist	2	4
	Speech Therapist	1	2
	Nutritionist	1	2
Work shift	Day	32	64
	Night	18	36
Hours of work	6h/day	5	10
	8h/day	2	4
	12h/day	43	86
Workplaces	Only 1	19	38
	2 or more	31	62
Risk factors	No comorbidity	40	80
	Cardiovascular disease / hypertension	6	12
	Asthma / Pneumopathy	2	4
	Diabetes Mellitus	1	2
	Obesity	1	2

laboratory assays, in the positive healthcare workers group, some people (53.6%) presented symptoms such as diarrhea (24%), sore throat (22%), cough (18%), fever (14%), dyspnea, and others (12%). Few symptoms, similar to flu, were also observed in 23% of negative healthcare workers. Aiming to evaluate the serological response against the SARS-CoV-2 virus, we performed two quick tests (for immunoglobulins anti-SARS-CoV-2 detection). The time interval between two tests was two months. There was an increase in frequency of positive IgG anti-SARS-CoV-2 blood testes overtime (Table II) and a decrease in IgM-positive tests.

The blood parameters of healthcare workers were evaluated by comparing the results of positive and negative individuals. Individuals with serology positive to the previous infection by SARS-CoV-2 presented a mild but significant increase in the levels of hematocrit, total leukocytes, neutrophils, and lymphocytes (Table III), although the results were within reference values. Likewise, CRP and fibrinogen were also increased in positive healthcare workers when compared to the negative group (Table III).

COVID-19 is an expressive immunological disease and inflammatory cytokines, associated with T lymphocytes response can lead to severe disease. We investigated immunological parameters in healthcare workers to evaluate if the immunological response could be changed after the continuous exposition. Regarding the results of cytokine production, we used a negative control (samples from volunteers who no are healthcare workers) to compare values among samples. We observed that TNF-α, IL-6, and IL-17 cytokines produced by negative controls were lower in relation to the healthcare professionals. In this sense, statistical differences were observed in IL-6 (Figure 1). Furthermore, we also could observe an increase in IL-17 values in the positive healthcare workers (p = 0.04) in relation to negative healthcare workers (Figure 1).

The immunophenotyping results showed that $CD56^{+}$ T lymphocytes were elevated in

Table II. Immunoglobulins anti-SARS-CoV-2 present in serology of the positive healthcare professionals.

	1st analysis			2nd analysis		
Immunoglobulin	IgM	IgG	IgM + IgG	IgM	IgG	IgM + IgG
Healthcare workers	28.6%	50%	21.4%	7.2%	71.4%	21.4%

The interval between analysis was two months.

Table III. Analysis of blood and biochemical parameters of health professionals.

	Reference values	Healthcare workers		p value
Hematological parameters		(+)	(-)	
Hematocrit (%)	36 - 54	41.5 ± 3.4	39.3 ± 1.5	*0.03*
Total Leucocytes (number)	4,000 – 11,000	8,402 ± 1,494	7,379 ± 1,013	*0.03*
Neutrophils (%)	50 - 70	55.58 ± 4.6	51.26 ± 5.7	*0.03*
Lymphocytes (%)	25 - 45	38.3 ± 7.5	31.87 ± 7.6	*0.002*
Biochemical parameters				
C-reactive Protein (mg/dL)	0 – 0.5	4.7 ± 2.6	2.4 ± 1.4	*0.01*
Fibrinogen (mg/dL)	200 - 400	366 ± 77	303 ± 57	*0.007*

(+) positive healthcare workers; (-) negative healthcare workers.

the positive group, reinforcing the counts observed in the hematological parameters (high lymphocyte values). However, $CD19^+$, $CD4^+$, and $CD8^+$ lymphocyte subsets did not show statistical differences between groups (Figure 2).

Results about intracellular cytokines showed that both $CD4^+$ and $CD56^+$ lymphocytes produced IL-10 and IL-17 in statistical values in positive healthcare professionals, being more enhanced in $CD56^+$ cells (Figure 3).

The pathways investigation was performed to understand the immune activation in T lymphocytes of virus-exposed and not virus-exposed healthcare workers. The Th2/Th17/Th22 was the most predominant immune response observed by positive healthcare workers (Figure 4). Through STAT6, STAT3, and FOXO4 it could observe that this immune response profile is high in relation to STAT4 (Th1 response) and JAK2 (a pleiotropic gene to both Th1 and Th17 responses).

