RESEARCH ARTICLE

REVISED Distribution of *Toxoplasma gondii* IgM and IgG antibody seropositivity among age groups and gestational periods in pregnant women [version 3; peer review: 2 approved]

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Abstract

Background: Toxoplasmosis is a globally distributed parasitic disease. The present study aimed to estimate the prevalence and geographic distribution of toxoplasmosis as well as determine the percentage of toxoplasmosis-associated IgM and IgG seropositivity among different age groups. In addition, it aimed to estimate the proportion of toxoplasma IgM seropositivity among pregnancy trimesters.
Methods: A total of 500 pregnant women were included in this study. From each participant, a 5-ml venous blood sample was collected and centrifuged to obtain serum that was tested for Toxoplasma gondii IgM and IgG antibodies using immunochromatographic testing and ELISA.
Results: The overall seroprevalence of toxoplasmosis was 24.8%. Out of the total of 500 participants, only 8% had a serological marker of acute toxoplasmosis). There is a statistically significant difference in the seroprevalence of disease among the study areas. Amongst positive cases of every trimester, 54.34% of first trimester positive cases had a serologic marker for acute toxoplasmosis.
Conclusions: In this study, there is a high prevalence of toxoplasmosis. Therefore, it is necessary to test every pregnant woman for toxoplasmosis and distinguish the type of infection, as well as the conduction of public health education programs to generate the awareness.

Keywords

Toxoplasma gondii, Toxoplasmosis, Seroprevalence, IgG, IgM, Pregnant women

Open Peer Review

Reviewer Status ✓ ✓

	Invited Reviewers	
	1	**2**
REVISED **version 3** published 18 Jun 2019	✓ report	✓ report
REVISED **version 2** published 25 Mar 2019	✓ report	? report
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Any reports and responses or comments on the article can be found at the end of the article.

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Author roles: Sadiqui S: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Project Administration, Resources, Software, Validation; **Shah SRH**: Data Curation, Formal Analysis, Investigation, Methodology, Project Administration, Writing – Review & Editing; **Almugadam BS**: Software, Writing – Original Draft Preparation, Writing – Review & Editing; **Shakeela Q**: Data Curation, Methodology, Project Administration; **Ahmad S**: Data Curation, Methodology, Project Administration, Supervision, Writing – Review & Editing

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REVISED **Amendments from Version 2**

In response to reviewer, we have removed the animal contact part from the abstract, results, discussion, conclusion and dataset. Consequently, authors made minor changes in some words and sentences of the abstract, discussion, and conclusion. Additionally, we changed the title for the dataset.

See referee reports

Introduction

Toxoplasmosis is a widely distributed zoonotic illness causes by *Toxoplasma gondii*, an obligate intracellular parasite[1,2]. Globally, the distribution of this disease is extremely variable even inside the countries[3,4]. In all host species, including humans, Toxoplasmosis is generally acquiring either vertically from mother to fetus (congenital infection), or through ingestion of oocysts in contaminated food or water[5]. Rarely, *T. gondii* can transmit through organ transplantation and the transfusion of infected blood[6,7]. Following ingestion, the intestinal epithelium is the primary portal of entrance for *T. gondii*; next, it spreads to other tissues, where it can cause more severe pathogenesis[8,9]. If toxoplasmosis is acquired during pregnancy, severe infection may develop, especially in immunocompromised individuals, such as those with defects in T-cell-mediated immunity[10]. In patients with AIDS, toxoplasmosis may lead to life-threatening disease[11]. For example, cerebral focal lesions are caused by cerebral toxoplasmosis (CT) in HIV-infected patients[12].

The signs and symptoms of this illness are markedly divergent and range from asymptomatic to serious infection[13]. This variation depends on several factors includes inoculums size, virulence of the strain of toxoplasma, the individual's genetic background and the status of the immune system of the infected individual[14]. In addition, since the organism has an affinity for muscular and neural tissues as well as the other visceral organs, many hosts harboring latent tissue cysts following toxoplasmosis[15].

Fetuses may acquire toxopasmosis through the placenta during pregnancy[16]. Early infection of the fetus may cause severe damage, or either pre- or post-natal death[17]. The clinical manifestations of congenital toxoplasmosis generally depends on the gestational stage, and can include seizures, mental retardation, severe neurological defects, chorioretinitis, epilepsy and blindness[10,16,18].

