Nigerian
ISSN 0794-1056
Biomedical Science Journal
Nigeria Foremost Journal of Biomedical Practice.
Aug. - Dec., 2022

Nigerian Biomedical Science Journal (NBSJ) is published bi-monthly by
MEDINET COMMUNICATIONS *in association with*
IBMS Publications (UK)
123 Ojuelegba Road, Akintan B/Stop, S/Iere, Lagos.
Email: nbsj50@gmail.com
nigbiomedjournal@yahoo.com
Website: www.nbsj.org.ng
Tel: 0803 697 4364, 0802 359 4150, 0809 631 4346

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Contents

Effects of Malaria Infectivity on Productivity among Patients Attending FCT Hospital Abuja

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ABSTRACT

Background: Malaria is known to contribute to reduction in productivity through absenteeism as worker-hours are lost thus impacting company productivity and performance. This paper analysed the impact and effects of malaria infectivity on productivity among workers through absenteeism.Methods: Three methods were employed for data collection namely: survey, field observation and the use of questionnaire. The questionnaire was designed to enable the researcher to assess and collect the required data in all aspects of effect of malaria on productivity and loss of man hour. Results: The distribution of respondents by sex the sample population is made up of 100 (48%) males and 108 (52%) females. The male makes 48% of the population while the female is 52%. All categories of age are affected by malaria, 33% from age group 4-5, 20% from 21-30, 22% from 31-40, 12% below 20 years and 31 7% from 51 and above. Malaria does not respect age group. From the respondents 204 (98%) respondents indicated that they have had malaria infection, while 4 (2%) said no. Also 58% of the respondents worked for 8 hours daily, 24% worked for 10 hours daily and 18% worked 12 hours daily. 63% of the respondent worked 5 days per week; 29% worked 6 days per week; while 8% worked 7 days per week. Conclusion: Malaria contributes significantly to worker absenteeism. Employers, therefore, ought to put measures that protect workers from malaria infections. Protecting workers can be done through malaria educative campaigns, providing mosquito nets, providing insecticide-treated work suits, providing repellents and partnering with different ministries to ensure protection of workers from mosquito bites.

Key Words:

INTRODUCTION

Malaria, which is a vector-borne disease, has been in the world for over thousands of years. There is hardly any country in the tropics that has not experienced malaria at one point in time or the other. It has therefore become a disease without borders and has become a major source in countries all over the world. The concern by all nations of the world can be appreciated from the background of the fact that malaria causes not only approximately 250 million cases of fever and approximately one million deaths annually but majority of the cases occur in children under 5 years old. Pregnant women are similarly highly vulnerable and liable to infection. The danger is that if the prevalence of malaria remains on its upward course, the death rate from malaria would double to 2 million annually within the next twenty years. Malaria brings to an end either suddenly or gradually, human existence. Malaria causes painful but preventable deaths all over the world. It is no wonder therefore, that governments at all level, and in countries all over the world do not sit down and fold their arms and allow the disease to decimate populations in their territories, (WHO, 2008).

At the international level, an organization, the World Health Organization (WHO) came into existence by the agreement of all United Nations members on the 19th of April 1948. The mandate of the WHO includes "the attainment by all peoples of the highest possible level of health". Its major task is to combat diseases, especially key infectious diseases, and to promote the general health of the people of the world. Hence it has taken seriously the issue of malaria prevention, control and treatment in all member states worldwide. The various experts in the organization have continuously carried out researches and developed treatment methods and drugs for the control and prevention of malaria. The WHO in about two decades ago embarked upon a programme of "Health for all by the year 2000", and one of the serious challenges facing the realization of this ambitious goal for health is no doubt malaria. No wonder the Global Fund for AIDS, Malaria and Tuberculosis was similarly established to promote funding and logistic support for nations in their bid to treat, control and probably eradicate malaria within their territories.

In Nigeria, the Public Health Department was responsible for health issues during the colonial period. They did their best with regards to the issue of malaria. Indeed, it was Britain's Sir Ronald Ross's work in General Hospital in Calcutta that finally proved in 1898 that malaria is transmitted by mosquitoes. Medicines and treatment pattern developed for territories under the British Empire were similarly brought to Nigeria and applied during the colonial period.

Malaria is a major health problem in Nigeria accounting for about 60 percent of out-patient attendance and 30 percent of hospital admission. Malaria transmission is high all year round with estimated incidence rates of 2.5 per person per year being attacked in the age groups 0 to 2 years. Malaria also attacks 1.5 persons per year in the age group of 2-5 years and 1.0 attack per person per year in the age group above 10years. Nigeria received two global fund Malaria Grants (Round 2 and 4) to implement malaria prevention programmes in 18 states out of 36 states including the Federal Capital Territory. The Yakubu Gowon Centre was the principal recipient.

According to the 1999 constitution of the Federal Republic of Nigeria, health has been placed on the concurrent legislative list. By implication therefore, the three tiers of government are vested with the responsibility to promote health and prevent disease.

In the Federal Capital Territory, which is the case study consideration has been placed on the secondary health care. This is in line with the 1999 constitution. Thus, the federal Capital Territory Administration has generally focused on four major areas of health as defined by the WHO namely:

the promotion of public health and vitality; the prevention of infectious diseases as well as injury; the organization and provision of services for diagnosis treatment of illnesses and the rehabilitation of sick and disabled people to the highest possible level of function. Malaria which forms this case study falls under three of the four main areas of concern for public health as enumerated by the WHO.

The Federal Capital Territory has been listed as one of the territories which the Global Fund for the malaria was chosen to be carried out under Malaria Grants (Rounds 2 and 4). The funds are for implementation of malaria prevention programmes. In what ways and to what extent the Federal Capital Territory has benefitted from this programme would be unraveled in this study.

Effect of Malaria on Productivity and Loss of Man hour

Ajani and Ashagidigbi (2008) in their study of effect of malaria on Rural Households' Farm Income in Oyo state, Nigeria, studied 100 respondents using stratified random sampling technique. They found such factors as low level of awareness (56%), use of modern preventive measures (12%), poor sanitation conditions, and large household size (8 persons) as major factors responsible for the high malaria incidence in the rural household. In addition, the increase in malaria incidence had a significant increase in the number of days of incapacitation of an average of 22 days and an income loss of N15,231.50 during the days of incapacitation. The Organization of African Unity (OAU) says that by the year 2000, it would cost African economy $3.6 billion in a year as a result of working hours lost and the cost of treatments. Rural households, unlike the fixed wage earners not only lose valuable working hours in treating the sickness but also lose income that would have been generated at this period. This poor health status thus directly affects the productive capacity of the households. This in turn translates into income loss and eventual poverty through the sick and the caregivers to the households. According to Medical Health (2011), Malaria is not just a disease commonly associated with poverty but also a cause of poverty and a major hindrance to economic development. In countries where malaria is common, they further argued, average per capita GDP has risen (between 1965 and 1990) only 0.4% per year, compared to 2.4% in other countries.

Poverty is both cause and effect. They compared average per capita GDP in 1995, adjusted for parity of purchasing power, between countries with malaria and countries without malaria and found a five-fold difference (1,526 USD versus 8,268 USD). The article concluded that the economic impact includes costs of health care, working days lost in education, decreased productivity due to brain damage from cerebral malaria, and loss of investment and tourism. WHO (2009) concluded that Malaria is the most significant health problem in Nigeria. The economic cost of malaria, arising from cost of treatment, loss of productivity and earning due to days lost from illness, may be as high as 1.3% of economic growth per annum. The study is aimed at evaluating relevant issues pertaining to the effect of malaria on productivity and also examine the impact of malaria on productivity in the Federal Capital Territory.

Materials And Methodology

Three methods were employed for data collection namely: survey, field observation and the use of questionnaire. The questionnaire was designed to enable the researcher to assess and collect the required data in all aspects of effect of malaria on productivity and loss of man hour.

The Study Area

The study area is located between latitudes 4^{o} 45^{1}N and 6^{o} 17^{1}N and longitudes 7^{o} and 8^{o} 10^{1}E. These constitute Federal Capital Territory which is supposed to be developed in four phases, Phase I comprises: Garki, Wuse, Maitama, Central Business District, Asokoro. Phase II comprises of Gudu, Durumi, Wuye, Utako, Jabi, Mabushi, Katampe, Phase III is Gwarimpa and beyond, while Phase IV comprise of Airport

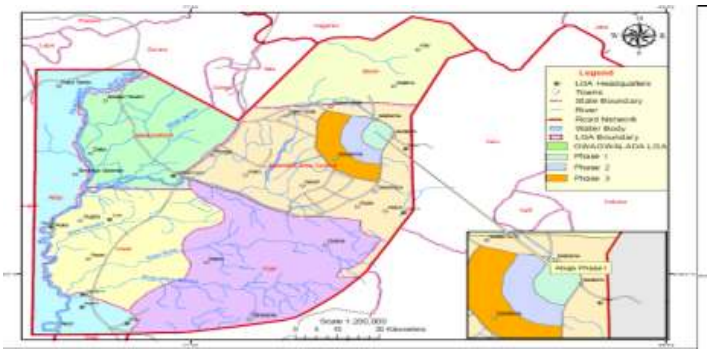


Fig 1.1: Map of Abuja, Showing the study area

Abuja Phase I

Research Design

The study used a sample population to collect primary data using instrument of structured questionnaire on the following: Self-employed that were admitted as in-patients in Maitama Hospital due to malaria.

(a) The next target groups were salary earners (civil servants/ company staff) who were admitted as in-patients in the wards due to malaria.
(b) Another category comprised of salary earners who were treated in the out-patients Department and discharged.
(c) Other groups were working class group who came to the hospital for treatments and complaints not related to malaria.

Sample Selection

In order to make the study comprehensive and to cover all the target groups identified, sample was drawn from three stratified groups. These were: malaria in-patients; malaria out-patients and non-malaria patients.

After the stratification of the samples, each group was randomly selected. The first fifty were selected for administration of questionnaire.

Randomly selected were the in-patients who have been classified into two groups namely: self-employed patients; salaried workers.

The Study Population

The population were patients who attended Maitama District Hospital, whose laboratory diagnosis showed positive parasitemia. They were divided into three categories.

The first target populations were patients who suffered malaria and were admitted into the ward of the hospital for treatment.

The second target groups were malaria sufferers who came for the treatment and were seen at the Out-Patient Department (OPD) and were not admitted into the wards.

The third category comprised of other patients who came to the hospital and were sent to the laboratory for ailment other than malaria. They were suffering from other diseases which they came to Maitama District Hospital to consult a doctor.

All the three categories were working class people. Some were salary earners, while others were self-employed people.

Determination of Sample Size

The population of this study is a finite one. This makes it possible to apply Yamane (1964) formula for determining sample size from a finite population. Onwe (1988) supports this principle.

formula states that

N = N

1 + N (e) 2

Where

n = Research population size

N = Population of the Study

I = Statistical Constant

E = Maximum margin of error at 0.125% level of confidence

The researcher decided to distribute the same size according to the strata of the population using the principle of

RESULT

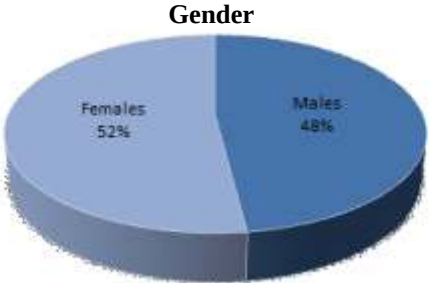
Table 1. AgeDistribution of Respondents

Below 20 years	**25**	**12**
21-30	55	26
31-40	45	22
41-50	68	33
51 and above	15	9
Total	**208**	**100**

Sex Distribution of Respondents

Figure 2. The distribution of respondents by sex

The sample population is made up of 100 (48%) males and 108 (52%) females. The male make 48% of the population while the female is 52%.



Educational Qualification of Respondents

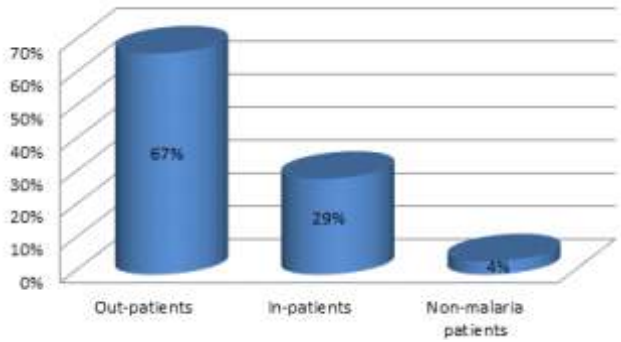
Table 3. Educational qualifications of respondents

Qualification	Number	Percentage (%)
School certificate and below	56	27
OND/NCE or equivalent	50	24
AHND/BSC or equivalent	54	26
MSc or equivalent	37	8
Ph.D	11	5
Total	**208**	**100**

In-patient / Out-patient category in the Hospital

Three categories of patients were involved in the study namely: 140 out-patients (67%); 60 in-patients (29%) and non-malaria patients (4%).

Figure 4.2: The malaria treatment category of the respondents in the hospital



Number of Hours and Days Respondents work

Hours	Number	Percentage (%)
8 hours	120	58
10 hours	50	24
12 hours	38	18
Total	208	100

Days per week	Number	Percentage (%)
5 days	130	63
6 days	60	29
7 days	18	8
Total	208	100

4.10 **If employed: salary per hour, day, week, and month**

Respondents who were salary earners gave their earnings per month, week, days and hour and these are analyzed in table 4.8. Also for senior staff, an average of N130,000 was considered, the middle income salary was N60,000, while the junior staff salary taken was N10,000 per month. 30 days a month, 7 days a week and 24 hours per day were considered as yard stick for the calculations.

Table 4 Average salary per month/week/day/hour

Period	Junior Staff ₦.k	Middle Staff ₦.k	Senior Staff ₦.k
Per hour	13.88	83.33	180.55
Per day	333.33	2,000.00	4,333.33
Per week	2,500.00	15,000.00	32,500.00
Per month	10,000	60,000.00	130,000.00
Total Respondents	150		

4.11**Income for self-employed patients**

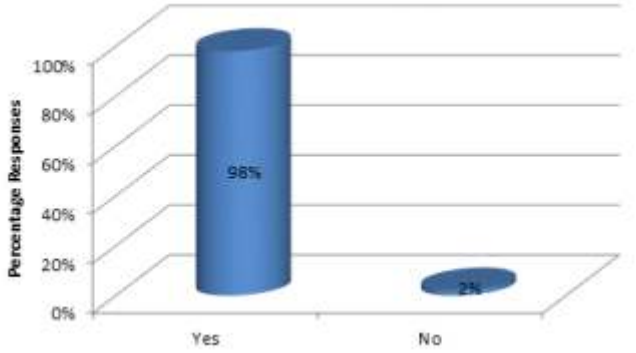
Table 5 The income for the self-employed patients are analyzed

Category	Salary per Month (₦.k)	Salary per Week (₦.k)	Salary per Day (₦.k)	Salary per Hour (₦.k)
Low income	50,000	12,500	1,666.66	69,44
Middle income	100,000	25,000	3,333.33	138.88
High income	200,000	50,000	8,666.66	277.77
Total number of 1respondents	58			

4.12 **Malaria and respondents**

204 (98%) respondents indicated that they have had malaria infection, while 4 (2%) said no. The results are shown in the figure 2 below.

Figure 4.3: Responses to infection by Malaria



Productivity/output of work lost to malaria

Productivity/ Output lost	Number	Percentage (%)
10	86	42
20	29	29
30	58	14
40	13	8
50	10	5
60	4	2
Total	**200**	**100**

Man hour lost this week to malaria

On the number of man hour lost their week to malaria responses received are in table

Man hour	Number	Percentage (%)
20	66	33
30	42	21
40	46	23
50	24	12
60	22	11
Total	**200**	**100**

Percentage of man-hour loss incurred as a result of their malaria

Respondents were asked the percentage of man hour loss as a result of this malaria and their responses.

Percentage of man hour loss	Number	Percentage (%)
Less than 20	35	18
20-30	58	29
31-40	44	21
41-50	30	15
51-60	22	11
Above 61	11	6
Total	**200**	**100**

Level of productivity/output in place of work before malaria attack

Respondents were asked to provide their level of productivity/output in their place of work before the malaria attack and their answers are in table 4.33

4.33 Level of productivity/output in place of work before malaria attack

Level of productivity/output	Amount
Average per hour	1,000
Average per week	
Total	**70,000**

Discussion of Finding

The study on the effect of malaria on productivity focused on patients in Maitama District Hospital, Abuja. It examined five different relevant issues to malaria and productivity namely: Malaria treatment, cost of treatment, diagnosis, prevention and control measures, productivity and man hour loss due to malaria and the Roll Back Malaria programmes and their effects.