Since we observed a group of exposed healthcare workers that did not contract SARS-COV-2 infection, we decided to investigate if genetic polymorphisms in immunological genes associated with an inflammatory response to the virus were enhanced in one of the groups. In fact, the possible immune polymorphisms in healthcare workers were evaluated to discard the possibility of genetic changes in the population of this study. Allelic frequencies for each polymorphism were made in accordance with the Hardy-Weinberg balance and are shown in Table IV.

The most common genotypes found among interleukins polymorphisms were GG from IL-6 (64.2%), AA from IL-10 (75%), TC from IL-1β (57.1%), AA from IL-17 (46.4%), GG from TNF-α (64.2%) in positive healthcare professionals. Negative healthcare professionals presented high polymorphism in GG IL-6 (72.7%), GA from IL-10 (81.8%), TT from IL-1β (50%), AA from IL-17 (63.6%), and GG from TNF-α (59%). However, no statistical differences were observed (Table V).

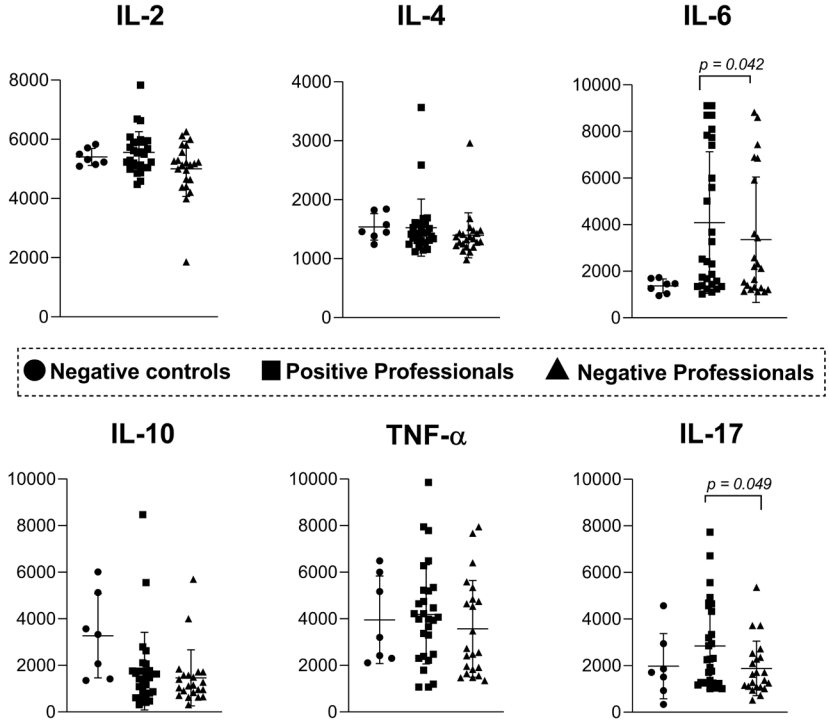

Figure 1. The levels of Th1, Th2, and Th17 cytokines measured in the healthcare workers and volunteers sera. Vertical bars represent a mean of the 57 voluntaries. Significant values were found in IL-6 and IL-17 cytokine to positive professionals. Values are represented as mean±standard deviation.