Approximately 90% of pregnant women infected with *T. gondii* are asymptomatic, and recover spontaneously[19,20]. Only a small percentage of pregnant women show the clinical symptoms of disease[19,21]. In pregnant women, the clinical signs are no more severe than in non-pregnant women, and typically an influenza-like illness is seen after an incubation period of 5 to 18 days[19,22,23]. Early diagnosis and treatment of mothers during pregnancy prevents fetal infection and minimizes the probability of complications[24,25].

Laboratory diagnosis of toxoplasmosis is usually performed by serological detection of *T. gondii*-specific IgG and IgM antibodies[26]. Worldwide, the screening of *T. gondii* infection in pregnant women is preferably performed during the first trimester and subsequently every month or trimester in seronegative women, as applied in many countries[27].

Our study was undertaken to determine the prevalence and geographic distribution of toxoplasmosis as well as to estimate the seropositivity of toxoplasma antibodies among different age groups. It also attempted to identify the percentage of toxoplasma IgM seropositivity (indicative of acute infection) among different pregnancy trimesters.

Methods

This a descriptive cross-sectional hospital-based study carried out in the District Head quarter Hospital (Mansehra, Hazara, Pakistan) and Ayub Medical Complex Hospital (Abbottabad, Khyber Pakhtunkhwa, Pakistan) over a period of 4 months (April to July 2015).

Study population and sample size

Our study included pregnant women of different trimesters, ages and ethnic groups who visited our study areas hospitals; the only eligibility criteria were pregnancy and visiting the hospitals in our study area. Patients were recruited by the researchers face-to-face. During this study duration, a total of 500 pregnant women (convenience sample) fulfilled the inclusion criteria. Out of the total of participants, 204 were recruited from Abbottabad and 296 from Mansehra district.

Laboratory analysis

A total of 5 ml venous blood was collected from each participant using a sterile syringe and transferred to a blood container without anticoagulant, allowed to clot at room temperature for 15 minutes, then centrifuged at 3000 rpm for 10 minutes to obtain serum, which was transferred into a 1.5ml microcentrifuge tube and stored at −80°C for further analysis. In this study, every sample was screened and confirmed for toxoplasmosis through the serological tests.

Screening

All sera samples were screened for *T. gondii* IgG and IgM antibodies using Rapid Diagnostic immunochromatographic test (Tox IgG/IgM Rapid Test Dip strip, CTK BIOTECH, San Diego, USA) according to manufacturer instructions. In order to avoid false-positive results due to the incomplete specificity of the screening test, every positive sample was further subject to confirmation step by ELISA. Each positive individual also answered a questionnaire concerning their age, trimester and whether they had been in recent contact with animals (Supplementary File 1).

Confirmation

Following the screening, all the positive samples (n=150) were further confirmed to toxoplasmosis using IgM and IgG ELISA kit (Monobind, San Diego, USA) according to the manufacturer protocol. The positive ELISA test for *T. gondii* IgG titers indicates the chronic infection, whereas with high IgM titers indicate the recent or acute infection. All ELISA tests were performed in triplicate.

Ethical statement

Our study was approved by the Ethics Review Committee of Hazara University. Further approval was provided by the administration of Ayub Medical Complex Hospital. From every participant, written informed consent was obtained for conduction of the study. In addition, all the performed steps in this study were completely in accordance with the Helsinki Declaration and the rules defined by the World Medical Association, including samples collection and processing.

Statistical analysis

The obtained results were analyzed by Graph Pad Prism 5 (Graph Pad Software, La Jolla, CA, USA). A χ^2 test was involved to check the statistical differences in seropositivity and negativity of anti-toxoplasma antibodies among the participants of different study areas and gestational periods, at 95% level of significance. Moreover, ANOVA has tested the statistical difference of these antibodies among the participants of every age group. The difference was considered statistically significant when $P < 0.05$.

Results

Seroprevalence of toxoplasmosis

Out of 500 women, using ELISA the overall seroprevalence of toxoplasmosis was 24.8% (124/500). Statistically significant differences were observed between the seroprevalence of disease in Abbottabad and Mansehra district (Figure 1). In addition, the prevalence of toxoplasma antibodies among pregnant women revealed out of the total of 500 participants, only 8% had a serological marker of acute toxoplasmosis (Figure 2).

Toxoplasma antibodies seropositivity among age groups and gestational periods in overall positive cases

Among the positive cases (n=124), the seropositivity of toxoplasma antibodies was shown to be statistically significant different among different age groups (Table 1). There was also a statistically significant difference in the seropositivity of toxoplasma IgM (indicating acute infection) between different gestational trimesters, the highest level of IgM seropositivity was observed in first trimester (54.34%) (Figure 3).