From the research finding, it is quite clear that malaria affects two critical issues as far as the working population is concerned. These are loss of man-hour and productivity. The study found that there is a positive correlation between loss of man-hour and productivity as far as malaria was concerned.

Characteristics of Respondents

The Study found that malaria affects people of different ages, sex, educational backgrounds, salary-earners and self-

employed people. There is no group that is left out. It affects both junior and high management personnel. Indeed, the study found that malaria is no respecter of persons, male, female, rich or poor. It attacks whoever is available without fear or favour.

Malaria Treatment Cost

The study found that different levels of cost were incurred for the treatment of malaria by the patients selected for the study. These costs involved: transportation to the hospital; consultation fee; laboratory test fees; cost for medication prescribed by the doctors; and cost for bed and admission fees for those patients who were admitted for malaria. The study found that for out-patients, depending on the medication prescribed by the doctors, the cost at Maitama District Hospital could be up to N3,500 (Three Thousand Five Hundred Naira). For those on admission, the costs are relatively higher because it depends on number of days they spent on admission and further treatment. The study found that those on admission could pay for the out patients, the study also found that the 160 patients spent N560,000, while for the in-patients, the total 60 of such patients could be with N456,000 (Four Hundred and fifty six thousand naira). These treatment cost did not involve other vitamin supplements and other essentials which they may be advised to buy outside the hospital and use to help them recover quickly. The study therefore found that Malaria which affects virtually everybody in the population is becoming expensive to treat. For example the estimated N3,500 spent by these out-patients to treat malaria in this hospital for just one day is ever higher than the income of many Nigerians. Hence they would rather go to pharmacists/chemists to treat themselves or at the worst do self-medication instead of going to the hospital to receive proper treatment against malaria.

Man-hour/days lost due to malaria

The study found that it is not only money that is required for proper treatment in the hospital that is used by the patient. Another source of loss found in this study is man-hour and can even last days, when the malaria persists or even becomes recurrent. The loss of time can be classified into two namely; loss of time spent in Maitama District Hospital to access treatment and loss of days thereafter before the patients could fully recuperate.

The study found that the least time they lost in the hospital while trying to access treatment was 1 hour. For most of the patients, who are even out-patients, they could spend up to 5 hours in the hospital for treatment. While those who were admitted into the wards could spend between 3 to 5 days before they are discharged. Some even spent 2 weeks as found in the study.

The second loss could be assessed in terms of number of days in which the patents suffering from malaria lose. For the in-patients, the study found that they could lose between one day and 2 weeks in the wards. While for the out-patents, they could lose between 3 to 10 days. Indeed, they may lose extra days because they have to come back to get their test results and see the doctors who will have to review their malaria situation. Sometimes, further laboratory investigations could be prescribed and the patient may still have to lose further time. The percentage of man-hour loss incurred from malaria attack varied from less than 20% to above 61%.

Loss of income due to malaria

The study found that it was not just man-hour and days that the patients lost, but they equally lose income. This has a much more serious effect on them especially if they were self-employed. While for salary earners, it is their employers that incur loss, especially if they are unable to go to work and perhaps a temporary hand is either hired to cover their schedule. If no temporary hand could be hired, their role in the whole production process may be lost, thereby affecting the overall income/revenue especially if it is a manufacturing outfit.

The study found that for the junior staff category, they lost N13.88 per hour; N333.33 per day out, N2500 per week and N10,000 per month. While for the senior management category, they lost N180.55 per hour, N333.33 per day, N32,500 per week and N130,000 per month. All these losses when considered together are quite enormous.

For the self-employed patients, the income the loose includes:

(a) For junior/hour-income, N69.44 per hour, N1,666.66 per day; N12,500 per week and N50,000 per month.
(b) For middle income earners, they lost N138.88 per hour, N3,333.33 per day, N25,000 per week, N100,000 per month.
(c) For the high-income earners, they lost N277.77 per hour, N8,666.60 per day, N50,000 per week and N200,00 per month.

Indeed to attend Maitama District Hospital for malaria treatment for one day, the income lost by self-employed patients, the amount varied from N100 per hour to N4,000 for the day. These losses are quite big and not encouraging especially if the person is running a one-man type of business. Their business or shop may have to be closed down completely for the entire period (hours or days) when they are away to seek medical attention for malaria and may indeed not be able to resume work in the shop for several reasons after attending the hospital. There is also the possibility for the person to suffer further loss, if the customers cannot afford to wait and go elsewhere to get attended to or get service. The percentage of income lost through money spent on malaria treatment was found to vary between 20 to 70 percent, which is rather high.

Loss of Productivity

A major type of loss as a result of malaria is loss of productivity by both employed and self-employed patients who came to access treatment for malaria at Maitama District Hospital. The study found that the productivity/output of work lost due to malaria varied between 10% and 60%. If one considers this along with impact on their income or revenue, one will find this to be quite high and unacceptable. When aligned with their income and revenue (for self-employed patients), it was found that the loss on average per hour was N1,000, per day N10,000 and per week was over N70,000. Such amount of productivity and revenue due to malaria attack is too exorbitant.

When this loss of productivity is juxtaposed with the number of days of "excuse duty" from work, one will begin to appreciate the magnitude of such colossal loss. The loss due to "excuse duty" ranged from 3 days to 30 days. This no doubt, is a great loss in level of productivity and output of work.

Malaria Prevention and Control

Another way to look at loss of productivity, income, man-hour and so on, is to inquire how much the patients spent for malaria prevention and control. It is a fact that malaria can be prevented and controlled. Malaria prevention and control measures as found in this study involved spending and incurring costs on measures such a; insecticide treated nets; insecticides such as mobil, raid, shelltox; construction of windows and door nets as well as costs of fumigation of their environment, including stagnant water and drainages.

We also found that many of the patients slept under insecticide treated nets which they purchased ranging from N500 to N2,000. Others who were lucky got theirs through the local receipts of the global fund sources. The insecticide

treated nets though quite important in preventing mosquito bites by anyone sleeping under it, have their own limitation too. Some of them are not so effective anymore. Besides, they need to be "maintained", washed and socked in certain chemicals to make sure that they are effective again. Their life span for efficacy may expire and needs proper treatment. Not many patients can afford them, while some don't like sleeping under them.

The study found that insecticides such as mobil, Raid, Shelltox and so on were used at home by the patients. The costs of these vary from N500 to N700. The insecticides though quite effective, have their own limitations as well. Rooms have to be closed and everyone evacuated while spraying is going on as they could be toxic to our health. In addition, apart from the fact that their costs are increasing daily, some of them are not effective and may not kill the mosquitoes even in the sprayed room.

Window and door nets were constructed in their homes by some of these patients, the research found. The costs of constructing these window/door nets are variable depending on the number of doors/windows in the house.

Some of the respondents spent between N500 to N5,000 to construct the nets. Apart from the fact that they have to be maintained from wear and tear, some patients complained that they restrict fresh air from entering the rooms.

Other patients used "Sunday Sunday" medicines to prevent malaria. They repeated that these medicines cost between N100 and N500. Perhaps some of them may not be too effective or if not regularly used may not be able to prevent malaria attack.

The fumigation of stagnant water and other drainages near the houses is another alternative which needs careful programming and may not be for cost effective. It requires efforts and intervention of governments to make them effective. It is even in the instant fumigation of the environment by government and their agencies that can make it work effectively. Without such programmes, it is quite doubtful if it can work.

This research found that the amount spent monthly by the patients to prevent mosquito bites is high and is doubtful if too many people can afford that on a regular basis. The percentage of money spent monthly by the patients to prevent malaria attack relative to their income/revenue ranged between 20% and 50%. Yet they still had malaria attack requiring them to go to hospital for treatment.

Roll Back Malaria and their programmes

Not many of the patients were aware of the National Strategic Target for Roll Back Malaria in Nigeria which ran between 2001-2005 and also 2006-2010. This research also found that majority of the patients were also not aware of efforts by the public sector, government and its agencies, Community Based Organizations (CBOs), Non-Governmental Organizations (NGOs) on malaria reduction in Nigeria. Similarly, many were not aware of Global Fund for HIV/AIDs/TB and malaria.

A lot more needs to be done so as to ensure that majority of the people feel the effects of Global Fund for Malaria and benefit more by way at reduction in malaria incidence in the country.

Recommendations and Conclusion

This research has established the effects of malaria on productivity. The impact of malaria on productivity among patients attending Maitama District Hospital malaria was found in this research to have a negative effect on productivity of the working class population of patients attending the hospital, and also to have adverse effect on the incomes and revenues through loss of man hour and money spent to procure treatment at the hospital.

Based on the dual effects of malaria on the patients, affecting their productivity, time and income, the issues of malaria diagnosis and treatment on one hand was compared to that of malaria prevention and control. The issues if understood can go a long way in reducing the effect of malaria on productivity for the working class population attending Maitama District Hospital.

Malaria is a disease that cannot ignore groups, educational qualification and professional achievement. Malaria can also be recurrent especially if not properly treated and completely addressed. It can lead to complicated health problems, even though it is preventable, treatable and curable.

The causes of malaria have been well treated in this study. The main source which is the biting of human being by infected female anopheles mosquito and transmitting of plasmodium parasites to humans. Therefore the most effective and critical remedy is the total eradication of mosquitoes since they are the main vectors of malaria parasites.

This research thus found that correct diagnosis plus appropriate and full treatment received in the hospital led to correct situation of receiving cure from malaria.

Recommendations

There is an urgent need to review properly in details all the past efforts amended to tackling malaria in the Federal Capital Territory in particular and in Nigeria as a whole. These effects should be practically reviewed as there are serious gaps and lapses, which have rendered them ineffective.

It is recommended that in advert to liberate the working class people in particular and indeed creative Nigerian people from the menace of malaria and its effect as found in this study, the Roll Back Malaria programme in Nigeria should be totally overhauled. The programme has been done in two cycles of 5 years each totaling 10 years, and yet there seem to be no-effect.

The monthly environmental sanitation should be strengthened to ensure that our environment remains clean and will not be a breeding ground for mosquito. Various area councils within the Federal Capital Territory, NGOs, and CBOs should include general fumigation of environment as part of Roll back malaria Programme.

Conclusions

Therefore, the total and effective elimination of mosquitoes and indeed emancipation of inhabitants of the Federal Capital territory, Abuja from malaria is through the constant effort of all. All hands should be on deck towards the fight against malaria as well orchestrated programme and a battle of mosquito eradication taken directly to the breeding places and homes of mosquitoes.

That is the only way out in Abuja, the Federal capital Territory. The fight against mosquitoes and the end of malaria is a task that must be done jointly by all inhabitants of Abuja city.

Contributions to Knowledge.

This study has made humble contributions to knowledge and stakeholders in the issue of malaria.

To the Federal Capital Territory Administration the study should provided them a better understanding on the way out of solving malaria health problems within the territory.

For the Area council in the FCT, especially Abuja Municipal Area Council, this study should be an eye-opener for them to know what programmes to effectively embark upon within the Area council to solve malaria menace.

As for the N.G.Os, C.B.Os and Civil Society Organizations operating within Abuja City, this study will be a wake-up call for them to now charge and focus on the proper way forward in the elimination of malaria in the city.

Communities too will benefit and a lot in the Abuja city by waking up to face the challenges posed by Malaria which makes individuals sick and even die if not properly treated.

Individuals would also benefit from this study by knowing that the cleaning up drainages, stagnant water and environmental cleanliness being anchored by the monthly sanitation progamme in the FCT is a programme that should be taken seriously. It will eliminate mosquitoes, the main cause of malaria.

For the hospitals within Abuja City such as Maitama District Hospital that are over worked and completely overwhelmed by malaria patients, they too will learn the need to embark on Advice programmes to the authorities within the city to wake up, kill mosquitoes and stop the scourge of malaria. This will enable the hospitals to concentrate on other more serious public health diseases and problems.

Areas for Further Research

This research does not claim to cover all aspect of effects of malaria on productivity. There is therefore, room for further research especially in areas that could not be comprehensively dealt with in this research because of time and fund limitations.

Such areas for further research may include effect of malaria on pregnant women and children under 5 years of age, effect of malaria on old-age people; effect of malaria on school children; effect of malaria on people living with HIV/AIDs and such. They may also include a programme for development of community involvement organised private sector involvement in tackling malaria.

References

Abu-Raddad L, Patnaik P, Kublin J (2006). "Dual infection with HIV and malaria fuels the spread of both diseases in Sub-Saharan Africa". Science 314 (5805):1603-6.doi:10.1126/science.1132338. PMID 17158329. (.

Alonso PL, Sacarial J, Aponte JJ et.al. (2004) "Efficacy of the RTS,S/AS02A vaccine against Plasmodium falciparum infection and disease in young African children: randomized controlled trial Lancet 364(9443):1411-20.

Aponte JJ, A, P, Renum M, etl. (November 2007) "Safety of the RTS, S/A S02D candidate malaria vaccine in infants living in a highly endemic area of Mozambique: a double blind randomized controlled phase 1/11b trial Lancet, 370(9598): 1543-51.

Achumba, I. C. (2000) Strategic "Marketing Management and Distribution services in the 21st century" Macmillan and Capital Publishers Inc. Charlotte,

Africa (2007) Malaria – Vaccine Expected in 2011 Accra Mail (http ://www.accra.mail.com) 9 January 2007.

Agues R, White LJ, Snow RW, Gomes MG (2008) "Prospects for malaria eradicationin sib-saharan Africahttp://www.pubmedcentral.nih.gov/article render fcgi PLOS ONE 3(3):e/1767 doi: 10, 1371 /journal.pine.0001767)

Asika, N. (1991) Research Methodology in the Behavioral Science, Lagos, Longman

Barat L (2006). "Four malaria success stories: how malaria burden was successfully reduced in Brazil, Eritrea, India, and Vietnam". Am J Trop Med Hyg 74 (1): 12-6. PMID 164073 (http://wwww.ncbi.nlm. nih.gov/pubmed/16407339).

BBC News (2005) "Fungus may help malaria fight"http://www.newsbbc.co.uk/hi/health/4074212st m. BBC News 2005-06-05.

Beare NA, Taylor TE, Harding SP, Lewallen S, Molhyneux ME (November 2006)."Malarial retinopathy: a newlyhestablished diagnostic sign in severe malaria". Am. J. Trop. Med. Hyg. 75(5): 790-7 . P M I D 1 7 1 2 3 9 6 7 . P M C : 2 3 6 7 4 3 2 . http:www.ajtmh.org/cgi/ pmid lookup? view= long & pmid=17123967.

Bell C, Devarajan S, Gersbach H (2003)(PDF). The long-run economic costs of AIDS: theory and an application to South Africa. World Bank Policy Research Working Paper No. 3152. http:www1. worldbank.org/hiv aids/docs/BeDeGe BP total2.pdf. retrieved on 2008-04-28

Boivin MJ (October 2002). "Effects of early cerebral malaria on cognitive ability in Senegalese children". J DevBehavPediatr 23 (5): 353-64. PMID 12394524.http://meta.wkhealth.com/pt/pt-core/templatejournal/lwwgateway/media/landingpage.htm?issn=0196206x&volume=23&issue=5&spage=353.

"Biography of Ronald Ross" the Nobel Foundation. http://nobelprize.org/nobelprizes/medicine/laureates/1902/ross-bio.html. Retrieved on 2007-06-15.

Breman J (2001). "The ears of the hippopotamus: manifestations, determinants, and estimates of the malaria burden". Am J Trop Med Hyg 64(1-2 Suppl): 1-11.PMID11425172. http://www.ajtmh.org /cgi/repring/64/1suppl/1-c.Centers for Disease Control. Fact Sheet: Tuberculosis in the United States. 17 March 2005, Retrieved on 6 October 2006.

Chernin E (November 1983). "Josiah Clark Nott, insects, and yellow fever". Bull N Y Acad Med 59 (9): 790-802. PMID 61.40039.

Chaisson RE, Martinson NA (2008). "Tuberculosis in Africa-combating an HIV-driven crisis". N Engl J Med 358 (11):1089-1092. doi: 10.1056/ NEJMp 080 0809. PMID 18337598. http:// content. nejm.org/ cgi/contemt/full/358/11/1089/ query=TOC.

Cox F (2002). "History of human parasitology". (http://pubmedcentral.nih.gov/articlerender.fcgi?tool =p mcentrez&artid=126866) Clin MicrobiolRev 15 (4):595-612.

