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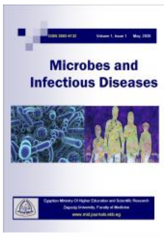

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Original article

The use of folic acid as a prophylaxis against COVID-19 among healthcare workers

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ABSTRACT

Background: Vaccines may be thought of as a reliable intervention to prevent SARS-CoV-2 infection. Prophylactic drugs may be a sound alternative. We aimed to assess the use of folic acid to protect against COVID-19 infection. **Methods:** This randomized controlled study was conducted in an isolation hospital at Cairo University Hospitals on three groups of nurses caring for COVID-19 patients: group I; the control group, group II whose participants received 500 micrograms of folic acid daily, and group III whose participants received 1000 micrograms daily. PCR conversion was tested for the three groups as a primary endpoint. **Results:** In total, 526 nurse were included. Group I comprised 139 nurse, group II comprised 163 nurse, and group III comprised 224 nurses. The rates of PCR conversion from negative to positive were 4.9% (8/163) in group II and 1.8% in group III (4/224) compared with 14.4% in the control group I (20/139). Statistically significant (p<0.005) and highly statistically significant (p<0.001) differences respectively were found. **Conclusions:** Prescribing folic acid daily may significantly decrease the risk of COVID-19 infection among exposed healthcare workers.

Introduction

Coronavirus has claimed the lives of more than million people worldwide at the time of writing these lines, and this death toll continues to rise rapidly daily. Before the introduction of COVID-19 vaccines, nonclinical preventative measures have been implemented as the principal means of limiting deaths. However, these measures could not stop the unprecedented disruption to daily lives and economic activity [1]. Although the most important pharmacologic interventions to prevent SARS-CoV-2 infection are likely to be vaccines, the

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repurposing of established drugs for short-term prophylaxis is another, more immediate option [2]. Some researchers have promoted chloroquine and hydroxyl-chloroquine for the treatment of COVID-19 [3]. Hydroxychloroquine can inhibit the replication of SARS-CoV-2 *in vitro* [4]. Although some observational studies have suggested the benefits of hydroxychloroquine in treatment of COVID-19, other reports have described contradictory results [5]. Ivermectin, which is a broad-spectrum antihelminthic with antiviral activities as well as other naturally used supplements, such as vitamin C, zinc and other supplements, have been suggested for prophylaxis of SARS-CoV-2 infection. Apart from Ivermectin which acts by decreasing the transport of the virus to the human cell nucleus, the mechanism of action of the above-mentioned medications and/or supplements in the treatment of COVID-19 patients was largely empirical and has not been studied academically [6]. Additionally, many public and scientific concerns about the safety of the vaccines being developed rapidly have been raised in addition to concerns about their efficacy and the durability of the immunity they are supposed to confer.

In addition to the aforementioned options, epigenetic approaches have been attempted successfully in the control of some virus infections such as AIDS, CMV and hepatitis viruses infections [7]. Epigenetics is the study of the modifications of gene expression that are not due to mutations or changes in the genetic sequence [7]. COVID-19 damages the immune system and organs through epigenetic and methylation pathways [8]. The epigenetic analysis conducted *by* **Menachery et al** reported that DNA methylation, rather than histone modification, plays a crucial role in MERS-CoV-mediated antagonism of antigen presentation gene expression [9]. Folic acid has been used to correct methylation defects through epigenetic pathways in preventing neural tube defects, such as spina bifida, and is recommended by the FDA to be taken as 600 micrograms daily in pregnant females, confirming the safety of the medication. It is also used to prevent precancerous colonic lesions such as polyps in addition to other therapeutic uses [10]. A recent study in Iran suggested using folic acid to prevent and treat COVID-19 cases and proved that it decreases the intracellular transport of the virus through a transmembrane protein called furin [11]. However, the researchers did not suggest the potential role of folic acid in the epigenetics of COVID-19 through the DNA methylation pathway, which is suggested in this work.

In the present study, the primary end point was to study the efficacy of folic acid as a chemoprophylaxis against COVID-19 among healthcare workers caring for COVID-19 patients in an isolation hospital in Cairo, Egypt. The second end point was to determine the best of the two used doses in this study: “500 and 1000 micrograms”.