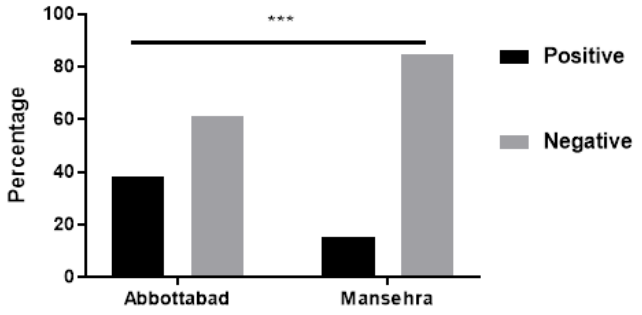

Figure 1. Seroprevalence of toxoplasmosis in different districts. Out of the total of participants in every district, 38.7% (79/204) had the serologic marker of toxoplasmosis in Abbottabad district and 15% (45/296) in Mansehra. ***P = 0.0002.

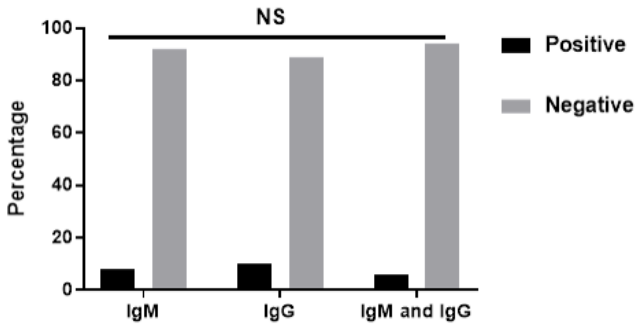

Figure 2. The overall prevalence of Toxoplasma IgM (acute infection) and IgG (chronic infection). Out of 500 pregnant women, 8% (40/500) were positive to IgM, 10.8% (54/500) to IgG, and 6% (30/500) to both antibodies. P = 0.567.

Table 1. Percentage of *Toxoplasma gondii* antibodies seropositivity among the total of positive cases in every age group.

Age, years	Positive cases	IgG	IgM	IgG and IgM
17–24	46	43.5% (20/46)	32.6% (15/46)	23.9% (11/46)
25–32	54	40.7% (22/54)	35.2% (19/54)	24.1% (13/54)
33–40	24	50% (12/24)	25% (6/24)	25% (6/24)
P value		0.003		

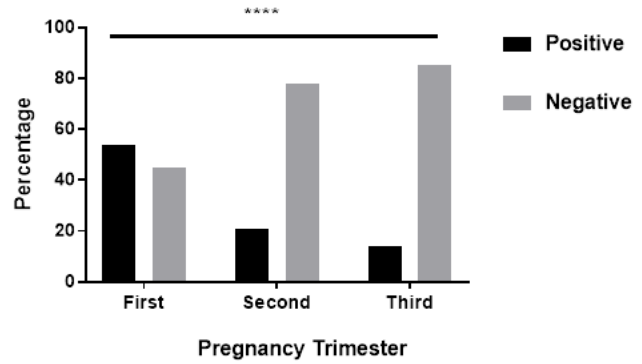

Figure 3. Percentage of IgM seropositivity among the total of positive cases in each pregnancy trimesters. Among the total of positive cases in every trimester, the seropositivity of IgM revealed statistically significant difference. Out of 46, 51, and 27 toxoplamosis infected cases in a first, second and third trimesters, respectively, 54.34% (25/46) were seropositive to IgM (acute infection) in first trimester, 21.56% (11/51) seropositive to IgM in second trimester, and 14.81% (4/27) seropositive to IgM in third trimester. ****P = 0.0001.

Dataset 1. The raw data associated with this study. Excel file includes the results of screening (ICT) and confirmatory tests (ELISA), plus the pregnancy trimesters of toxoplasmosis positive cases

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Discussion

Toxoplasmosis in pregnancy can predispose the fetus to serious complications[28]. The fetus can be severely damaged when the infection is acquired during pregnancy[29]. Therefore, testing the serum of pregnant women for toxoplasma IgG and IgM is important to avoid intrauterine infection and complications. The current study was conducted on 500 blood samples collected from pregnant women in Mansehra and Abbottabad district of Pakistan, and examined for *T. gondii* IgM (acute infection) and IgG (chronic infection) antibodies. Out of the total of 500 pregnant women, 24.8% (124 women) had a serologic marker of toxoplasmosis. Among the 124 positive cases, 54 were seropositive for toxoplasma IgG antibody, 40 cases for Toxo-IgM and 30 cases for both IgM and IgG antibody. In addition, out of 500 participants, 8% had a serologic marker of acute toxoplasmosis. In 2007, Obeed reported the prevalence of IgG (chronic infection) and IgM (acute infection) antibodies were 36% and 26.6%, respectively, which are greater than those seen in our study results[30]. In addition, the seroprevalence of toxoplasmosis in Saudi Arabia was reported as 21.8%[31]. In pregnant women from South Korea, a low prevalence was observed (0.79%)[32], with rates of 20% reported in Finland[33] and 24% in Prague[34]. These findings indicate the prevalence of toxoplasmosis is markedly difference in different countries.