Doi:10.1128/CMR.15.4.595-612.2002. (http://dx.doi.org/10.1128/CMR.15.4.595-612.2002). PMID 12364371. (http://ncbi.nlm. nih.gov/ pubmed/12364371).

Cecil, Russel (1988). Textbook of Medicine. Philadelphia: Saunders. Pp. 1523, 1799. ISBN 0721618480.

Davies PDO, Yew WW, Ganguly D, et al. (2006). "Smoking and tuberculosis: the epidemiological association and pathogenesis". TrajsR Soc Trop Med Hyg 100: 291-8. doi:10.1016;j.trstmh.2005.06.034. PMID 16325875.

Divisions of HIV/AIDS Prevention(2003). HIV and its transmission". Centers for Disease Control & Prevention.http://www.cdc.gov/HIV/pubs/facts/trans mission.htm. Retrieved on 2006-05-23.

Emergence of Mycobacterium tuberculosis with extensive resistance to second-line drugs-worldwide, 2000-2004". MMWR Morb Mortal Wkly Rep 55 (11): 301-5. 2006. PMID 16557213. http://www.cdc.gov/mmwr/preview/mmwrhtml/mm551 1a2.htm.

"Ettore Marchiafava: http:// www. whomnamedit. com/ doctor.cfm/2478.html Retrieved on 2007-06-15.

Fine P, Floyd S, Standford J, Nkhosa P, Kasunga A, Chaguluka S, Warndorff D, Jenkins P, Yates M, Ponnighaus J (2001). "Environmental mycombacteria in northern Malawi: implications for the epidemiology of tuberculosis and leprosy". Epidemiol Infect 126 (3): 379-87.Doi.10.1017/S095026880100553 PMID 11467795. http:// www. cbi. nih.gov/pubmed/11467795)

FMOH (2005) National Policy on Integrated Disease Surveillance and Response (IDSR) September 2005.

FMOH (2008) National Malaria Control Programme: Annual Report.

FMOH (Aug. 2008) Federal Ministry of Health: National Malaria and Vector Control Programme – Manual for Health workers on Monitoring and Eradication for Malaria Control in Nigeria Participant's Manual.

FMOH (Sept. 2008) National Malaria and Vector Control Programme: Training Manual for Management of Malaria in Nigeria.

FMOH (2009) Strategic Plan 2009 – 2003: A road map for Malaria control in Nigeria.

Gallo RC (2006). "A reflection on HIV/AIDS research after 25 years".Retrovirology3:72.doi:10. 1186/1742-4690-3- 72.

PMID 17054781.http://www.retrovirology.com/content/3//72

Gao F, Bailes E, Robertson DL, et al (1999). "Origin of HIV-1 in the Champanzee Pan troglodytes troglodytes".Nature 397(6718):436-441. doi:10.1038/17130 PMID 9989410.

Gardner M. Hull N, Fung E. et.al. (2002) "Genome sequence of human malaria parasite Plasmoldium Falciparum" Nature 370(6906):1543

Gladwell, Malcolm. (2001-07-02)."The Mosquito Killer". The New Yorker. http:// www.gladwell.com /2001/2001 07 02 a ddt.htm.

Greenwood BM, Bojang K, Whitty CJ, Targett GA (2005). "Malaria". Lancet 365: 1487-1498. doi.10.1016/S0140-6736(05)66420-3.PMID 15850634. (http:// www.ncbi.nlm. nih.gov/ pubmed/ 15850634).

Hay S, Guerra C, Taterm A, Noor A, Snow R (2004). "The global distribution and population at risk of malaria: past, present, and future". Lancet Infect Dis 4 (6): 327-36. doi:10.1016/S1473-3099(04)01043-6. PMID 15172341.(http://www.ncbi.nlm.nih.gov/pubmed/15172341.

Health (2009) you can get paid to catch malaria SeattleTimes Newspaper.http://www.seattletimes.nwsource.com/html)malaria05m.html.

Heppner DG, Kestr KE, Ockenhouse CF, et. Al. (2005) "Towards an RTS, S-based, multi-antigen vaccine against falciparum malaria: progress at the Water Reed Army Institute of Research Vaccine 23 (17-18): 2243-50.

Hollingdale M, Nardin E, Tharavani JS, Schwartz A, Nussenzweig R (1984)"Inhibition of entryu of Plasmodium Falciparum and P. vivaxsporozoites into cultured cells: an in vitro assay of protective antibodies" J. Immunol 132 (2): 909-13.

Hoffman SL, Goh LM, Luke TC et. Al. (2002) "Protection of humans against malaria by immunization with radiation-attenuated Plasmodium Falcipanum Sporozoites"J.Infect Dis 185(8):1155-64.

Holding PA, Snow RW (2001). "Impact of Plasmodium falciparum malaria on performance and learning: review of the evidence". Am. J. Trop. Med. Hyg. 64 (1-2 Suppl): 68-75. PMID 11425179. http://www.ajtmh.org/cgi/pmidlookup?view=long&pmid=11425179.

Hull, Kevin. (2006) "Malaria: Fever Wars". PBS Documentary Idro, R; Otieno G, White S, Kahindi A, Fegan G, Ogutu B, Mithwani S, Maitland K, Neville BG, Newton CR. (2007) "Decorticate, decerebrate and opisthotonic posturing and seizures in Kenyan children with cerebral malaria". Malaria Journal4 (57):571475-28754-57.PMID16116645. http:// www. ncbi. nlm.nih.gov/pubmed/16336645).

Imperial Collège, London, "Scientistscreate first transgenic malaria mosquito", (http://www. ic.ac.uk/templates/text).2000-06-22.

Ito J, Ghosh A, Moreira LA, Wimmer EA, Jacobs-Lorena M (2002). "Transgenicano pheline mosquitoes impaired in transmission of a malaria parasite". Nature 417:387-8. (http://www. ncbi. nlm.nih.gov / pubmed/12024215).

Jha P, Jacob B, Gajalakshmi V, et al. (2008). "A nationally representative case-control study of smoking and death in India". N Engl J Med 358(11): 1137-1147. http:// content.nejm.org /cgi/contnt /full /358/11/1137?query=TOC.

Joy, D., Feng, X., Mu J et al (2003). "Early origin and recent expansion of Plasmodium falciparum". Science 300 (5617): 318-21. (http:// www.ncbi.nlm. nih.gov/pubmed/12690197).

Kallings, L.O. (2008). "The first postmodern pandemic: 25 years of HIV/AIDs".J> Intern Med 263 (3): 218-243.

Korenromp, E., Williams, B., de Vlas, S., Gouws, E., Gilks, C., Ghys.P. andNahlen, B. (2005). "Malaria attributable to the HIV-1 epidemic, sub-Saharan Africa".Emerg Infect Dis 11(9):1410-1419. (http://www.ncbi.nlm.nih.gov/pubmed/16229771).

Killeen, G., Fillinger, U., Kiche,I., Gouagna, L. and Knols, B. (2002). "Eradication of Anopheles gambiae from Brazil: lessons for malaria control in Africa?". Lancet Infect Dis 2 (10):http:// www.ncbi. nlm. nih.gov/pubmed/12383612).

Lademarco, M.F. and Castro, K.G. (2003). "Epidemiology of tuberculosis". Seminars in respiratory infections 18 (4): 225-40. doi: 10.1017/S0950268801005532. PMID 14679472.

"Malaria (2008): Disease Impact and Long-Run Income Differences" (http:// ftp.iza.org /dp2997. pdf).
Institutefor the Study of Labor.http:// ftp.iza. org /dp2997.pdf.Retrievedon2008-12-10.

Malaria control (2007) "What is Malaria control net" Africa-at-home.Web.com.ch/AfricaAfrica-at-home/malaria control html 2007-3-11.s

Matuschewski, K. (2006) "Vaccine development against Malaria" CurrOpinImmunol 84(4):449-57.

Nussenzweig, R., Vanderberg, J., must H. and Orton, C. (1967). "Protective immunity produced by the injection of x-irradiated sporozoites of plasmodium berghen" Nature 216 (5111): 160-2.

Onwe, O. J. (1988) Elements of Project Dissertation Writing: A Guide to Effective Dissertation Reports, Lagos Impressed publishers.

Osuala, E. C. (1993). Introduction to Research Methodology, Onitsha, Africana Press Samaria Press and Publications http://www.samaria.com/press /publications.html)

SFI (2008) Scaling Up Malaria Control in Nigeria: Technical Report Mid-Term Evaluation of Phase Grand for Scaling Up Malaria Control in Nigeria. Society for family Health, Federal Ministry of Health and Yakubu Gowon Centre.

WHO (2008) World Malaria Report2008(http:// www.who.int/malaria/mediacentre/wmr2008/)

Yamane, T. (1964). Statistics. An Introductory Analysis, New York, Harper & Row.

Zavala, F., Cochrane. A., Nardin, E., Nussenzweig, R. andNussenzweig, V. (1983) "Circumsporozoite proteins of malaria parasites contain a single immunodominant region with two or more identical epitopes" J. Exp Med 157 (6): 1947-57.

Comparison of POCT Glucose Meters and Analysis of the Interference Factor

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ABSTRACT

Objectives: Not many reports have covered large-scale point of care testing (POCT) blood glucose comparisons, and many interfering factors affect detection. This study aims to verify the performance of POCT blood glucose meters and discusses the factors that interfere with detection. Methods: Accuracy and precision verification in five glucose concentration groups-high 1 (H1), High 2 (H2), medium 1 (M1), medium 2 (M2), and low (L); comparison of different test methods and specimens; and also the influence of iodophor was investigated in a dilution experiment. Results: A total of 58 out of 64 Accu-Chek Inform II POCT blood glucose meters (ACI II) qualified for testing. A proportional significant difference in the relative bias was observed with the POCT instruments in the intermediate and high glucose concentration groups (H=15.364, p=0.02). There were significant differences among the five groups with compliance rates (?2=21.03, p=0.00); Group L showed higher values than groups H1 and H2. The precision verification met the requirements issued by the Consensus. Significant differences were found between the three detection methods. The measurement of the Glucose Oxidase Method (Cobas B 123) was lower than that of the HITACHI Plasma Hexokinase Method and the Glucose Dehydrogenase Method on the ACI II (p=0.005 and 0.003) in the preliminary study. No differences were seen among the three types of specimens (p>0.05). The glucose results were incorrect in the presence of iodophor interference. Conclusions: The ACI II and Cobas B123 (with a slightly negative bias) provide sufficiently accurate measurements, and all types of blood specimens can be applied. Iodophor, a disinfectant, interferes with glucose measurement.

Keywords: iodophor; methodological comparison; performance verification; POCT blood glucose meter; specimen type.

INTRODUCTION

As one of the "five parts" of diabetes management in China, blood glucose monitoring is an essential index for clinicians to diagnose and observe the progress of diabetes mellitus (DM). Moreover, because the early symptoms of DM are not noticeable and are likely to be ignored, accurate detection of blood glucose is of vital importance. Convenient and easyto-operate features allow the point of care testing (POCT) to be widely adopted in the clinic; furthermore, the POCT is suitable for almost all kinds of blood samples [1]. At present, the automated biochemical analyzer and POCT blood glucose meter are mainly used to detect glucose (GLU). Being widely used in Emergency Departments, Intensive Care Units, and Operating Rooms, the blood gas analyzer is also categorized as a POCT instrument that simultaneously detects blood gases and analyzes the biochemical components in blood.

The quality assessment plan among clinical departments of the Chinese Experts Consensus on Clinical Operation and Quality Management Norms of the Portable Blood Glucose Meters (hereinafter "Consensus") mentions the following: "it is suggested thatmedical institutions may carry out comparison activities between portable blood glucose meters and their laboratory biochemical meters once a year [1, 2]." Meanwhile, the Consensus also clearly points out that "75% ethanol should be used to disinfect the blood sampling sites, rather than other reagents, such as iodophor, that may interfere with its detection" [1]; however, no specific reasons for the interference are given.

Considering that the POCT blood glucose analyzers and blood gas analyzers are widely scattered across wards, it is difficult to collect various blood specimens; thus, it is challenging to carry out a large-scale comparison work.

Consequently, more domestic studies on the subject are needed. Furthermore, this study is undertaken to compare the clinical reliability of the POCT instrument in detecting GLU and to evaluate its interference factors in the accuracy of GLU detection, i.e., iodophor.

Materials and methods

Samples

In brief, 2mL of arterial blood, venous blood, and capillary blood from 53 surgical patients were collected over a 3-month period in 2021 with heparin tubes and tested with multiple instruments. The study was performed with the approval of the Ethics Committee of General Hospital of Tianjin Medical University (No. IRB2021-WZ-035), and was exempted the requirement of obtaining informed consent.

Instruments and reagents

The Roche Accu-Chek Inform II POCT blood glucose meter (Roche, Switzerland) uses the glucose dehydrogenase (GDH) detection method. Being a mutant of quinone protein glucose dehydrogenase (MUT. Q-GDH), the enzyme in the GLU test paper can convert the GLU in the blood into gluconolactone; thus, it can produce direct current and judge the GLU concentration. This method is suitable for GLU concentration detection in fresh arterial blood, venous blood, finger capillary blood, and neonatal heel stick capillary blood. The Hitachi 008AS automated biochemical analyzer (Hitachi, Japan), uses the Hexokinase (HK) detection method. While using HK as a catalyzer, GLU and ATP undertake phosphorylation reactions, and nicotinamide adenine dinucleotide phosphate (NADP) is

reduced to reduced nicotinamide adenine dinucleotide phosphate (NADPH) simultaneously.

NADPH production was measured at 340 nm to calculate the GLU concentration. We used a Cobas B 123 POC System blood gas analyzer (Roche, Switzerland) with a glucose oxidase (GOD) detection method.

GLU is oxidized to gluconolactone in the sensor; theH2O2 generated by the manganese dioxide/carbon electrode was measured at 350 MV tocalculate the GLU concentration.

Methods

Accuracy verification

The tests results of 64 Roche Accu-Chek Inform II POCT blood glucose meters (ACI II) in each ward of Tianjin General Hospital were selected and compared with those of Hitachi 008AS biochemical analyzer.

Original reagents and test strips were used. As per the requirements of the Consensus, internal quality control (IQC) of POCT blood glucose meters was conducted with quality control solution on a daily basis, and instrument maintenance was also scheduled periodically. Five groups of heparin anticoagulant venous blood with different glucose concentrations were selected as specimens, including two high concentrations of groups H1 and H2 (about 11.0 mmol/L), two medium concentrations of groups M1 and M2 (about 7.0 mmol/L), and one low concentration of group L (about 2.8 mmol/L). Heparin anticoagulant venous blood with the same glucose concentration was divided into two portions. The first was measured on each POCT blood glucose meter, and the latter was tested on biochemical analyzer after centrifugation. To avoid potential errors caused by glycolysis, the interval between the two instruments was less than 30 min. The bias of each POCT blood glucose meter and biochemical analyzer was calculated and compared as per the Consensus evaluation criteria (tolerance as following: when GLU<5.5 mmol/L, the error was within ±0.83 mmol/L; when Glu≥ 5.5 mmol/L, the error was within ±15%) [1,3]. The overall specimen qualification rates of each instrument needed to reach 80% [1].

Precision verification

Two heparin anticoagulant venous blood specimens (high concentration: about 10 mmol/L, low concentration: about 4 mmol/L) were collected for repeated experiments. Each specimen was well mixed before the experiment, and was tested 20 times on the POCT blood glucose meter to calculate the SD and CV as per the Consensus evaluation criteria (when GLU<5.5 mmol/L, SD<0.42 mmol/L; and when GLU=5.5 mmol/L, CV<7.5%) [1, 3]. If the test results failed to meet the requirements, the POCT blood glucose meter should be maintained and calibrated, and the above-mentioned precision performance verification checking should be re-conducted.

Comparison of different test methods

Fifty-three heparin anticoagulant arterial blood specimens were divided into two tubes; the first tube was tested on the POCT blood glucose meter and blood gas analyzer, and the biochemical analyzer measured the second tube after centrifugation. The experimental interval was controlled within 30 min, and the operations complied withthe Trial Implementation for Clinical Operation and Management of Portable Blood Glucose Detector in Medical Institutions (hereinafter referred to as "Implementation").

Comparison of different specimens

Fifty-three heparin anticoagulant arterial blood, venous blood, and corresponding finger capillary blood specimens were collected and immediately measured by the POCT blood glucose meter. The abovementioned operation complied with Implementation by the designating staff.