Subjects and Methods

Study design

This was a cluster randomized controlled study performed from May 17th 2020 until June 30th 2020, during the peak of the COVID-19 pandemic in Egypt.

In total, 526 nursing staff members (nursing supervisors, nurses and nursing assistants) were included in the study. They were distributed over three intervals (waves), each of which had a 15-day duration.

Information and consent

All the participants consented for the trial and agreed to participate.

Trial Registration: The study was approved by Pan African Clinical Trial Registry (www.pactr.org). Unique identification number for the registry is PACTR202005599385499. The study was also approved by the Ethical Review Committee of Faculty of Medicine, Cairo University.

Setting

The new Kasr Alainy Teaching Hospital, one of Cairo University Hospitals, comprises 12 floors and approximately 800 beds.

During the pandemic, it served as an isolation center for COVID-19 cases. All health care workers in areas occupied by COVID-19 patients were instructed to wear full personal protective equipment (PPE). The PPE included coverall suits, filtrating facepiece respirators [N95, KN95 or FFP2 mask], and 2 nonsterile surgical gloves in addition to protective glasses or a face shield. On floors designated for the housing of health care workers with no patient contact, the health care workers were allowed to wear only surgical masks and adhere to social distancing.

Personnel

Health care workers, including nursing staff (nursing supervisors, nurses and nursing assistants), were admitted to isolation every two weeks.

All the nursing staff attended a lecture on the principles of infection control before starting the isolation period.

Inclusion criteria

- Age between 20 and 60 years.
- Male or non-pregnant, non-lactating female.
- Availability for follow up by phone.
- Willing to participate and provide verbal informed consent.

Exclusion criteria

- Subjects with a history of comorbidities (e.g., DM, hypertension, cardiac or respiratory diseases).
- Current symptoms of fever, cough, or shortness of breath.
- PCR-confirmed positive test of COVID-19.

PCR testing protocol using nasopharyngeal swabs

Swabs were performed from all participants before entering isolation, and individuals who tested positive were not allowed to work in the isolation hospital.

Swabs taken from the departing team were performed at the end of their last shift before leaving to detect those who acquired infection. All the teams were further home isolated for 14 days after leaving the isolation hospital before restoring their healthcare activities in the non-isolating settings at Cairo University hospitals.

According to the policies of the Egyptian Ministry of Health at the time of the study, all health care workers were given a prophylactic dose of hydroxychloroquine as follows: two hydroxychloroquine 200 mg tablets twice at the start of the isolation period, followed by one hydroxychloroquine 200 mg tablet once weekly for two weeks from the last date of exposure.

Folic acid

Folic acid was given to the study participants according to this protocol:

Group 1 comprised 139 nursing staff who served as a control group and did not receive folic acid.

Group 2 comprised 163 nursing staff who received folic acid (Mepaco, Medifood) 500 mcg dietary supplement from day 1 in the isolation period for 30 days.

Group 3 comprised 224 nursing staff who received folic acid (Mepaco, Medifood) 1000 mcg from the day 1 in the isolation period for 30 days.

Randomization

Each wave of health care workers was randomized for each group during its given time (with 14 days of isolation in the hospital while working) using the closed envelope technique. We used cluster randomization of our study because randomizing the intervention among the participants was more important than randomizing the participants from our point of view.

Statistical methods

The data were coded and entered using the SPSS statistical package and were summarized using the mean, standard deviation, median, minimum and maximum in quantitative data and frequency (count) and relative frequency (percentage) for categorical data. Comparisons between quantitative variables were performed using the non-parametric Kruskal-Wallis and Mann-Whitney tests. To compare categorical data, the chi squared ($\chi 2$) test was performed. The exact test was used instead when the expected frequency was (Mepaco, Medifood) less than 5.

Correlations between quantitative variables were performed using the Spearman correlation coefficient. *p-values* less than 0.05 were considered statistically significant.

Results

During the period from 17/5/2020 until 30/6/2020, 526 nursing staff were included in our study. Of which, 338 (64.3%) were females and 188 (35.7%) were males, with a mean age of 37.5 years ± 8.85. The youngest was 20 years old, and the oldest was 57 years old. PCR was performed at baseline for all. The participants were divided into group I (control; n=139), group II (n=163 who received 500 mcg of folic acid), and group III (n=224 received 1000 mcg of folic acid). Each group worked for two weeks continuously at the isolation hospital, and PCR was performed at the end of the period for all participants. **Tables 1 & 2** shows the demographic distribution among the 3 groups with no significant difference regarding age or sex (*p-value* for age was 0.40 and that for sex was 0.047).