Moreover, our study revealed that the geographic distribution of toxoplasmosis is significantly different among the study areas. Out of the 296 participants analyzed from Mansehra and 204 from Abbottabad, the overall prevalence of toxoplasmosis was 15% and 38.7%, respectively. The higher prevalence in Abbottabad when compared with Mansehra may because Abbottabad is an area where agricultural practices are common, and domestic animals like cats and goats were generally kept in or near the homes. Thus, contact with these animals may be the main risk factor of the disease. In addition, low educational and socioeconomic level may have contributed.

In our study, a high percentage of IgM seropositivity was reported in the 1st trimester, which indicated a high prevalence of acute toxoplasmosis or recent infection in this trimester compared with the others. Furthermore, as reported in this study, there is a mild difference in the seropositivity of toxoplasma antibodies among age groups, which requires further study to assess whether, is there any significant association exists between toxoplasmosis and age.

Usually *T. gondii* does not cause clinical illness in the majority of animal species[35]. Human often acquires this infection from animals by ingestion of improperly cooked or raw animal meat, or via consumption of contaminated food and water with animal's waste[14]. However, there is a need for detailed knowledge about the risk factors of toxoplasmosis. Previously, it was reported that some risk factors are associated with toxoplasmosis, such as owning cats[36]. Additionally, the previous study revealed that that contact with domestic animals may associate with this disease[37,38]. Therefore, the next study studies should evaluate the role of cats contact in disease development.

In this study, a high prevalence of toxoplasmosis was revealed. Moreover, in the first and second trimester of pregnancy, the prevalence of acute toxoplasmosis seems to be higher compare with a third. Thus it is necessary to test every pregnant woman for toxoplasmosis and distinguish the type of infection. In addition, urgent treatment and medicine are essential to decrease the risk of intra-uterine infection and congenital toxoplasmosis. Additionally, there is a need to conduct public health education to create greater awareness about the disease, its transmission, symptoms, and prevention. In addition, screening of *T. gondii* infection and maternal care should be considered as the main stratagem to reduce the risks of congenital toxoplasmosis.

Data availability

Dataset 1. The raw data associated with this study. Excel file includes the results of screening (ICT) and confirmatory tests (ELISA), plus the pregnancy trimesters of toxoplasmosis positive cases.

DOI: **https://doi.org/10.5256/f1000research.15344.d249392**[39].

Grant information

The author(s) declared that no grants were involved in supporting of this work.

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Supplementary material

Supplementary File 1. Study questionnaire

Click here to access the data

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Open Peer Review

Current Peer Review Status: ✔ ✔

Version 3

Reviewer Report 01 July 2019

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✔ **Wafa Babiker**
Faculty of Medical Laboratory Sciences, University of Khartoum, Khartoum, Sudan

It's an acceptable improvement.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 19 June 2019

https://doi.org/10.5256/f1000research.21380.r50028

✔ **Asghar Fazaeli**
Department of Medical Parasitology & Mycology, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran

I read the new version of the Sadiqui *et al.* article. Almost all my comments were addressed and corrections have been made by the authors. So, I can confirm the latest version of the article.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 2

Reviewer Report 08 April 2019

https://doi.org/10.5256/f1000research.20454.r46160

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Asghar Fazaeli
Department of Medical Parasitology & Mycology, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran

The authors' response about association of animal contact and toxoplasmosis is not convincing. The following descriptions may help to this discussion: 1) Cat is the only animal as the definitive host for Toxoplasma gondii which can shed Toxoplasma oocysts becoming infective after a few days in environment; so this is the only animal species with possible association of its contact with toxoplasmosis. 2) Other animals like caw, buffalo, goats, and dogs may harbor the latent parasite (tissue cysts) inside their body tissues and cannot transmit to human or other animal by outer contact. 3) Even if this idea, possible association between these animal spp and Toxoplasma infection, could be proposed, the method of the present study is not suitable for this evaluation, as the study population contained only seropositive women, whereas no seronegative control groups with animal contacts were included in the study. So, the authors' aim to measure the association of these animal contact with toxoplasmosis is not basically scientific and the relevant data and discussion are not acceptable.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 03 April 2019

https://doi.org/10.5256/f1000research.20454.r46159

✔

Wafa Babiker
Faculty of Medical Laboratory Sciences, University of Khartoum, Khartoum, Sudan

I think, it is an acceptable improvement.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 12 February 2019

https://doi.org/10.5256/f1000research.16719.r43385

?