Influence of iodophor on blood glucose testing

Diluted samples of various concentrations were prepared by mixing one heparin anticoagulant venous blood specimen (B) with alcoholic free iodophor (I) per the volume ratios of 1I:9B, 2I:8B 3I:7B, 4I:6B, and 5I:5B. Each sample was repeatedly tested three times with the POCT blood glucose meter. The linear relationship of each diluted sample was analyzed. The variation of each dilution ratio was calculated by comparing the theoretically calculated results of each diluted concentration against the measured results. Thus, the disinfection influence of iodophor on GLU detection by the POCT blood glucose meter was evaluated.

Table 1: GLU Comparison using POCT blood glucose meter and biochemical analyzer ($\bar{x} \pm s$) (mmol/L) (n=64).

	Group L	Group M1	Group M2	Group H1	Group H2
$GLU_{biochemical\ analyzer}$	2.89 ± 0.04	6.69 ± 0.10	8.61 ± 0.16	11.48 ± 0.18	14.31 ± 0.25
GLU_{POCT}	3.27 ± 0.12	7.34 ± 0.31	9.48 ± 0.41	12.54 ± 0.39	15.95 ± 0.56
TEa	<±0.83 mmol/L	<±15%	<±15%	<±15%	<±15%
Bias	0.39 mmol/L	9.93%[a]	9.39%[a]	10.26%[ab]	11.61%[b]
Qualification (%, n/n)	100 (64/64)[a]	93.8 (60/64)[ab]	95.3 (61/64)[ab]	87.5 (56/64)[b]	81.3 (52/64)[b]

Comparison between two groups: [ab]represents no difference between the two groups; [a] or [b] represents a difference between two groups.

Statistical analysis

SPSS 22.0 statistical software was adopted for data analysis. Continuous variables were tested for normality, and they were expressed as x ± s (normal distribution) or M (P25-P75) (non-normal distribution).

The Kruskal-Wallis H test was used for multi-group data comparison. K-W single-factor ANOVA was used for comparison between groups, the ?2 test was used for comparing the compliance rate between groups, and Multi-group ?2 test was used for comparison between groups. p<0.05 was considered statistically significant.

Results

Comparison between POCT and biochemical analyzers (accuracy verification) According to the Consensus evaluation criteria, the overall qualification rate of the 64 ACI II Meters against the test samples was 80%, i.e., 58 instruments qualified for the comparison study, and six instruments failed. On comparing the relative bias of 64 ACI II in M/H Groups, significant differences among the four concentration groups became apparent (M1, M2 vs. H1 and H2) (H=15.364, p=0.02) (because Group L required an absolute bias, it was not included in the inter-group comparison); Groups M1 and M2 showed significantly lower values than Group H2 (p=0.043 and 0.002). The bias gradually

Table 2: POCT accuracy verification (mmol/L).

GLU	$\bar{x} \pm s$	CV (%)	Evaluation criteria
L	4.56 ± 0.16	4.30	SD<0.42 mmol/L
H	10.68 ± 0.48	4.93	CV<7.5%

increased along with the increase in the GLU concentration.

On comparing each group's compliance rate of the 64 ACI II, significant differences were observed among the five groups (?2=21.03, p<0.01). Group L showed obviously higher values than Groups H1 and H2. With the increase in the GLU concentration, the qualification ratio declined. The details are shown in Table 1.

POCT accuracy verification

The accuracy complied with the evaluation criteria of Consensus. The details are shown in Table 2. Comparison of various detection methods The differences in blood glucose values were notable among the three detection methods (p=0.000). Group GLUGOD showed significantly lower values than Group GLUGDH and Group GLUHK (p=0.005 and 0.003); no difference was found between Group GLUGDH and Group GLUHK. The details are shown in Table 3.

Table 3: Comparison of GLU values by various detection methods (mmol/L) (n=53).

Test methods	GLU_{GDH} (ACI II) M (P25, P75)	GLU_{GOD} (Cobas B123) M (P25, P75)	GLU_{HK} (HITACHI 008AS) M (P25, P75)	Kruskal-Wallis H-Value	p-Value
GLU	5.500 (5.2, 6.0)[a]	4.900 (4.50, 5.60)[b]	5.530 (5.10, 6.10)[a]	23.862	0.000

Comparison between two groups: [ab]represents no difference between the two groups; [a] or [b] represents a difference between two groups.

Comparison of multiple specimen types

No difference was seen in GLU among the three blood types (p=0.13). The details are shown in Table 4.

Table 4: Comparison of GLU concentration against multiple specimen types. By ACI II POCT blood glucose meters (mmol/L) (n=53).

Specimen types	$GLU_{arterial\ blood}$ M (P2, P75)	$GLU_{capillary\ blood}$ M (P25, P75)	$GLU_{venous\ blood}$ M (P25, P75)	Kruskal-Wallis H-Value	p-Value
GLU	5.50 (5.2, 6.0)	5.60 (5.2, 6.2)	5.30 (5.1, 5.8)	3.960	0.13

Table 5: Influence of iodophor on blood glucose detection (mmol/L).

(Iodophor I: Blood B)	0I:10B	1I:9B	2I:8B	3I:7B	4I:6B	5I:5B
Measured result	4.73	6.63	5.53	5.00	4.47	3.70
Theoretical result	4.73	4.26	3.78	3.31	2.84	2.37
Variation (%)	0.0	30.81	26.62	28.76	31.48	30.99

The influence of iodophor on blood glucose detection

The variation in the detected GLU after diluting whole blood with iodophor in different proportions was notable.

The measured results were higher than the theoretical results, and as the dilution increased, the GLU gradually decreased. Thus, the analysis showed that iodophor, a disinfectant, had a serious influence on the detection results of POCT blood glucose meters. The details are shown in Table 5.

Discussion

According to the requirements of the Consensus, methodological evaluations are mandatory before clinical usage of the POCT blood glucose meters, which mainly include accuracy verification, precision verification, detectable range verification, and anti-interference performance. The POCT quality control scheme is determined by risk assessment, and it is designed based on the consideration of the complexity of the project [3]. IQC must be carried out for all items at all institutions that perform POCT testing [4, 5]. For portable blood glucose meter testing, we recommend the IQC frequency at least once every test day and recording the analysis and quality control results. In cases of out of control, analyze and record test results promptly and take corrective measures before performing patient test [6]. It is worth noting that the common problem of unqualified instruments found in this study was the non-standardized performance of the IQC, i.e., the tests were incomplete. In conclusion, a strict IQC is essential and crucial for EQA assessment. Different detection systems and POCT instruments of the same model must be compared to each other regularly to ensure the comparability of detection results [7]. The EQA assessment is based on the requirements of WS/T 415-2013 Laboratory Testing and Evaluation Method with no External Quality Evaluation and CNAS-CL02-A004. The projects that cannot participate in the EQA assessment can be completed with reference to the relevant articles of EQA. By analyzing the tested results of the ACI II, 58 instruments were in line with the requirements.

At least two concentration values surpassed the bias with six blood glucose meters. Information about unqualified instruments has been fed back to each concerned ward immediately. Furthermore, after recalibration and retesting, the unqualified instruments were replaced.

In the daily work, significant differences are often found between the detection results of the POCT blood glucose meters and those of the biochemical analyzers. In this study, it was also found that with the increase in the blood glucose concentration, the deviations of the POCT blood glucose meters gradually increased, or the compliance rate decreased. The bias overrun appeared at the H1 and/or H2 levels with six instruments listed as unqualified; even if the bias overrun appeared only at one concentration, it was basically concentrated at a high concentration with partial instruments listed as qualified. Given that the two instruments conduct tests based on different methodologies, ±15% TEa is relatively loose. Hence, in the clinical work, if the blood glucose detected by the POCT blood glucose

meter is abnormally high, a re-examination of venous blood through the biochemical analyzer is suggested.

Clinical studies on GLU detection by blood gas analyzers are relatively limited. A study by Luo [8] has found that although the test results of blood gas analyzer and biochemical analyzer vary, the differences are acceptable.

Our study also indicated that the GLU detected by the blood gas analyzer is generally lower than that detected by the biochemical analyzer. The pressure of oxygen (PO2) in blood specimens may partially affect the detection results of some blood glucose meters based on the GOD methodology [9]. The change in PO2 can have a greater impact on the results of blood glucose meter through the GOD methodology, and that the increase in PO2 will lead to a lower GLU and vice versa. GLU measurement by blood gas analyzer is carried out through sensors. The sensors can reduce the oxygen consumed in the enzymatic reactions, and the GLU has no correlations with the oxygen concentration in the blood. Thus, it is suitable for the GLU measurement of arterial blood and can avoid the influence of intra-operative oxygen inhalation on the GLU detection results. In contrast, the determining factor of GLU determination by the blood gas analyzer is glycolysis in red blood cells. The metabolism of red blood cells in a short time will also reduce the concentration of GLU. Therefore, the blood specimen has to be analyzed immediately. In this study, the GLU level detected by the blood gas analyzer was significantly lower than that detected by the biochemical analyzer and POCT blood glucose analyzer. Because plasma was collected from surgical patients in this experiment, during the operation, all patients inhaled high flow pure oxygen, which was speculated to affect the experimental results, resulting in lower results. In future research, we will collect the plasma of patients without oxygen inhalation, and we expect results will be more comparable. In conclusion, the reliability of blood gas analyzers for clinical biochemical detection has not been unanimously confirmed, and it still needs to be investigated further [10, 11].

It is worth emphasizing that the detection results of the POCT instruments are only for the screening assessment purpose, and they are not applicable for clinical decision support. The biochemical analyzer remains the primary choice and the "gold standard" for GLU detection. In case abnormal results are found during the screening assessment, venous blood needs to be collected and tested to reconfirm the accuracy of the POCT instruments. Generally speaking, the accuracy and specificity of the HK methodology are prioritized over the GOD methodology. At present, the HK methodology is widely adopted as the reference standard for the determination of fasting blood glucose and 2 h postprandial blood glucose.\

The study results showed that despite a slight difference in arterial blood, venous blood, and capillary blood detected by the POCT blood glucose meter, there was no statistically significant difference. In addition to the factors, such as improper operations, electromagnetic interference, and inadequate maintenance, the GLU detection result may also be affected by shock, tissue hypoperfusion, and drug application (such as dopamine) [12]. Furthermore, when the arterial blood flows through the capillaries, the tissue cells of the peripheral microcirculation histiocytes consume GLU. Hence, the GLU of capillary blood is generally lower than that of arterial blood, which is a physiological phenomenon. In addition, because of the GLU difference in the arterial and venous blood, including the difference in tissue incorporation, there is a theoretical GLU difference among various tissues. However, the experiment did not show much difference, which further proves that the blood glucose test results of the POCT blood glucose meter are consistent with different blood specimens.

In order to cover different types of blood specimens, surgical patients of this study had stable vital signs, were in a calm condition, and were operated in warm environments.

When the fingertip blood was taken, the blood drop fell naturally rather than being squeezed to avoid the interstitial fluid. The sample sizes were moderate, and the nonparametric test method (with weaker statistical power than the parametric test) was adopted. In addition, the GLU of selected patients were within the range of 3.3–9.6 mmol/L, which can cover the blood glucose range of the majority of people. Larger sample size and parametric test would be required if extremely high or low blood GLU level specimens were selected. Meanwhile, whether any different results will be obtained if the parametric test adopted is worth a further discussion.

The Consensus also states the following: "try to avoid disinfection with iodophor when using the POCT blood glucose meter," which was also discussed in this study.

Significant deviation had been noticed when the specimen was mixed with a tiny amount of iodophor. The reason for this is that blood GLU can be easily oxidized. In addition to quinones used as oxidants in POCT blood glucose meters, more potent oxidants or even weak oxidants can also oxidize GLU. The enzyme on the blood glucose test paper is Mut. Q-GDH is an improved glucose dehydrogenase. Thus, it is presumed that there are quinone residues or side chains from a certain residue in the tertiary or multi-level structure of dehydrogenase or catalytic site, which oxidize glucose into glucosamine, and the subsequent reaction continues. Especially when encountering strong oxidants, such as iodine, blood GLU is oxidized by iodine before reacting with the quinone protein glucose dehydrogenase.

Pyrroloquinoline quinone (PPQ) is the most potent redox biocatalytic molecule ever found [13]. When introducing the redox reagent, the redox potential of the entire electrochemical reaction changes. If the potential of the redox reagent is located in a particular step of the reaction cycle, in that case, it will directly interfere and lead to the increase in the blood GLU detection result. Therefore, redox substances, including iodophor and hypochlorous acid, which may cause oxidation-reduction reactions, should be avoided in disinfection. In addition, iodine will react with water and produce hypoiodous acid, which will generate additional electrical signals. After the electrode collects the redox reaction electrical signal, it will become negative iodine ions or hypoiodous acid (positive iodine ions) through oxidation and reduction. At the same time, iodine will also react with quinones. These complex chemical changes may result in collection of false-positive electrical Wang et al.: POCT glucose meter comparison 199 signals by the instrument; thus, it may interfere with the test results. Moreover, common substances with redox properties, such as vitamin C and uric acid, also possibly raise the determination results [14]. The Consensus suggests that alcohol should be adopted for disinfection because both alcohol and GLU are alcohol substances and do not react with each other. Moreover, alcohol is volatile; both its physical and chemical properties are adequate for blood glucose meters.

Most of the samples used for POCT blood glucose meters are whole blood. The accuracy was partly affected by hematocrit (HCT). When HCT decreases, the blood glucose level appears high, and vice versa [15]. This is because the results produced by POCT blood glucose meters are based on electrochemical biosensor technology. HCT determines the mass transfer coefficient. The higher the HCT, the smaller the mass transfer coefficient and the lower the detection value, and vice versa [16]. When HCT exceed 35 and 55%, the doctors must pay attention to the accuracy

of the detection value of POCT blood glucose meter. The HCT of newborns can be between 43 and 63%, which can lead to low detection values. The decrease of HCT in patients with anemia can increase the numerical value of the results [17]. The blood glucose meter with HCT correction function adds a serum separation membrane on the surface of the electrode. The plasma obtained from the whole blood after separating red blood cells by the serum separation membrane reacts with the enzyme on the electrode surface. This approach minimizes differences. In the meantime, further separation of high purity blood into cells and plasma or serum can improve the sensitivity of various clinical analysis to prevent the degradation of analytes in plasma caused by long-term contact with blood cells, thus releasing pollutants and causing deviations within the detection results of the biochemical analyzer [18].

Conclusions

In this experiment, the performance of POCT blood glucose meters was analyzed. The influences of different test methods and blood specimen types on blood GLU detection were discussed, and in addition, the influence of interference by iodophor was also discussed.

The detection results of ACI II are accurate, and they can be applied to all types of blood specimens. It is expected that more scientific and accurate conclusions will be drawn and the POCT blood glucose meter gap in clinical application will be filled. This investigation provides new ideas and methods for the scientific management and application of POCT.

Acknowledgments:

Thanks to Prof. Yu Zhilin from Department of Chemistry, NanKai University; Mr. Chen Qiang and Lu Yuan from Company FLIGHT. We thank LetPub (https:// www.letpub.com/) for its linguistic assistance during the preparation of this manuscript.

Research funding: Tianjin General Hospital of Medical University Youth Incubation Foundation (ZYYFY201604).

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission. QianWang: paperwriting, experiment operation. Jin Du: Collection of clinical specimens. Lin Chen: Literature review, Statistical collation data. Yu-diDu: Correct translation, experiment operation.

Wei Luo: Experimental instruction

Competing interests: Authors state no conflict of interest.

Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: Research involving human subjects complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013), and has been approved by the authors' institutional review board (Ethical Committee Tianjin Medical University General Hospital) (IRB2021-WZ-035).

References

1. The Laboratory Medical Society of the Chinese Medical Association. The Clinical Inspection Center of the National Health and Family Planning Commission. Chinese expert consensus on the clinical operation and quality management norms of portable blood glucose meter. Natl Med J China (Peking) 2016;96:2864–7. (Chinese).

2. Zhou R, Wang Q. Opportunities and challenges of point-of-care testing in clinical application. Chin J Lab Med 2019;42:323–7. (Chinese).

3. ISO. In vitro diagnostic test systems-requirements for bloodglucose monitoring systems for self-testing in managing diabetes mellitus [S/OL]. 2013-05-14, 2015-08-20.

4. International Organization for Standardization (ISO). ISO2287: 2016(en) Point-of-care testing (POCT)-requirements for quality and competence [DB/OL]. 2021-04-01.

5. Nichols JH, Alter D, Chen Y, Isbell TS, Jacobs E, Moore N. AACC Guidance document on management of point-of-care testing. J Appl Lab Med 2020;5:762–87.

6. International Organization for Standardization. ISO/TS22583:2019(E) Guidance for supervisors and operators [DB/OL]. 2021- 05-01.