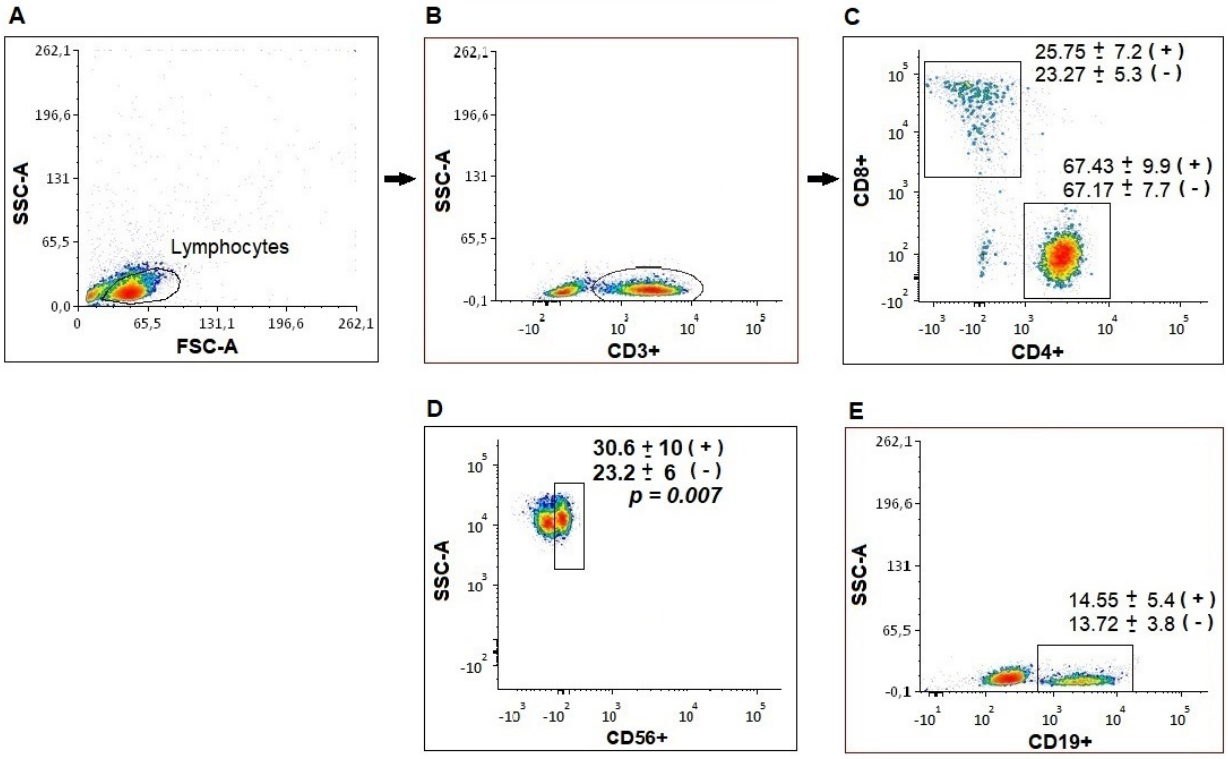

Figure 2. Immunophenotyping of lymphocytes from healthcare workers. The average of each dot plot was represented in perceptual values. **a** – Dot plot with lymphocytes gate definition. **b** – Isolation of $CD3^+$ T lymphocytes population. **c** – Isolation of $CD3^+CD4^+$ and $CD3^+CD8^+$ T lymphocytes population. **d** and **e** – Isolation of Natural killers and B lymphocytes population, respectively. The figures represent an average of 50 volunteers. Symbols on graphics (+) and (-) are related to positive and negative healthcare workers, respectively.

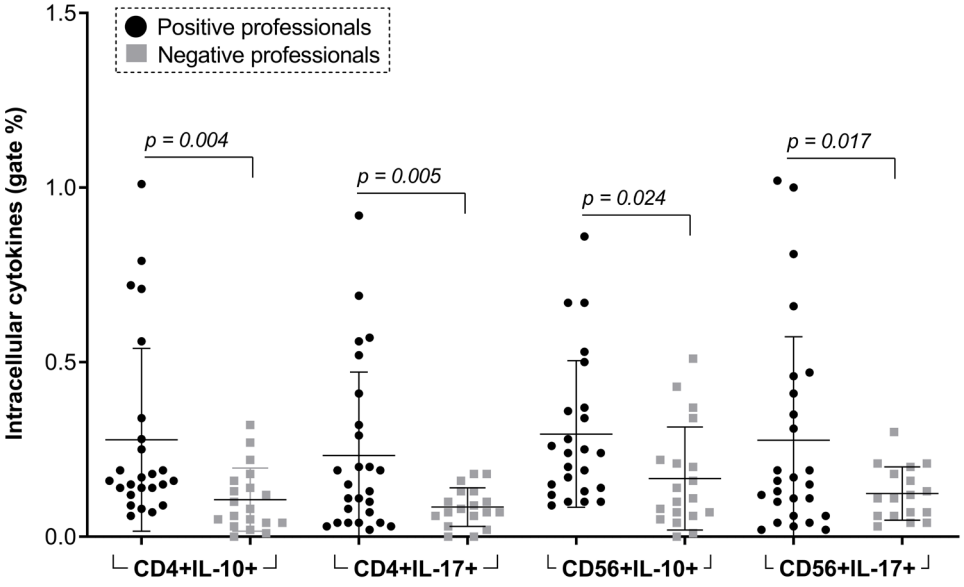

Figure 3. Immunophenotyping of intracellular cytokines from $CD4^+$ and $CD56^+$ lymphocytes of the healthcare workers. lack scatters means positive healthcare workers and gray scatters are negative healthcare workers. The values represent about 50 volunteers. *P* values are represented in the graph.