Participants of the control group; group 1 had the highest rate of PCR conversion where 20 participants converted to PCR positive out of 139 nurse (14.4% conversion rate).

Participants of group II, who received 500 mg of folic acid, had a significantly lower PCR conversion rate than group I where only 8 participants out of 163 got the infection (4.9%). Difference was found to be statistically significant (*p-value* = 0.005) **(Table 3)**. Relative risk reduction for subjects of this group was 0.66. Those who took folic acid (500 mcg) had a 66% lower risk for getting SARS-CoV-2 infection than controls.

Participants of group 3, who received 1000 mg of folic acid, had a much lower PCR conversion rate. It was found that PCR conversion was found only among 4 out of 224 participant (1.8%). Difference was statistically highly significant (*p-value* <0.001) **(Table 4).** Relative risk reduction for subjects of this group was 0.87. Those who took folic acid (1000 mcg) had an 87% lower risk for getting SARS-CoV-2 infection than controls.

Table 1. Age distribution among studied groups.

	N	**Mean**	**Std. Deviation**	**Minimum-maximum**	**Range**	***p* value**
Group I	139	37.1	8.36	20-56	36	0.40
Group II	163	37.0	9.05	20-54	34	
Group III	224	38.12	9.0	20-57	37	
Total	526	37.5	8.85	20-57	37	

Table 2. Sex distribution among studied groups

Groups			**Sex**		**Total**	***p* value**
			F	**M**		
	Group I	**N**	98	41	139	
		%	70.5%	29.5%	100.0%	
	Group II	**N**	109	54	163	0.047
		%	66.9%	33.1%	100.0%	
	Group III	**N**	131	93	224	
		%	58.5%	41.5%	100.0%	
Total		**N**	338	188	526	
		%	64.3%	35.7%	100.0%	

Table 3. Comparison of PCR results among group I and group II.

Groups			**PCR**		**Total**	***p* value**
			negative	**positive**		
	Group I	**N**	119	20	139	
		%	85.6%	14.4%	100.0%	
	Group II	**N**	155	8	163	0.005
		%	95.1%	4.9%	100.0%	
Total		**N**	274	28	302	
		%	90.7%	9.3%	100.0%	

Table 4. Comparison of PCR results among group I and group III.

Groups			PCR		Total	*p* value
			negative	positive		
	Group I	N	119	20	139	
		%	85.6%	14.4%	100.0%	
	Group III	N	220	4	224	<0.001
		%	98.2%	1.8%	100.0%	
Total		N	339	24	363	
		%	93.4%	6.6%	100.0%	

Discussion

The coronavirus disease 2019 (COVID-2019) outbreak originating in December 2019 in Wuhan, China, has emerged as a global threat to human health. Highly contagious SARS-CoV-2 infection and transmission endangers diverse human hosts and increase disease risk with advancing age, highlighting the importance of an in-depth understanding of its biological properties [8].

In July 2020, WHO warned that up to 10,000 health care workers (HCWs) were infected with COVID-19 in Africa [12]. In a study performed in Washington State in the USA from March 12, 2020, to April 23, 2020, 3477 symptomatic employees were tested for COVID-19 PCR at two employee testing centers; 185 (5.3%) employees tested positive for COVID-19. The prevalence of SARS CoV-2 infection was similar when comparing frontline HCWs (5.2%) with non-frontline staff (5.5%) [13].

Epigenetics is the study of the modifications of gene expression that are not due to mutations or changes in the genetic sequence. Epigenetic approaches have been attempted successfully in the control of many viruses, such as HIV, CMV and hepatitis viruses [7].

SARS-CoV-2 damages the immune system and organs through epigenetic and methylation pathways. A recent study highlighted the relationship between the methylation of two important genes that play a pivotal role in the pathophysiology of COVID-19. The first is the ACE 2 gene responsible for the production of ACE, which serves as a receptor for SARS-CoV-2. The second is the interferon gene, which encodes a crucial inflammatory cytokine. Increased interferon production can explain the dilemma of cytokine storm observed among these patients. The authors postulated, based on many lines of available clinical evidence, that hypermethylation of both ACE 2 and interferon genes and thus their decreased expression, is protective against COVID-19 and vice versa [8].