7. Yip PM, Venner AA, Shea J, Fuezery A, Huang Y, Massicotte L, et al. Point-of-care testing: a position statement from the Canadian society of clinical chemists. Clin Biochem 2018;53:156–9.

8. Luo K, Liang S, Liu Y, He H, He Y, Huang X. Correlation and consistency of electrolytes, blood glucose and lactic acid test results detected by POCT blood gas analyzer and central laboratory. Internet J Lab Med 2019;40:1938–40+44. (Chinese). 200 Wang et al.: POCT glucose meter comparison

9. Vote DA, Doar O, Moon RE, Toffaletti JG. Blood glucose meter performance under hyperbaric oxygen conditions. Clin Chim Acta 2001;305:81–7.

10. Shah R, Harding J, Brown J, McKinlay C. Neonatal glycaemia and neurodevelopmental outcomes: a systematic review and metaanalysis. Neonatology 2019;115:116–26.

11. Quinn LM, Hamnett N, Wilkin R, Sheikh A. Arterial blood gas analysers: accuracy in determining haemoglobin, glucose and electrolyte concentrations in critically ill adult patients. Br J Biomed Sci 2013;70:97–100.

12. Zhang N, Fu R, Chu H, Ye H, Xu R. Comparison of capillary blood glucose from the 1st and 2nd drop of blood drawn from the finger with venous blood glucose. J Nurs Sci 2020;35:40–2. (Chinese).

13. Stites TE, Storms D, Bauerly K, Mah J, Harris C, Fascetti A, et al. Pyrroloquinoline quinone modulates mitochondrial quantity and function in mice. J Nutr 2006;136:390–6.

14. Tang Z, Du X, Louie RF, Kost GJ. Effects of drugs on glucose measurements with handheld glucose meters and a portable glucose analyzer. Am J Clin Pathol 2000;113:75–86.

15. Dungan K, Chapman J, Braithwaite SS, Buse J. Glucose measurement: confounding issues in setting targets for inpatient management. Diabetes Care 2007;30:403–9.

16. Tang Z, Lee JH, Louie RF, Kost GJ. Effects of different hematocrit levels on glucose measurements with handheld meters for point-of-care testing. Arch Pathol Lab Med 2000; 124:1135–40.

17. Ginsberg BH. Factors affecting blood glucose monitoring: sources of errors in measurement. J Diabetes Sci Technol 2009;3:903–13.

18. Baillargeon KR, Murray LP, Deraney RN, Mace CR. High-yielding separation and collection of plasma from whole blood using passive filtration. Anal Chem 2020;92:16245–52.

The changing epidemiology of human monkeypox - A potential threat? A systematic review

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ABSTRACT

Monkeypox, a zoonotic disease caused by an orthopoxvirus, results in a smallpox-like disease in humans. Since monkeypox in humans was initially diagnosed in 1970 in the Democratic Republic of the Congo (DRC), it has spread to other regions of Africa (primarily West and Central), and cases outside Africa have emerged in recent years. We conducted a systematic review of peer-reviewed and grey literature on how monkeypox epidemiology has evolved, with particular emphasis on the number of confirmed, probable, and/or possible cases, age at presentation, mortality, and geographical spread. The review is registered with PROSPERO (CRD42020208269). We identified 48 peer-reviewed articles and 18 grey literature sources for data extraction. The number of human monkeypox cases has been on the rise since the 1970s, with the most dramatic increases occurring in the DRC. The median age at presentation has increased from 4 (1970s) to 21 years (2010–2019). There was an overall case fatality rate of 8.7%, with a significant difference between clades—Central African 10.6% (95% CI: 8.4%– 13.3%) vs. West African 3.6% (95% CI: 1.7%– 6.8%). Since 2003, import- and travel-related spread outside of Africa has occasionally resulted in outbreaks. Interactions/activities with infected animals or individuals are risk behaviors associated with acquiring monkeypox. Our review shows an escalation of monkeypox cases, especially in the highly endemic DRC, a spread to other countries, and a growing median age from young children to young adults. These findings may be related to the cessation of smallpox vaccination, which provided some cross-protection against monkeypox, leading to increased human-to-human transmission. The appearance of outbreaks beyond Africa highlights the global relevance of the disease. Increased surveillance and detection of monkeypox cases are essential tools for understanding the continuously changing epidemiology of this resurging disease.Monkeypox, a zoonotic disease caused by an orthopoxvirus, results in a smallpox-like disease in humans. We conducted a systematic review to assess how monkeypox epidemiology has evolved since it was first diagnosed in 1970 in the Democratic Republic of the Congo. In total, human monkeypox has now appeared in 10 African countries and 4 countries elsewhere. Examples include Nigeria, where the disease re-emerged in the last decade after a 40-year hiatus, and the United States, where an outbreak occurred in 2003. The number of cases has increased at a minimum of 10-fold and median age at presentation has evolved from young children (4 years old) in the 1970s to young adults (21 years old) in 2010–2019. This may be related to the cessation of smallpox vaccinations, which provided some cross-protection against monkeypox. The case fatality rate for the Central African clade was 10.6% versus 3.6% for the West African clade. Overall, monkeypox is gradually evolving to become of global relevance. Surveillance and detection programs are essential tools for understanding the continuously changing epidemiology of this resurging disease.

INTRODUCTION

Monkeypox, currently a rare zoonotic disease, is caused by the monkeypox virus, which belongs to the Poxviridae family, Chordopoxvirinae subfamily, and Orthopoxvirus genus [1]. The variola virus (smallpox virus) is closely related [1], and monkeypox disease results in a smallpox-like disease. Historical data have indicated that smallpox vaccination with vaccinia virus (another orthopoxvirus) was approximately 85% protective against monkeypox [2]. However, following the eradication of smallpox in 1980, routine vaccination against smallpox was no longer indicated [3], and it has now been four decades since any orthopoxvirus vaccination program.

The name monkeypox originates from the initial discovery of the virus in monkeys in a Danish laboratory in 1958 [4]. The first case in humans was diagnosed in 1970 in a 9-monthold baby boy in Zaire (now the Democratic Republic of the Congo, DRC) [5]. Since that time, monkeypox has become endemic in the DRC, and has spread to other African countries, mainly in Central and West Africa. Outside of Africa, the first reported monkeypox cases were in 2003 [6] and, at the time of this systematic review, the most recent cases were in 2019 [7,8].

A previous systematic review, which evaluated the literature through summer 2018, described the epidemiology of monkeypox outbreaks [9]. In view of the recent increase in reports from Nigeria and elsewhere, we initiated a new systematic literature review with a focus on the changes in the evolution of the epidemiology of human monkeypox since the first cases in the 1970s through the present day.

Methods

This systematic review was performed in accordance with international standards for conducting and reporting systematic reviews, including guidelines from the Cochrane Collaboration [10] and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [11]. The review is registered with PROSPERO (CRD42020 208269).

Searches were performed in MEDLINE (accessed using PubMed), Embase, African Journals Online (AJOL) and the Internet Library sub-Saharan Africa (ilissAfrica), with no language restrictions. All published literature reported through September 7, 2020, the date of last search, was considered for eligibility. In PubMed, searches included Medical Subject Headings (MeSH) and limits to title and abstract (tiab). The search string used in PubMed was Monkeypox[MeSH] OR "Monkeypox virus"[MeSH] OR monkeypox[tiab] OR "monkey pox"[tiab] OR "variole du singe"[tiab] OR "variole simienne"[tiab] and in Embase was 'monkeypox'/exp OR 'monkeypox virus'/exp OR monkeypox:ti,ab OR "monkey pox":ti,ab. In AJOL and illissAfrica, separate searches were conducted for each of

the following terms: monkeypox, variole du singe, and variole simienne. We aimed to explore how monkeypox epidemiology has evolved regarding incidence, case characteristics, clades, transmission, and case fatality rate.

We also sought to explore the risk factors for acquiring human monkeypox.

After all articles were identified from the four databases and duplicates removed, screening of the title and abstract was performed in duplicate by two researchers (EMB [author] and BVD). Articles that seemed to contain relevant data for the review objectives, which included all age populations, were selected for full-text screening. Excluded were non-human studies, modelling studies that did not provide original data, articles that focused primarily on smallpox, and articles with data not related to the topics of interest. In cases of doubt, the article was selected for full-text screening. Full text articles were then reviewed to determine whether at least one of the review objectives was met. At this stage, other articles such as conference abstracts or narrative reviews were also excluded. The first 10% of full text articles was critically evaluated in duplicate by two researchers (EMB and BVD), and the remaining 90% were reviewed by EMB. Each article was then further reviewed during data-extraction. Some additional exclusions occurred in this step. For example, for articles with similar results from largely identical data sets, only one article was included, generally the most recent. In some instances, there was a partial overlap of data, such that different articles included the same cases plus some unique cases. In these situations, only the unique cases in each article were included in the data extraction sheet. One researcher (EMB) created the data extraction sheet for the eligible articles, and these were reviewed by a second researcher (RVH). A random check of 10% of the data extraction was performed by RVH.

Articles suitable for extraction from the literature searches were case reports, outbreak investigations, epidemiological studies, and surveillance studies. For these types of articles, no formal checklists for critical appraisal are available, so no formal quality assessments were performed.

Information on study quality as reported by the authors of the selected articles were added as comments in the data extraction sheet. In addition to the four primary search sources, seven sources of grey literature and Google were searched during weeks 41–44 of 2020. These sources were the websites of the World Health Organization (WHO), specifically a review of the Weekly Bulletins on Outbreaks and other Emergencies, United States Centers for Disease Control and Prevention (CDC), Africa CDC, Nigeria CDC, African Field Epidemiology Network, Epicentre, and ProMed. The Google search was performed on the African countries known to have monkeypox cases, including a check on the websites of their ministries of health. No formal search strategy was employed, and therefore no denominator on number of reports is described. One researcher performed the search of the grey literature (LM) and a second researcher (EMB) reviewed the findings and added the relevant information to the data extraction sheet.

Pooled analysis For age at monkeypox infection, a weighted average of the median ages per decade was calculated, based on investigations where median age was reported and on single cases where age was presented. In each respective decade, these single cases were treated as a unit, and median
age determined.

Data on case fatality rate (CFR) were pooled, and 95% confidence intervals (CIs) were calculated using the binomial (Clopper-Pearson) exact method. Both overall CFR and CFR per clade were calculated. Since specific clade data were not always reported in the literature, we used the geographical spread of the clades as described by WHO [12] to assign the clade variant.

Monkeypox cases from the DRC, Gabon, Central African Republic (CAR), South Sudan, and Republic of the Congo were assumed to be of the Central African clade, while cases in all other countries were assumed to be of the West African clade. Cameroon was not included in the number of cases per clade or the calculation of CFR by clade, as WHO has reported that both clades have been detected there [12].

Case Definitions

Case definitions were not standardized across sources, but in general the definitions displayed in Table 1 were used.

Results

The search strategy yielded a total of 1,995 publications, 129 of which were selected for fulltext screening. Of these, 48 articles were suitable for data extraction. An additional 18 records from the grey literature (primarily the WHO website) were also included for data extraction.

The PRISMA flowchart of the selection process for the systematic review is in shown in Fig 1.

Number of reports by country

Monkeypox data from the DRC accounted for approximately one-third of the eligible articles [5,13,14,16–28]. The remaining articles had monkeypox data from the CAR [29–35], United States (US) [36–41], Nigeria [5,42–44], Republic of the Congo [15,45–47], Sierra Leone [42,48,49], Cameroon [5,50], Coˆte d'Ivoire [51,52], Gabon [53,54], United Kingdom (UK) [55,56], Israel [57], Liberia [42], Singapore [8], and South Sudan [formerly Sudan] [58]. (Note: two articles [5,42] described data for more than one country, therefore the total number of articles per country exceeds 48.) The 18 grey literature reports were from the CAR [59–62], DRC [63–66], Cameroon [65,67,68], Republic of the Congo [69–71], Liberia [72,73], Nigeria [68,74], UK [7], and US [6]. All but two sources [17,45] reported on the epidemiology of monkeypox; these two were peer-reviewed articles on risk factors for acquiring monkeypox.

Table1. MonkeypoxCaseDefinitions.

Typeof case	Definition
Suspected	Suddenonsetofhighfever,followedbyavesicular-pustuleeruptionpresentingpredominantlyonthe face,palmsofthehands,andsolesofthefeet;orthepresenceofatleast5smallpoxtypescabs.
Confirmed	Suspectedcasewithlaboratoryconfirmation(PositiveIgMAntibody,PCR,orvirusisolation).
Probable	Suspectedcasewithnopossibilityoflaboratoryconfirmation,butwithepidemiologicallinktoa confirmedcase.
Possible	Vesicular,pustularorcrustedrash,notdiagnosedaschickenpoxbythefamilyorthehealth-care provider[13]. Historyoffeverandvesicularorcrustyrash[14]. Individualmetoneoftheepidemiologiccriteriaordemonstratedelevatedlevelsoforthopoxvirus-specificIgMandhadunexplainedrashandfeverand≥2othersignsorsymptomsfromtheclinical criteria[15].

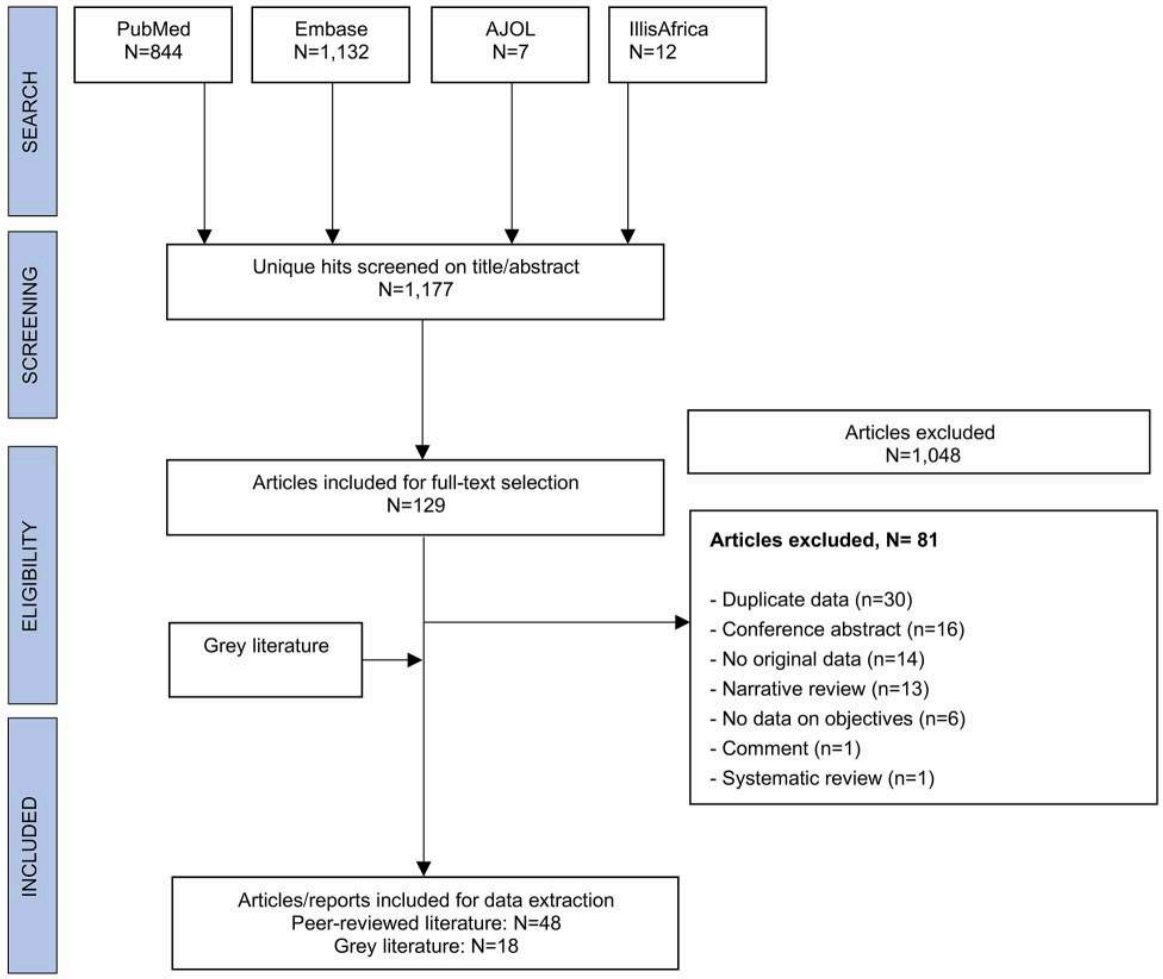

Fig1.PRISMAflowchart.