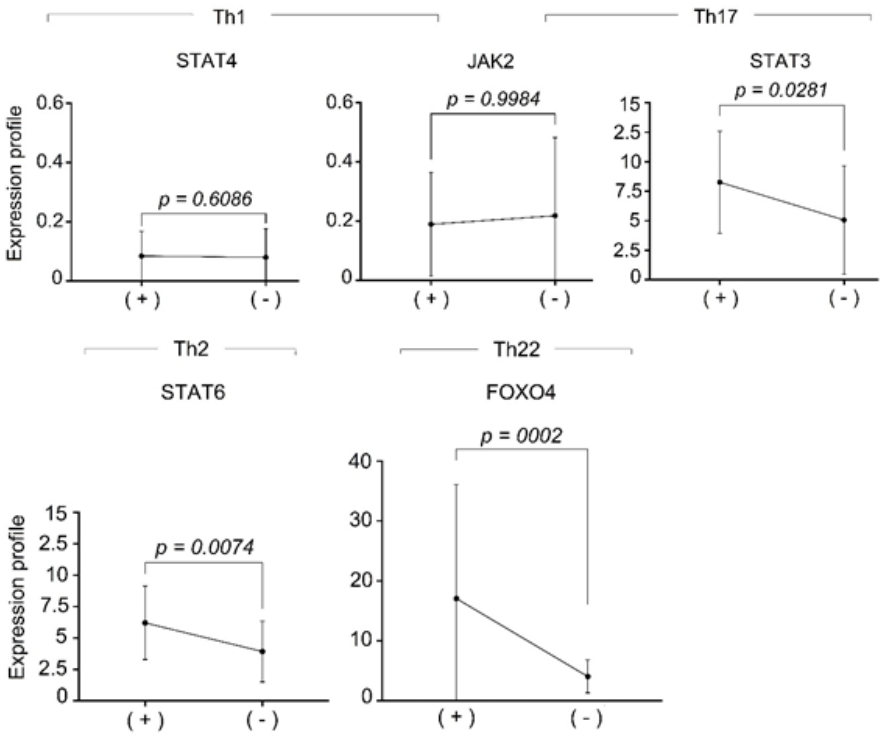

Figure 4. **Results about immune response pathways in 50 healthcare workers. Genes associated with Th2/Th17/Th22 response (STAT3, STAT6, and FOXO4) were observed in high values to positive healthcare workers. Values of *p* were found through Mann-Whitey test and are on graphics. Symbols on graphics (+) and (-) are related to positive and negative healthcare professionals, respectively.**

Table IV. **Hardy-Weinberg balance to the IL-6, IL-10, IL-1β, IL-17 and TNF-α polymorphisms among healthcare workers.**

Interleukins	Healthcare workers (negatives)		Healthcare workers (positives)	
	X^2	*p*-value	X^2	*p*-value
IL-6	0.3835	0.5357	0.6625	0.4157
IL-10	0.1592	0.6899	2.7778	0.0956
IL-1β	1.5774	0.2091	0.2681	0.6046
IL-17	0.8400	0.3594	2.4931	0.1143
TNF-α	0.0021	0.9631	0.2870	0.5922

DISCUSSION

This is a study of healthcare workers, exposed to the COVID-19 pandemic, performed in Brazil during a period before the start of vaccination against SARS-CoV-2. In 2020, those workers with different actions and abilities helped the public and private health units to treat and control the disease, saving a lot of lives. Similar to our results, other studies with healthcare workers have shown a high number of women on the front line of health services, above 50 years old, and with technical training levels. Such findings are reinforced by the great prevalence of nursing due to the degree of dependence on patient care when compared to other professional categories (Lombardi et al. 2020, Teixeira et al. 2020, Baumann et al. 2018).