Asghar Fazaeli
Department of Medical Parasitology & Mycology, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran

The manuscript is written in a good structure and presents useful data including the rate of anti-*Toxoplasma* antibodies (IgG and IgM) in sera of pregnant women referred to hospitals of two districts in Pakistan. The manuscript however requires some corrections and modifications as follows:

1. The authors should consider that “toxoplasmosis” is not necessarily “illness or disease” as such they have mentioned in many parts of the manuscript. Toxoplasmosis is an infection which is mostly latent and does not lead to disease or illness in most of immunocompetent individuals. Also, some other issues in the Introduction are scientifically susceptible, e.g. toxoplasma acquisition in fetuses during delivery (mentioned in the 3rd paragraph of the Introduction); to my knowledge, this issue is not documented in the literature.

2. The basis of data arrangement, analysis and discussion about percentage of *Toxoplasma gondii* antibodies in different age groups (Table 1) is not valid. For this issue, all 500 study cases must be primarily divided into different age groups, then the percentage of seropositivity (IgG, IgM, IgG+IgM) in all initial samples of each group should be calculated and consequently compared and analyzed.

3. There is no scientific reason to indicate possible relation between *Toxoplasma* infection and contact with animals like caw, buffalo, goats, and dogs, except for cats which are a definitive host of *Toxoplasma* and shed oocysts. The only way of passing *Toxoplasma* infection from these animals to humans, is ingestion of undercooked meats or rarely through contact of open wounds with meats contaminated with bradyziotes, but not other types of contact with these animals or their wastes. So, this variable (animal contact) is not wise to be included in the manuscript and is recommended to be removed from all parts of the manuscript.

4. Miss-citation is the case for some *Toxoplasma* facts in the manuscript. For example, cerebral toxoplasmosis is referred to Kristiah (2009), which is not the right citation; while it was originally reported and discussed by other researchers, i.e. Luft and Remington (1988[1]) and Luft and Remington (1992[2]).

5. English writing correction is required in some parts of the manuscript text.

References

1. Luft BJ, Remington JS: AIDS commentary. Toxoplasmic encephalitis.*J Infect Dis*. 1988; **157** (1): 1-6 PubMed Abstract
2. Luft BJ, Remington JS: Toxoplasmic encephalitis in AIDS.*Clin Infect Dis*. 1992; **15** (2): 211-22 PubMed Abstract

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 16 Mar 2019

Syed Rafiq Hussain Shah, Hazara University, Mansehra, Pakistan

Dear Dr. Asghar Fazaeli,

Most of your suggestions have been addressed in Version 2, while we didn't address points 2 and 3 due to the following reasons:

1. Because we aimed to estimate the percentage of toxoplasma antibodies seropositivity among positive cases. Therefore, we calculate them by dividing the frequency of Ab seropositivity/Number of positive cases.
2. Because toxoplasmosis is a zoonotic disease, thus it can transmit to humans from animals as we mentioned in Version 1. In addition, in past research, scholars have found that there is an association of cat contact with disease development, while the association of other kinds of animals with disease is poorly understood. Therefore, we aimed to evaluate the role of different kinds of animals in disease development.

Thank you.

Competing Interests: No competing interests were disclosed.

Reviewer Report 10 December 2018

https://doi.org/10.5256/f1000research.16719.r40932

Wafa Babiker
Faculty of Medical Laboratory Sciences, University of Khartoum, Khartoum, Sudan

This article has studied toxoplasmosis, which is considered as one of the most common causes of abortion, stillbirth, intrauterine death, and congenital abnormalities. In addition, it's a globally distributed disease.

The paper is well designed and clearly written; the results were presented accurately and the conclusion is supported by study findings. In my opinion, it's perfect and scientifically acceptable. However for further improvement of the article, the authors should revise the English grammar.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 19 Mar 2019

Syed Rafiq Hussain Shah, Hazara University, Mansehra, Pakistan

Dear Dr. Wafa Babiker,

We have addressed your suggestion in Version 2. Thank you.

Competing Interests: No competing interests were disclosed.