Number of cases by country

We identified 28 peer-reviewed articles [5,8,14,15, 18,20, 21,29–35,42,46–58] and 15 reports from the grey literature [6,7,59–65,67–69,72–74] with data on the number of confirmed, probable, and/or possible monkeypox cases, for a total of 1,347 cases, and an additional 28,815 suspected cases from the DRC. These data are displayed in Figs 2 – 6 (and S1 Table) by decade, starting with the 1970s, when the first cases were detected [5,42,51]. During the 1970s, a total of 48 confirmed and probable monkeypox cases were reported in six African countries, namely the DRC, Cameroon, Coˆte d'Ivoire, Liberia, Nigeria, and Sierra Leone, with most cases occurring in the DRC (n = 38) (Fig 2). In the 1980s compared to the 1970s, a 9-fold increase in the number of confirmed and probable monkeypox cases was observed in the DRC (n = 343). In addition, 14 other cases were spread among four other African countries (Fig 3). Cases continued to increase in the 1990s, with 511 confirmed, probable, and/or possible monkeypox cases reported in DRC and 9 confirmed cases in Gabon (Fig 4).

Fig2.Numberofconfirmed,probable,and/orpossiblemonkeypoxcasesbetween1970–1979.[5,42,51](baselayer ofthemap:https://datawrapper.dwcdn.net/W7k0L/4/).

Between 2000 and 2009, monkeypox cases were reported in three African countries (DRC, Republic of the Congo, and South Sudan) (Fig 5), but between 2010 and 2019, cases were found in seven African countries (Cameroon, CAR, DRC, Liberia, Nigeria, Sierra Leone, and Republic of the Congo) (Fig 6). Compared to the last three decades of the 20th century, outbreaks as of the year 2000 were greater in total number of cases and fewer in singular case reports.

The DRC is the country most affected by monkeypox, and no other country has reported monkeypox cases continuously during the past five decades. As of the year 2000, however, the number of suspected cases, rather than confirmed, probable, and/or possible cases, was primarily reported, as shown in Fig 5 (2000–2009) and Fig 6 (2010–2019). More recently, between January and September 2020, another 4,594 suspected cases were reported for the DRC [66].

The second most affected country is Nigeria, due to the 181 confirmed and probable cases from the outbreak that started in September 2017 [74]. (Note: 183 cases are noted in the Nigeria CDC report [74], but two cases originating from Nigeria were diagnosed in Israel [57] and Singapore [8] and considered travel-related events for these respective countries. The three UK cases that originated in Nigeria [7,55] are not among the 183 cases in the Nigeria CDC report.) The third and fourth most affected countries with confirmed, probable, and/or

Fig3.Numberofconfirmed,probable,and/orpossiblemonkeypoxcasesbetween1980–1989. [20,21,31,34,50,52,54](baselayerofthemap:https://datawrapper.dwcdn.net/lGHEu/1/).

Fig 4. Number of confirmed, probable, and/or possible monkeypox cases between 1990–1999. [14,53] (base layer of the map: https://datawrapper.dwcdn.net/ EAn8M/1/).

possible cases of monkeypox are the Republic of the Congo (n = 97) and the CAR (n = 69). All other African countries had less than 20 confirmed and probable monkeypox cases each in total over the past five decades.

Monkpox was not reported outside Africa until 2003, when an outbreak of 47 confirmed or probable cases occurred in the US following exposure to infected pet prairie dogs, which had acquired monkeypox virus from infected exotic animals imported from Ghana [6,40]. In recent years, there have been several travel-associated cases of monkeypox, all following exposures in Nigeria. There was one case in Israel in 2018 [57], three in the UK (two in 2018 [55]; one in 2019 [7]), and one in Singapore in 2019 [8]. A fourth case in the UK (2018) was the result of nosocomial transmission to a healthcare worker [56].

Number of cases by clade

There are two distinct genetic clades of monkeypox, the Central African (or Congo Basin) clade and the West African clade. Only 10 peer-reviewed articles [8,23,29,35,40,44,48,49,57,58] and one report from the grey literature [63] described specific data on these variants.

Fig 5. Number of confirmed, probable, and/or possible monkeypox cases between 2000–2009. [6,18,46,58,69] ? Number reflects suspected cases, since as of the year 2000, the number of suspected cases was primarily reported by the DRC. (base layer of the map: https://datawrapper.dwcdn.net/SXvj7/1/).

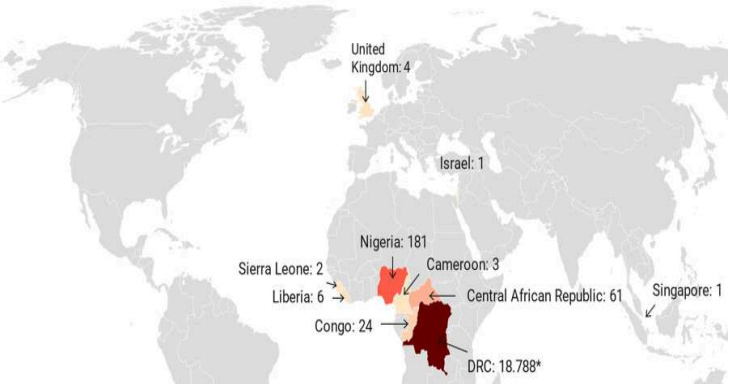

Fig 6. Number of confirmed, probable, and/or possible monkeypox cases between 2010–2019. [7,8,15,18,29,30,32,33,35,47–49,55–57,59–67,72–74] ? Number reflects suspected cases, since as of the year 2000, the number of suspected cases was primarily reported by the DRC. (base layer of the map:

Therefore, as noted above in the Methods section, in our calculation of data by clade, we separated the clades according to the geographical division as described by WHO [12]. Table 2 shows the number of cases per clade per decade. A plot of these data (Fig 7) reveals a similar pattern of evolution in the number of cases for both clades. By far,

most cases were infected with the Central African clade, which was found in the CAR [29,35], DRC [23,63], and South Sudan [58]. The outbreak in the US (2003) [40] and the outbreak in Nigeria (which started in 2017) [44] cover the largest part of the West African clade cases. This latter clade was also found in Sierra Leone [48,49] and the travel-related cases in Israel [57] and Singapore [8]. Preliminary sequencing data of two UK cases were also determined to be consistent with the West African clade [55].

Number of suspected cases versus confirmed, probable and/or possible cases Fifteen peer-reviewed articles and 12 reports from the grey literature described the number of suspected vs. confirmed, probable, and/or possible cases from the various outbreaks (S2 Table). One grey literature source [63] and all but two peer-reviewed articles [15,25] described the number of individuals tested among the suspected cases, and this varied widely from 5% to

Table2. NumberofCasesperClade [1].

Decade	CentralAfricanClade(N)	WestAfricanClade(N)	TotalCases
1970–1979	38	9	47
1980–1989	355	1	356
1990–1999	520	0	520
2000–2009	92confirmed 10,027suspected [2]	47	139 10,027
2009–2019	85confirmed 18,788suspected [2]	195	280 18,788

1 The five cases from Cameroon are not included in this table, as clade was not reported in any of the articles and WHO reported that Cameroon is the only country in which both clades have been detected [12].
2 Suspected cases are from the Democratic Republic of the Congo, as number of suspected cases rather than confirmed cases were primarily reported. Suspected cases for other countries are not reported since testing of suspected cases was generally undertaken.

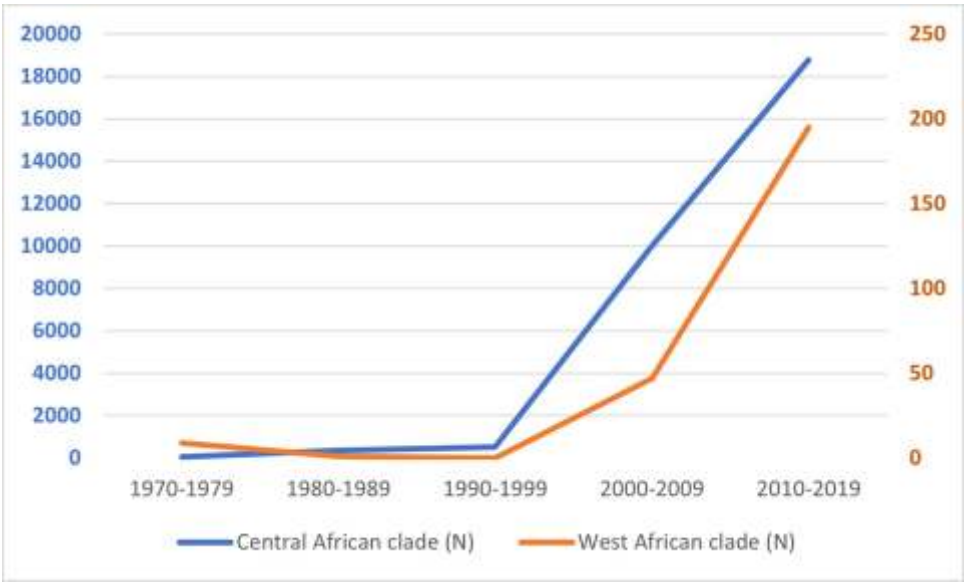

Fig7.Evolutionofnumberofcasesperclade. For2000–2019,thenumbersfortheCentralAfricancladearebased largelyonsuspectedcases,perthereportingsystembytheDemocraticRepublicoftheCongo.

100%, with the proportion of tested cases found to have confirmed or probable monkeypox ranging from 37.5% to 91.7%. In comparison, none of the 10 WHO reports describe the number suspected cases tested, and in seven of these reports [60,62,65–67,71,73], the percentage of confirmed cases among all tested and non-tested suspected cases was less than 15%.

Incidence of monkeypox

The incidence rate of monkeypox was reported in only six articles, all peer-reviewed, three with data from the DRC [13,18,28] and three from the CAR [32–34]. Surveillance data of suspected monkeypox cases in the DRC showed that the incidence increased from 0.64/100,000 in 2001 to 2.82/100,000 in 2013 (Fig 8) [18]. Even with the removal of cases from areas of active surveillance, including the Sankuru district of the DRC, the investigators found that the increases remained substantial [18]. Between November 2005 and November 2007, the average annual cumulative incidence of confirmed monkeypox from nine health zones in the Sankuru district was 5.53 per 10,000, ranging from 2.18 to 14.42 per 10,000 [28]. An overall attack rate of confirmed or probable monkeypox in a 2015 outbreak in the CAR was calculated to be 2 per 10,000 persons [32], while an outbreak in 2016 had a reported attack rate of 50 per 10,000 for suspected and confirmed cases [33].

Secondary attack rate

Only 16 peer-reviewed articles reported secondary attack rates (SARs). Details are presented in S3 Table. A review of these articles did not establish any evolution in SAR over time. More than half of the articles (9/16) reported an SAR of 0% [8,23,30,42,49–52,57], and this spanned the decades from the 1970s through 2010–2019. Similarly, over those same five decades, the SAR ranged from 0.3–10.2% in 6/16 articles [5,14,20,22,54,56]. In the remaining article, a

median SAR of 50% was reported in an outbreak among 16 households [25].

Demographic characteristics

Data on age and sex of confirmed, probable, and/or possible monkeypox cases in Africa are presented in S4 Table. Age was described in 31 peer-reviewed articles and in one report from the grey literature and the sex of individuals was presented in 27 peer-reviewed articles. As shown in Fig 9, the weighted average of the median age of monkeypox infection in Africa has evolved from 4 and 5 years old in the 1970s and 1980s to 10 and 21 years old in the 2000s and

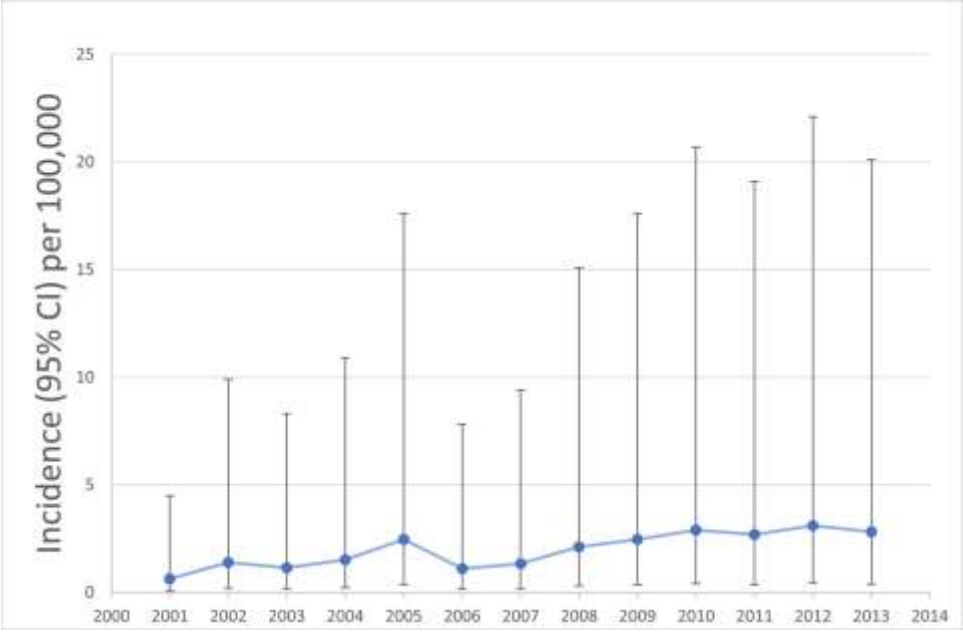

Fig8. Incidence rate of suspected monkeypox cases per 100,000 (95% CI) individuals in the DRC, 2001–2013. Data from Hoffet al [18].

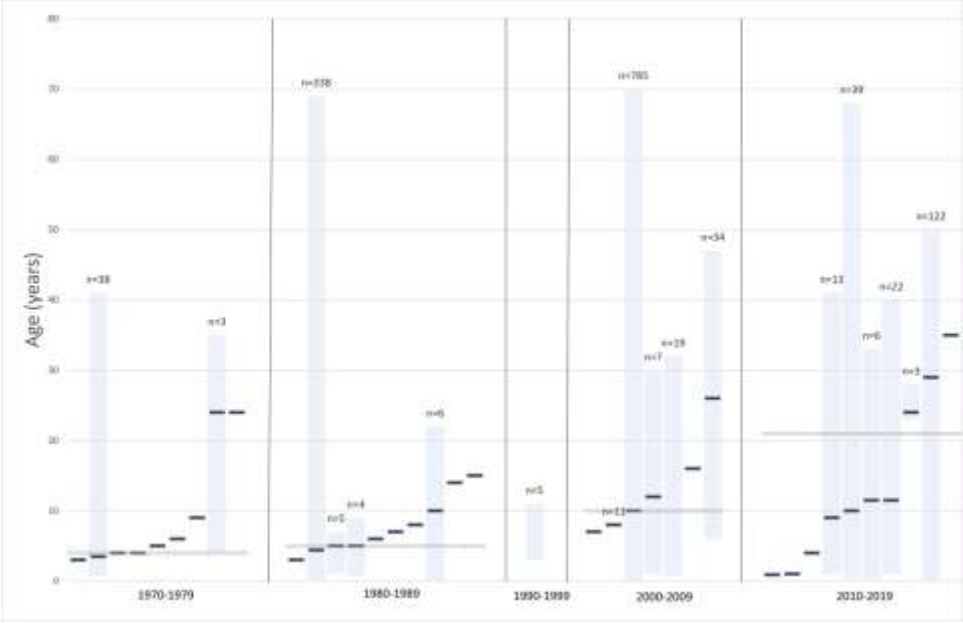

Fig9. Median age and range of confirmed, probable and/or possible monkeypox cases in Africa per decade. Blue bars without range refer to the age of a single case. The grey horizontal line represents the weighted median. No data on median ages could be retrieved for the 1990s.

2010s. Overall, males represented ≥50% of cases in most outbreaks of two or more cases as well as in singular case reports. Cases outside of Africa also occurred more frequently in males and primarily in adults [8,40,55–57]. Only nine peer-reviewed articles [8,15,23,35,44,49,55–57] reported data on occupation in confirmed, probable and/or possible cases. Commonly reported occupations included traders [44], students [44], artisans [44], healthcare professionals [35,44,56], farming [23,44,49], hunting [15], and transportation [35]. Children was listed as an occupation in 8% of 91 cases that reported occupation during the first year of the recent Nigerian outbreak (i.e., Sep 2017 –Sep 2018) [44]. Among the confirmed and further characterized US cases 10 of 34 (29%) were less than 18 years of age [40].