Studies report that a long working day in health services can compromise the surveillance of patients, reflecting negatively on the assistance offered, and increasing the risk of adverse events and contamination of workers

Table V. Analysis of associations between IL-6, IL-10, IL-1β, IL-17 and TNF-α polymorphisms in healthcare workers.

Polymorphism	HW Positives	HW Negatives	X^2	OR	CI	*P*
Genotype IL-6						
GG	18	16		1	ND	ND
GC	7	5	1.0000	0.8036	0.2124-3.0403	0.9883
CC	0	0		-	-	-
GC/CC	-	-		-	-	-
Trend Test						
Allele						
G	43	37		1	ND	ND
C	0	0		-	-	-
Genotype IL-10						
GG	4	3		1	ND	ND
GA	7	11	0.2339	20.952	0.3563-12.3219	0.7063
AA	14	7		0.6667	0.1158-3.8382	10.000
GA/AA	21	18		11.429	0.2253-5.7980	0.8020
Trend Test						
Allele						
G	15	17		1	ND	ND
A	35	25		0.6303	0.2658-1.4945	0.4059
Genotype IL-1β						
TT	9	10		1	ND	ND
TC	13	7	0.4525	0.4038	0.0697-2.3391	0.5624
CC	3	4		0.8333	0.1452-4.7810	0.8113
TC/CC	16	11		0.5156	0.0959-2.7731	0.7250
Trend Test						
Allele						
T	31	27		1	ND	ND
C	19	15		0.9064	0.3870-2.1232	0.9925
Genotype IL-17						
AA	13	14		1	ND	ND
AG	12	7	1.0000	0.5417	0.1632-1.7975	0.4804
GG	0	0		-	-	-
AG/GG	-	-		-	-	-
Trend Test						
Allele						
A	38	35		1	ND	ND
G	0	0		-	-	-
Genotype TNF-α						
GG	18	13		1	ND	ND
GA	6	7	0.7909	16.154	0.4389-5.9456	0.6950
AA	1	1		13.846	-	0.6070
GA/AA	7	8		15.824	0.4579-5.4690	0.6805
Trend Test						
Allele						
G	42	33		1	ND	ND
A	8	9		14.318	0.4981-4.1150	0.6902

***P*, G test (Williams); OR - Odds Ratio; CI - Confidence interval; ND – Not Determined, HW – Healthcare workers.**

(Magno et al. 2020, Pasila et al. 2017). Here, we could observe that most healthcare workers work 12-hour shifts during the day and have two or more employment relationships. This routine can be associated with low salary payments in many health professional categories in Brazil (Andrade et al. 2017, Dall'Ora et al. 2015). In addition, some surveys show that healthcare workers have reported being afraid of hospital contamination, widespread shortages, and frequent reuse of personal protective equipment during the COVID-19 pandemic (Tabah et al. 2020, Hoernke et al. 2021).

The clinical manifestations of COVID-19 can vary from asymptomatic conditions to symptoms such as fever, dry cough, fatigue, severe dyspnea, and in atypical cases, dermatological reactions (Niquini 2020, WHO 2020). In addition to these manifestations, digestive disorders have been reported in less severe cases, with diarrhea present in the initial phase of the disease, which may last from 1 to 14 days (Guan 2020, WHO 2020). Approximately half of the healthcare workers investigated here reported flu-like and other symptoms as possible signs of contamination. Similar to our findings, some studies have been demonstrating that, when healthcare workers report any symptoms, diarrhea stood out (Guan 2020, Niquini 2020, WHO 2020).

Studies have been indicating that the presence of COVID-19 symptoms is needed to induce a detectable immune humoral response (for both cytokines and immunoglobulins) and its serological prevalence (Lan et al. 2020, Long et al. 2020, Mardani et al. 2020, Wu et al. 2020b). Here, the presence of both IgM and IgG in positive healthcare workers exposed to the virus, in the hospital, is corroborated by the findings of Ko et al. (2020). They showed that the majority of mild cases of COVID-19 produced detectable antibodies such as IgG^{+} after the symptomatic period. Rusakaniko et al. (2021) showed that the major exposure to SAR-CoV-2 occurred across the spectrum of COVID-19 patient-facing staff, such as nurses and nursing assistants with the highest seroprevalence. Our findings support a preoccupation related to negative workers who are exposed daily yet do not show signs of infection. Al-Zoubi et al. (2020) also showed similar results when investigating 370 healthcare workers, a majority of whom were nurses followed by physicians and other personnel, and did not find contamination among them. In fact, some studies with healthcare workers have shown a high prevalence of asymptomatic individuals, with a progressive decrease of IgG titers and opening an immunological window to reinfection (Al-Zoubi et al. 2020, Korth et al. 2020, Mandié-Rajčević et al. 2020, Zhao et al. 2020).