However, current evidence supports the idea that viruses use epigenetics to modulate host cell susceptibility to infection through antagonizing host innate immune mechanisms and antiviral defense programs to enhance viral replication and infection efficiency [9].

In 2012, **Creder et al.** stated that "DNA methylation is an epigenetic modification critical to normal genome regulation and development. The vitamin folate is a key source of the one-carbon group used to methylate DNA. Because normal mammalian development is dependent on DNA methylation, there is enormous interest in assessing the potential for changes in folate intake to modulate DNA methylation both as a biomarker for folate status and as a mechanistic link to developmental disorders and chronic diseases including cancer" [10].

The function of DNA methylation occurs through cytosine methylation, which has been hypothesized to be an ancient component of the immune system designed to recognize and inactivate parasitic viral DNA sequences that infiltrate the genome [14].

To our best knowledge, none of the published papers till the date of writing these lines elucidated the role of folic acid as a prophylactic agent against COVID-19 among healthcare workers assuming its methylation activity and epigenetics. However, a group of researchers from Iran have postulated a very interesting hypothesis assuming a protective role of folic acid against COVID-19 through a molecular dynamic simulation study. The researchers concluded that both folic acid and its metabolite folinic acid can inhibit the action of furin; a protease enzyme that is essentially required to cleave the spike protein of SARS-CoV-2. This cleavage is a preliminary step to the virus entry into the cells [11].

Our preliminary results suggested a protective role of folic acid among healthcare workers who were subjected to a high viral load being on duty in the frontlines in an isolation hospital belonging to Cairo University hospitals.

In the present study, we demonstrated a relative risk reduction that ranged between 66% and 87% based on 500 and 1000 mcg daily supplementation, respectively.

Accordingly, we recommend the use of folic acid in a 500–1000 microgram daily dose among health care workers for the time of their exposure to COVID-19 patients in addition to the standard infection control measures.

The safety of up to 1000 micrograms of folic acid per day has been revised by NCBI for the general population [13], and a diet that contains a daily amount of folic acid below the established upper intake level of 1000 mcg has not been demonstrated to result conclusively in any adverse health outcomes. Previous concerns, including cognition (related to vitamin B12 deficiency), cancer, diabetes, thyroid-related disorders, and hypersensitivity-related outcomes, were based on the reports of patients who received more than 400 mcg per day. The U.S. National Toxicology Program (NTP) published a report concluding that, for the areas considered, no definitive evidence exists for adverse effects due to folic acid. [15,16] However, they reported rare instances of gastrointestinal upset [17].

In the present study, we were obliged by the Ministry of Health guidelines to use hydroxychloroquine as a prophylaxis for all health care workers in the 3 groups.

None of the nurses who were infected in groups 2 & 3 have experienced severe COVID. Infection was only detected at the end of their isolation rounds upon routine PCR screening. However, no data was available about the condition of the participants of the control group regarding the severity of COVID. Moreover, we were not notified about any side effects or negative interactions between the standard protocol of therapy or prophylaxis and folic acid intake. A point that was beyond the scope og our work.

Conclusions and recommendations

Using folic acid in the mass protection of communities in conjunction with infection control practices may be a step towards protecting HCWs.

We recommend constructing trials to study the role of folic acid in treatment of COVID-19 cases in the ICU. We also recommend studying the effect of folic acid oral supplementation in prophylaxis of influenza type A and B. We finally recommend conducting epidemiologic studies correlating the incidence and severity of the COVID-19 pandemic with daily folic acid consumption in food and drinks such as green leafy vegetables, beans and orange juice as this may elucidate differences observed in the severity of the pandemic the difference between countries.

Declarations

Ethical committee approval: The study was approved by the Research Ethics Committee of Cairo University as well as the Pan African Clinical Trial Registry.

Competing interests: The authors declare that they have no competing interests.

The study was funded by Cairo University Hospitals.

Authorship

All authors have made contributions to the following: (1) the conception and design of the study and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, (3) final approval of the version to be submitted.

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Conflicts of interest: None.

Financial disclosure: None

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