Smallpox vaccination status

In 21 peer-reviewed articles, information about smallpox

vaccination status was reported for confirmed, probable, and/or possible monkeypox cases. In 11 articles, describing outbreaks from 10 different countries, investigators reported that none of the 49 cases were vaccinated. These countries were Cameroon [5,50], Liberia [42], Nigeria [42], Sierra Leone [42], CAR [30,31,34], Republic of the Congo [46], DRC [23], Coˆte d'Ivoire [51], South Sudan [58], and the UK [56]. The outbreaks in these countries were small, with one to six cases per outbreak, except for the Republic of the Congo with 11 cases [46] and South Sudan with 19 cases [58]. In the 10 other articles, which reported data from outbreaks in the DRC [1981–2013] and US [2003], the proportion of monkeypox cases with a history of prior smallpox vaccination ranged from 4–21% [13,14,19–21,25,26,28,40,41], illustrating that the majority of cases (approximately 80–96%) occurred in unvaccinated individuals. The highest percentage of vaccinated cases (21%) was found in the US outbreak [40]. In a study of confirmed and suspected cases in the CAR, 19.2% (5/26) had a smallpox vaccination scar, and the overall attack rate was lower among vaccinated individuals (0.95/1000) compared to unvaccinated individuals (3.6/1000) [33].

Case fatality rates

Case fatality rates (CFR) of confirmed, probable, and/or possible cases of human monkeypox were described in 28 peer-reviewed articles and 10 reports from the grey literature, and age at death in 11 peer-reviewed articles and one grey literature report. The details of these data are described in S5 Table. Across all countries, the calculated pooled estimate CFR was 8.7% (Table 3). When the data were separated by clade, the CFR for the Central African clade

Table3. Pooledcasefatalityrateinconfirmed,probable,and/orpossiblemonkeypoxcases.

Countries/Clade	CaseFatalityRate	95%CI[1]
Allcountries [2]	78/892=8.7%	7.0%–10.8%
CentralAfricanclade [3]	68/640=10.6%	8.4%–13.3%
WestAfricanclade [4]	9/247=3.6%	1.7%–6.8%
WestAfricanclade,Africancountriesonly	9/195=4.6%	2.1%–8.6%

1 Exact binomial method (Clopper-Pearson).
2 The five cases from Cameroon are included in the "all countries" case fatality rate (CFR) calculation, but not in the calculation of CFR by clade, since WHO reported that Cameroon is the only country in which both clades have been detected [12]. The CFR without the inclusion of Cameroon is also 8.7% (77/887).
3 Central African clade includes the following countries: Central African Republic, Democratic Republic of the Congo, Republic of the Congo, and South Sudan.
4 West African clade includes the following countries: Coˆte d'Ivoire, Liberia, Nigeria, Sierra Leone, Israel, Singapore, United Kingdom, and United States.

(10.6%, 95% CI: 8.4–13.3%) was significantly higher than that of the West African clade (3.6%, 95% CI: 1.7–6.8%). When only African countries were included for the West African clade, the trend remained. All nine deaths reported for the West African clade occurred in the recent Nigerian outbreak (which had 181 confirmed or probable cases) [74]. There were no deaths in the cases outside of Africa [6,8,55–57]. Among the reports that included information on age at death, there were a total of 63 deaths (S5 Table). From the 1970s – 1990s, 100% of deaths (47/47) were in children younger than 10 years of age. Over the last two decades (2000–2019), only 37.5% (6/16) of deaths occurred in children <10 years old. A mean age of 27 years was reported for seven deaths among the 122 confirmed or probable cases of monkeypox reported in the first year of the Nigerian outbreak (September 2017-September 2018) [44].

Mode of transmission and risk factors

In 29 peer-reviewed articles, attempts were made to establish the mode of transmission for confirmed, probable, and/or possible monkeypox cases. The transmission details, by country, including number of cases and mode of transmission, are summarized in S6 Table.

A study from the 1980s involving 338 monkeypox cases from the DRC concluded that an animal source was suspected in 72.5% (245/338) of cases and a human source in 27.5% (93/338) of cases [21]. In contrast, in an investigation of 419 cases from the DRC in the 1990s, only 22% were primary cases (i.e., a person who reported no contact with another person with monkeypox), while 78% were secondary cases (i.e., monkeypox in a person who had contact with an infected person 7–21 days before onset of disease) [14]. Data from the Nigerian outbreak (Sept 2017-Sept 2018) found that transmission was unknown for 62.3% (76/122) of cases [44]. Of the remaining 46 cases, 36 or 78.3% had an epidemiological link to people with similar lesions before the onset of monkeypox and 10 or 8.2% reported contact with animals [44].

All but one of the cases outside of Africa were the result of confirmed or suspected animalto-human transmission [6,8,41,55,57]. This exception was a human-to-human transmission in the UK in a healthcare worker who provided care to one of the UK confirmed monkeypox cases [56].

The risk factors or risk behaviors for contracting monkeypox were reported in only five studies from three countries (DRC [17,22,26], US [41], Republic of the Congo [45]), and in general reinforced what has been suspected factors. For example, sleeping in the same room or bed, living in the same household, or drinking or eating from the same dish were risk behaviors associated with human-to-human transmission [22,26]. On the other hand, sleeping outside or on the ground or living near or visiting the forest were identified as factors that increase the risk for exposure to animals and subsequent risk for animal-to-human transmission of monkeypox [17,45]. Unexpectedly, assisting with toileting and hygiene and laundering clothes did not have a significant association with acquiring monkeypox, and preparing wild animal for consumption or eating duiker were identified as protective factors [26].

After adjusting for smallpox vaccination status, daily exposure to sick animals (adjusted odds ratio [aOR]: 4.0 (95% CI: 1.2–13.4) or cleaning their cages/bedding (aOR: 5.3 (95% CI: 1.4–20.7) were identified as risk factors for

acquiring monkeypox in the 2003 outbreak in the US [41].

Touching or been scratched by an infected animal sufficient to sustain a break in skin were each found to be both significant and nonsignificant risk factors [41].

Discussion

This systematic review provides a comprehensive overview of the evolution of the epidemiology of monkeypox since it was first detected in humans in 1970. Using a structured format, we describe the greater than 10-fold increase in confirmed, probable, and/or possible monkeypox cases over the past 5 decades, from 48 cases in the 1970s to 520 cases in the 1990s. Increases in the recent two decades may be confounded by the numbers coming out of the DRC, the country with the most reported cases. Beginning in the year 2000, the DRC started reporting primarily the number of suspected cases, and these have increased from >10,000 cases in 2000–2009 [18] to >18,000 in 2010–2019 [18,63–65]. In the first nine months of 2020 alone, another 4,594 suspected cases were reported in the DRC [66], and the WHO bulletin of the 12-month data for 2020, which was available following completion of this systematic review, reported a total of 6,257 suspected cases [75].

As a result of the recent outbreak, the number of confirmed and probable cases in Nigeria has dramatically escalated as well, from 3 cases in the 1970s [5,42] to 181 cases in 2017–2019 [74]. The surge in cases in the DRC from the 1990s (n = 511) through 2000–2019 (>28,000) is of a similar magnitude. The data from these two countries therefore suggest that the trend is not due to improved reporting alone. This is consistent with the analysis by Hoff and colleagues [18] who found that the rise in monkeypox cases in the DRC from 2001–2013 were likely actual disease increases and not merely a result of improved surveillance, since the reporting system was considered stable by 2008.

There are mounting concerns about the geographical spread and further resurgence of monkeypox. Over the past 5 decades, monkeypox outbreaks have been reported in 10 African countries and 4 countries outside of Africa. In addition to the re-emergence of monkeypox in Nigeria after nearly 40 years, in the years between 2010 and 2019, cases also re-emerged in Liberia and Sierra Leone (after 4 decades) and in the CAR (after 3 decades). First outbreaks emerged in the Republic of the Congo in 2000–2009 and in South Sudan (first appearance in East Africa) in 2005. From 2003, cases of monkeypox have occurred outside of Africa. Infected rodents from Ghana, a country that has not reported any human cases as of this review, were imported into the US. Animal-to-animal transmission then led to animal-to-human transmission, ultimately resulting in an outbreak of 47 confirmed or probable cases [6]. Beginning in 2018 through 2021, adults travelling from Nigeria were diagnosed with monkeypox in Israel [57], the UK [7,55,76], Singapore [8], and the US [77]. These cases were suspected to be the result of animal-to-human transmission. Three additional cases, one resulting from a nosocomial infection and two via transmission to a family member, occurred in the UK [56,76,78].

Of the four monkeypox cases imported into the UK, two have been associated with local transmission and each has resulted in either one or two subsequent cases, illustrating that infected travelers can act as index cases of local outbreaks. Interestingly, the infection imported to the UK in May 2021 [76] and to the US in July 2021[77] occurred at a time where the reported cases of monkeypox in Nigeria were at a very low level. Only 32 suspected cases of disease had been reported to the authorities since the beginning of 2021 [79]. Significant human-to-human transmission has been reported as well in the CAR [30,33,35], DRC [14,21,25], Republic of the Congo [46], South Sudan [58], and Nigeria [5,44], demonstrating the susceptibility of both clades to this type of transmission.

Mathematical modelling of human-to-human transmission found that monkeypox has epidemic potential, with R0 >1 [80].There has been much discussion about the reasons for the resurgence in monkeypox cases, the most prevailing being waning immunity, although deforestation may be a factor or can even act in potentiation [81–83]. Monkeypox virus, variola virus (smallpox), and vaccinia virus (smallpox vaccination) are closely related orthopoxviruses [1]. At the time when smallpox was rampant, no cases of monkeypox were reported. This could have been either because the focus was on smallpox and the presentation of the two diseases are similar or the lack of laboratory confirmation of the etiologic agent led to an assumption of smallpox [84].

Historical data have shown that smallpox vaccination was approximately 85% protective against monkeypox [2].

Following the successful vaccination campaign against smallpox, the disease was declared eradicated in 1980 by the World Health Assembly, and routine vaccination was halted [3].Using statistical modeling, Nguyen and colleagues [81] estimated that in 2016, the year before the outbreak in Nigeria began, only 10.1% of the population was vaccinated, and the population immunity, which takes into account waning individual-level immunity, was 2.6%, down from 65.6% in 1970. By 2018, the vaccinated population had decreased to 9.3% and the estimated population immunity had declined to 2.2%. In our review of the literature, we found that unvaccinated individuals accounted for approximately 80–96% of monkeypox cases.

One other possible factor influencing the resurgence of monkeypox might be the genetic evolution of the monkeypox virus.

An analysis of the virus genome diversity of 60 samples obtained from humans with primary and secondary cases of infection from Sankuru District, DRC, led to the detection of four distinct lineages within the Central African clade and revealed a gene loss in 17% of the samples that seemed to correlate with human-to-human transmission [85].

Our analysis shows that in the early years (1970–1989), monkeypox was primarily a disease of young children, with a median age at presentation of 4 to 5 years old; this increased to 10 years of age in 2000–2009 and 21 years in 2010–2019. Regarding age at death in monkeypox cases, 100% of deaths were in children <10 years of age in the early years, while for the years 2000–2019, age <10 years accounted for only 37.5% of deaths. These data appear to be consistent with the global intensified smallpox eradication program that began in 1967 [86] and the cease of routine smallpox vaccination by the 1980s following its eradication [3]. In the 2000s, only adults older than 20–25 years would have had a history of smallpox vaccination, leaving the age groups below 20 years vulnerable. Interestingly, the median age of monkeypox cases increased from 10 to 21 years in the next decade. Indeed, most cases were either likely too young to have been vaccinated or were born after the cessation of routine smallpox

vaccination, as in the more recent outbreaks.

Strengths and limitations

The strengths of this review are that basing on Cochrane [10] and PRISMA [11], it included a broad search strategy on monkeypox worldwide, without time or language limits, which reduced selection bias. In addition, there was a thorough review of the grey literature. Overall, more than 60 relevant sources were identified for comprehensive data extraction. There are also limitations.

First, our ability to present a complete picture of the number of confirmed, probable, and/ or possible cases was sometimes limited, as data quantity and quality varied across regions.

This is especially true for Central African countries, and in particular the DRC, where a systematic accounting and reporting of the number of cases per year is lacking. The number of cases presented in the maps in this review, especially after 1986 when WHO stopped their surveillance program in the DRC, are likely lower than the actual number of cases [28] and underreporting is quite probable, despite implementation of the Integrated Disease Surveillance and Response in the DRC in 2000 [18]. Furthermore, no national estimates of the number of confirmed cases in the DRC can be made, as polymerase chain reaction testing is infrequently performed in the field [18,25]. Second, there is a paucity of data on the age of cases, which could call into question the results of our analysis of median age at monkeypox diagnosis. For example, a report from the DRC, published after completion of this systematic review, found a median age of 14 years for confirmed cases in the Tshuapa Province during 2011–2015 [87].

These investigators did note, however, that median age at onset has increased over time [87], which corresponds to our findings. Furthermore, our review of the literature found that age at death from monkeypox has also increased, which is then consistent with our age-at-diagnosis findings. Third, since specific data on clades was infrequently reported, we assigned clades based upon the geographical spread described by WHO [12] and drew conclusions about both number of cases per clade and mortality per clade. These results did not allow for the possibility that one clade may invade other geographies or consider that factors other than clade (e.g., access to healthcare) might account for the mortality differences. Fourth, although more than half of the included articles presented data on transmission of monkeypox, in many studies not all cases could be definitively attributed to either animal-to-human transmission or human-to human transmission.

Therefore, an in-depth analysis about the proportion of cases being infected by human-to-human transmission could not be performed. Fifth, while a changing epidemiology of monkeypox could be linked to the genetic evolution of the monkeypox virus, a review of the literature on the latter was not within scope of our work. Lastly, data on risk factors for acquiring monkeypox are rather scarce, and some incongruous results were found. In one study, for example, eating duiker and preparing wild animal for food were identified as protective factors [26], which appears in contrast to factors identified in animal-tohuman transmission cases [15,29]. Thus, more formal studies of risk factors are warranted.

Conclusions

The waning population immunity associated with discontinuation of smallpox vaccination has established the landscape for the resurgence of monkeypox. This is demonstrated by the increases in number of cases and median age of individuals acquiring monkeypox as well as the re-emergence of outbreaks in some countries after an absence of 30–40 years. Further, the appearance of cases outside of Africa highlights the risk for geographical spread and the global relevance of the disease. The possibility for human-to-human transmission is a concern not just among household members, but also among providers of care to diseased individuals. In light of the current environment for pandemic threats, the public health importance of monkeypox disease should not be underestimated. International support for increased surveillance and detection of monkeypox cases are essential tools for understanding the continuously changing epidemiology of this resurging disease.

Supporting information

S1 Table. Number of monkeypox cases by decade by country. (DOCX)

S2 Table. Number of suspected cases versus confirmed, probable, and/or possible cases. (DOCX)

S3 Table. Secondary attack rate. (DOCX)

S4 Table. Age and sex of confirmed, probable, and/or possible and hospitalised cases from Africa. (DOCX)
S5 Table. Case fatality rate in confirmed, probable, and/or possible monkeypox cases. (DOCX)
S6 Table. Transmission of monkeypox. (DOCX)

Acknowledgments
The authors would like to thank Babette van Deursen (BVD), Rosa van Hoorn (RVH), Lauren Mason (LM), and Femke van Kessel (FVK), current (LM and FVK) and former (BVD and RVH) employees of Pallas Health Research and Consultancy, for assistance with performing the literature searches (BVD, LM), quality review of the findings (RVH), and creating figures and maps (FVK).

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References

1. (ICTV) ICoToV. Virus Taxonomy: 2020 Release. Available from: https:// talk. ictvonline.org/taxonomy

2. Fine PE, Jezek Z, Grab B, Dixon H. The transmission potential of monkeypox virus in human populations. Int J Epidemiol. 1988; 17(3):643–650. https:// doi.org/ 10.1093 /ije/ 17.3.643 PMID: 2850277

3. Jezek Z, Khodakevich LN, Wickett JF. Smallpox and its post-eradication surveillance. Bull World Health Organ.1987; 65(4):425–434. PMID: 3319266

4. von Magnus P, Andersen EA, Petersen KB, Birch-Andersen A. A pox-like disease in cynomolgus monkeys. Acta Path Microbiol Scand. 1959; 46:159.