Laboratory results showed an important inflammatory status to the positive healthcare workers. The increase of fibrinogen, C-reactive Protein, hematocrit, leukocytes, neutrophils, and lymphocytes (especially Natural Killer cells) showed a possible inflammatory persistence associated with the daily virus exposition. In fact, an increase in fibrinogen levels can be a predisposing factor for increased blood hypercoagulation and fibrin formation, functioning as an inflammatory marker for the risk of thrombosis and as an indicator of tissue inflammation (Iba et al. 2020). In addition, the presence of neutrophils at high values in blood and lung tissue due to SARS-CoV-2 presence can promote inflammatory damage in target organs (Tay et al. 2020, Wang et al. 2020b).

Host genetics as well as changes in immune status or environment can promote different illnesses. The polymorphism analyses of individuals in this study aimed to understand if genetic conditions, such as upregulation or downregulation of cytokines, could promote a specific immune response (McAleer & Kolls 2014). Our results showed that occurred no genetic

change in the analyzed genes (IL-1β, IL-6, IL-10, IL-17A, and TNF-α), and, possibly, the inflammatory findings of healthcare workers were specifically associated with the virus exposition.

Interestingly, when we associated the laboratory findings with the immunological response (by immunophenotyping and immune pathways), we can observe an inflammatory status in the positive healthcare workers, triggered by high production of IL-2, IL-6, TNF-α, and IL-17 associated with highlighted Th2/Th17/Th22 response.

Our results showed a high prevalence of NKT cells in the blood of positive healthcare workers. Those cells are involved in antiviral responses, helping to control viral load, and boosting the innate and adaptive immune response (Diana & Lehuen 2009, Vogt & Mattner 2021). Beyond, activated NKT cells can rapidly secrete high amounts of Th1, Th2, Th10, or Th17 polarizing cytokines and chemokines (Constantinides & Bendelac 2013, Sag et al. 2014, Cameron & Godfrey 2018). However, NKT cells aberrant activation can also perpetuate tissue damage (Mattner 2013) such as observed in COVID-19 patients.

The IL-17 cytokine, found in high values in positive healthcare professionals, also must be highlighted due to its immunological mechanisms in respiratory illness (promoted by allergic reactions or exogenous antigens). In asthma, IL-17 mediates neutrophil recruitment to the lungs, in COVID-19 promotes viral persistence by protecting virus-infected cells from apoptosis and can induce hyperinflammation (Megna et al. 2020, Peiser 2013, Sadeghi et al. 2021). In experimental and human models, Th17 cytokine is upregulated in laboratory-confirmed Influenza (H3N2) and Parainfluenza virus, indicating severe disease with respiratory involvement, and associated with modulation of inflammation and clearance of influenza infection (Kudva et al. 2011, Bermejo-Martin et al. 2009, Antalis et al. 2019).

Together, these findings are the first evidence set of possible lung inflammation in healthcare workers due to their daily virus exposition. Furthermore, the Th2/Th17/Th22 response is a known allergic response, associated with individuals who present asthma and chronic obstructive pulmonary disease (COPD) leading to an increase in fibrinogen and mucins production in pulmonary tissue (Alcorn et al. 2010, Zenewicz 2018).

CONCLUSIONS

Although this study was performed before the vaccination period, nowadays the Brazilian reality still shows low antigen testing in the population. Moreover, daily a lot of workers use crowded subways and buses to move to their jobs and are exposed to SARS-CoV-2 infection. Likewise, due to the asymptomatic evolution of the disease, the majority of those people do not suspect that may be infected by the virus and, consequently, cause transmission to their patients. Furthermore, recurrent laboratory and immunological investigations, as performed here, can be adopted in healthcare services to help workers (exposed to patients infected with SARS-CoV-2), as prevention therapy in those individuals. Due to the results observed in this study, some investigations should be performed to evaluate if the high exposure to the antigen results in lung tissue inflammation or chronic activation of the immune system in this target group.

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