5. Breman JG, Kalisa R, Steniowski MV, Zanotto E, Gromyko AI, Arita I. Human monkeypox, 1970–79. Bull World Health Organ. 1980; 58(2):165–182. PMID: 6249508

6. Centers for Disease Control and Prevention. Monkeypox.Available from:https: // www. cdc.gov/poxvirus/monkeypox/index.html

7. Public Health England. Monkeypox case confirmed in England 2019. Available from: https://www.gov.uk/government/news /monkeypox-case-confirmed-in-england

8. Yong SEF, Ng OT, Ho ZJM, Mak TM, Marimuthu K, Vasoo S, et al. Imported monkeypox, Singapore. Emerg Infect Dis. 2020; 26(8):1826–1830. https://doi.org /10.3201/eid2608.191387 PMID: 32338590

9. Beer EM, Rao VB. A systematic review of the epidemiology of human monkeypox outbreaks and implications for outbreak strategy. PLoS Negl Trop Dis. 2019 13(10):e0007791. https://doi.org/10.1371/ journal.pntd.0007791 PMID: 31618206

10. Higgins JPT TJ, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors). Cochrane Handbook for Systematic Reviews of Interventions version 6.1 (updated September 2020) 2020. Available from: www.training.cochrane.org/handbook

11. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst Rev. 2015; 4:1.https: // doi. org /10.1186/2046-4053-4-1 PMID: 25554246

12. World Health Organization. Monkeypox 2019 [updated December 9, 2019]. Available from: https://www.who.int/news-room/fact-sheets/detail/monkeypox

13. Mwanbal PT, Tshioko KF, Moudi A, Mukinda V, Mwema GN, Messinger D, et al. Human monkeypox in Kasai Oriental, Zaire (1996–1997). Euro Surveill. 1997; 2(5):33–35. https://doi.org/10.2807/esm.02.05.00161-en PMID: 12631813

14. Aplogan A, Mangindula V, Muamba PT, Mwema GN, Okito L, Pebody RG, et al. Human monkeypox—Kasai Oriental, Democratic Republic of Congo, February 1996-October 1997. MMWRMorb Mortal Wkly Rep. 1997; 46(49):1168–1171. PMID: 9408046

15. Doshi RH, Guagliardo SAJ, Doty JB, Babeaux AD, Matheny A, Burgado J, et al. Epidemiologic and ecologic investigations of monkeypox, Likouala Department, Republic of the Congo, 2017. Emerg Infect Dis. 2019; 25(2):281–289. htt ps:// doi. org /10.32 01/ eid2502.181222 PMID:30666937

16. Eltvedt AK, Christiansen M, Poulsen A. A case report of monkeypox in a 4-year-old boy from the DR Congo: challenges of diagnosis and management. Case Rep Pediatr. 2020; 20 20:8572596. https://doi. org /10 .11 55 /2020 /8 572596 PMID: 32328334

17. Fuller T, Thomassen HA, Mulembakani PM, Johnston SC, Lloyd-Smith JO, Kisalu NK, et al. Using remote sensing to map the risk of human monkeypox virus in the Congo Basin. Ecohealth. 2011; 8 (1):14–25. https: //doi. org/ 10.1007/s10393-010-0355-5 PMID: 21 069 4 25

18. Hoff NA, Doshi RH, Colwell B, Kebela-Illunga B, Mukadi P, Mossoko M, et al. Evoluti on of a disease surveillance system: An increase in reporting of human monkeypox disease in the Democratic Republic of the Congo, 2001–2013. Int J Trop Dis Health. 2017; 25(2). https: //doi. org/ 10.9734 /IJTDH /2017/35885 PMID: 30123790

19. Hoff NA, Morier DS, Kisalu NK, Johnston SC, Doshi RH, Hensley LE, et al. Varicella coinfection in patients with active monkeypox in the Democratic Republic of the Congo. Ecohealth. 2017; 14(3):564–574. https: //doi . org/10.1007/s10393-017 12 66-5 PMID: 288 9 4977

20. Jezek Z, Arita I, Mutombo M, Dunn C, NakandH, Szczeniowski M. Four generations of probable person-to-person transmission of human monkeypox. Am J Epidemiol. 1986; 123(6):1004–1012. https:// doi. org/ 10.10 93/ oxford journals. aje. a114328 PMID: 3010703

21. Jezek Z, Grab B, Szczeniowski M, Paluku KM, Mutombo M. Clinico-epidemiological features of monkeypox patients with an animal or human source of infection. Bull World Health Organ. 1988; 66 (4):459–464. PMID: 2 844428

22. Jezek Z, Grab B, Szczeniowski MV, Paluku KM, Mutombo M. Human monkeypox: secondary attack rates. Bull World Health Organ. 1988; 66(4):465–470. PMID: 2844429

23. McCollum AM, Nakazawa Y, Ndongala GM, Pukuta E, Karhemere S, Lushima RS, et al. Case report: Human monkeypox in the Kivus, a conflict region of the Democratic Republic of the Congo. Am J Trop Med Hyg. 2015; 93 (4): 718–721. https://doi.org/10.4269/ajtmh.15-00 95 PMID: 26283752

24. Meyer H, Perrichot M, Stemmler M, Emmerich P, Schmitz H, Varaine F, et al. Outbr eaks of disease suspected of being due to hum an monkeypox virus infection in the Democrat ic Republic of Congo in 2001. J Clin Micro biol 2002; 40(8):2919–2921. https: //doi. org/ 10.1128/JCM.40.8.2919-2921.2002 PMID: 12149 352

25. Nolen LD, Osadebe L, Katomba J, Likofata J, Mukadi D, Monroe B, et al. Extended human to -human transmission during a monkeypox out break in the Democratic Republic of the Congo . Emerg Infect Dis. 2016; 22 (6): 10 14–1021. https:// doi.org/10.3201 /eid2206. 150579 PMI D: 27191380

26. Nolen LD, Osadebe L, Katomba J, Likofata J, Mukadi D, Monroe B, et al. Introduction of mo nkeypox into a community and household: Ris k factors and zoonotic reservoirs in the Demo cratic Republic of the Congo. Am J Trop Med Hyg. 2015; 93(2):410–415. https://doi .org /10. 4269/ajtmh.15-0168 PMID: 26013374

27. Rimoin AW, Kisalu N, Kebela-Ilunga B, Mukaba T, Wright LL, Formenty P, et al. Endemic human monkeypox,Democratic Republic of Congo, 2001–2004. Emerg Infect Dis. 2007; 13(6):934–937. https:// doi.org/ 10.3201/eid1306.061540 PMID: 17553242

28. Rimoin AW, Mulembakani PM, Johnston SC, Lloyd Smith JO, Kisalu NK, Kinkela TL, et al. Major increase in human monkeypox incidenc e 30 years after smallpox vaccination campaig ns cease in the Democratic Republic of Congo. Proc Natl Acad Sci U S A. 2010; 107(37) :1626 2–16267. https://doi.org/ 10.1073/ pnas .1005 769107 PMID: 20805472

29. Berthet N, Nakoune´ E, Whist E, Selekon B, Burguière AM, Manuguerra JC, et al. Maculop apular lesions in the Central African Republic. Lancet. 2011; 378(9799):1354. https: //doi. org /10.1016/S0140-6736(11) 61142-2 PMID: 219 82097

30. Besombes C, Gonofio E, Konamna X, Selekon B, Grant R, Gessain A, et al. Intrafami ly transmission of monkeypox virus, Central African Republic, 2018. Emerg Infect Dis. 2019; 25(8):1602–1604. https:// doi.org /10.32 01/eid2508.190112 PMID: 31216261

31. Herve VMA, Belec L, Yayah G, Georges AJ. Monkeypox in central Africa. About two strains isolated Central African Republic. Med ecine et Maladies Infectieuses. 1989; 19(5) :32 2–324.

32. Kalthan E, Dondo-Fongbia JP, Yambele S, Dieu-Creer LR, Zepio R, Pamatika CM. [Twelve cases of monkeypox virus outbreak in Bangassou District (Central African Republic) in December 2015]. Bull Soc Pathol Exot. 2016; 109(5):358–363. https:// doi.org /10. 1007/s13149-016-0516-z PMID: 27783372

33. Kalthan E, Tenguere J, Ndjapou SG, Koyazengbe TA, Mbomba J, Marada RM, et al. Investigation of an outbreak of monkeypox in an area occupied by armed groups, Central Afri can Republic. Med Mal Infect.2018; 48(4):263–268. https://doi.org/10 . 1016 /j. medmal.2018.02.010 PMID: 29573840

34. Khodakevich L, Widy-Wirski R, Arita I. Monkeypox in the Central African Republic. Bulletin de la Societe de Pathologie Exotique et de ses Filiales. 1985; 78(3):311–320. PMID: 2992830

35. Nakoune E, Lampaert E, Ndjapou SG, Janssens C, Zuniga I, Van Herp M, et al. A nosocomial outbreak of human monkeypox in the Central African Republic. Open Forum In fect Dis. 2017; 4(4):ofx168. https: //doi. org/ 10.1093/ofid/ofx168 PMID: 29732376

36. Centers for Disease Control and Prevention. Update: multistate outbreak of monkeypox—Illinois, Indiana,Kansas, Missouri, Ohio, and Wisconsin, 2003. MMWRMorb Mortal Wkly Rep. 2003; 52(27) :642–646. PMID: 12855947

37. Centers for Disease Control and Prevention. Update: multistate outbreak of monkeypox—Illinois, Indiana,Kansas, Missouri, Ohio, and Wisconsin, 2003. MMWRMorb Mortal Wkly Rep. 2003;52(25) :589–590. PMID: 12836628

38. Centers for Disease Control and Prevention. Update: multistate outbreak of monkeypox—Illinois, Indiana,Kansas, Missouri, Ohio, and Wisconsin, 2003. MMWRMorb Mortal Wkly Rep. 2003; 52(26) :616–618.

39. Centers for Disease Control and Prevention. Update: multistate outbreak of monkeypox—Illinois, Indiana,Kansas, Missouri, Ohio, and Wisconsin, 2003. MMWRMorb Mortal Wkly Rep. 2003; 52(24) :561–564. PMID: 12816106

40. Huhn GD, Bauer AM, Yorita K, Graham MB, Sejvar J, Likos A, et al. Clinical characteristics of human monkeypox, and risk factors for severe disease. Clin Infect Dis. 2005; 41(12) : 1 742–1751. https://doi.org/10.1086/498115 PMID: 16288398

41. Reynolds MG, Davidson WB, Curns AT, Conover CS, Huhn G, Davis JP, et al. Spectrum of infection and risk factors for human monkeypox, United States, 2003. Emerg Infect Dis. 2007; 13(9):1332–1339. https:// doi. org/ 10.3201/eid1309.070175 PMID: 18252104

42. Foster SO, Brink EW, Hutchins DL, Pifer JM, Lourie B, Moser CR, et al. Human monkeypox. Bull World Health Organ. 1972; 46(5):569–576. PMID: 4340216

43. Ogoina D, Iroezindu M, James HI, Oladokun R, Yinka-Ogunleye A, Wakama P, et al.

Clinical course and outcome of human mon keypox in Nigeria. Clin Infect Dis. 2020; 71 (8) :e210–e214. https://doi.org/ 10.1093 /cid /cia a143 PMID: 32052029

44. Yinka-Ogunleye A, Aruna O, Dalhat M, Ogoina D, McCollum A, Disu Y, et al.Outbre ak of human monkeypox in Nigeria in 2017–18: a clinical and epidemiological report Lancet Infect Dis. 2019; 19(8):872–879. https://doi.org/10.1016/S1473-3099 (19) 3029 4-4 PMID: 31285143

45. Guagliardo SAJ, Doshi RH, Reynolds MG, Dzabatou-Babeaux A, Ndakala N, Moses C, et al. Do monkeypox exposures vary by ethnici ty? Comparison of Aka and Bantu suspected monkeypox cases. Am J Trop Med Hyg. 2020; 102(1):202–205. https://doi.org/ 10.4269 /ajt mh.19-0457 PMID: 31769405

46. Learned LA, Reynolds MG, Wassa DW, Li Y, Olson VA, Karem K, et al. Extended inter human transmission of monkeypox in a hospital community in the Republic of the Congo, 2003. Am J Trop Med Hyg. 2005; 73 (2):428–434. PMID: 16103616

47. Reynolds MG, Emerson GL, Pukuta E, Karhemere S, Muyembe JJ, Bikindou A, et al. Detection of human monkeypox in the Repu blic of the Congo following intensive commu nity education. Am J Trop Med Hyg. 2013; 88(5):982–985. https://doi.org/ 10.4269 /ajt mh.12-0758 PMID: 23400570

48. Reynolds MG, Wauquier N, Li Y, Satheshkumar PS, Kanneh LD, Monroe B, et al. Human monkeypox in Sierra Leone after 44-Year absence of reported cases. Emerg Infect Dis. 2019; 25(5):1023–1025. https: //doi .org/10.3201/eid2505.180832 PMID:307531 25

49. Ye F, Song J, Zhao L, Zhang Y, Xia L, Zhu L, et al. Molecular evidence of human monkeypox virus infection, Sierra Leone. Emerg Infect Dis. 2019; 25(6):1220–1222. https://doi.org/ 10.3201/eid2506.180296 PMID: 30900976

50. Tchokoteu PF, Kago I, Tetanye E, Ndoumbe P, Pignon D, Mbede J. [Variola or a severe case of varicella? A case of human variola due to mon keypox virus in a child from the Cameroon]. Ann Soc Belg Med Trop. 1991; 71(2):123–128 PMID: 1656900

51. Breman JG, Nakano JH, Coffi E, Godfrey H, Gautun JC. Human poxvirus disease after smallpox eradication. Am J Trop Med Hyg. 1977; 26(2):273–281. https://doi.org/10.4269/ ajtmh.1977.26.273 PMID: 192091

52. Merouze F, Lesoin JJ. [Monkeypox: second human case observed in Ivory Coast (rural health sector of Daloa]. Med Trop (Mars). 1983; 43(2):145–147. PMID: 6306382

53. Monkeypox, 1991. Gabon. Wkly Epidemiol Rec. 1992; 67(14):101–102. PMID: 1314067

54. Meyer A, Esposito JJ, Gras F, Kolakowski T, Fatras M, Muller G. [First appearance of mon keypox in human beings in Gabon]. Med Trop (Mars). 1991; 51(1):53–57. PMID: 1649373

55. Vaughan A, Aarons E, Astbury J, Balasegaram S, Beadsworth M, Beck CR, et al. Two cases of monkeypox imported to the United Kingdom, September 2018. Euro Surveill. 2018; 23(38).

56. Vaughan A, Aarons E, Astbury J, Brooks T, Chand M, Flegg P, et al. Human-to-human transmission of monkeypox virus, United Kingdom, October 2018. Emerg Infect Dis. 2020; 26(4):782–785. https://doi. org/10 32 01 /eid2604.191164 PMID: 32023204

57. Erez N, Achdout H, Milrot E, Schwartz Y, Wiener-Well Y, Paran N, et al. Diagnosis of im ported monkeypox, Israel, 2018. Emerg Infect Dis. 2019; 25(5):980–983. https://doi.org / 10.3201/eid2505.190076 PMID: 30848724

58. Formenty P, Muntasir MO, Damon I, Chowdhary V, Opoka ML, Monimart C, et al. Human monkeypox outbreak caused by novel virus belonging to Congo Basin clade, Sudan, 2005. Emerg Infect Dis. 2010; 16 (10):1539–1545. https://doi.org/10.3201/ eid1610.100713 PMID: 20875278

59. World Health Organization. Regional Office for Africa. 2019. Weekly Bulletin on Outbreak and other Emergencies: Week 31: 29 July—04 August 2019. Available from: https://apps. who.int/iris/handle/ 10665/326159

60. World Health Organization. Regional Office for Africa, Health Emergencies Programme. 2017. Weekly Bulletin on Outbreaks and other Emergencies: Week 21: 20–26 May 2017. Avai lable from: https://apps. who.int/iris/ handle/ 10665/255579

61. World Health Organization. Regional Office for Africa, Health Emergencies Programme. 2017. Weekly Bulletin on Outbreak and other Emergencies: Week 31: 29 July– 04 August 2017. Available from: https://apps. who. int/ iris/handle/10665/258688

62. World Health Organization. Monkeypox in Central African Republic [press release]. Relie fweb, October 14, 2016. Available from: https://reliefweb.int/report/central-african republic/monkeypox-centralafrican-republic

63. Laudisoit A, Komba M, Akonda I. Scientific report research bushmeat and monkeypox Yahuma health zone–Aketi health zone Bombongolo health area. DRC. 2016. Avail able from: https://www. researchgate . net/ profile/Anne Laudisoit/publication/ 312332722_Monkeypox_Outbreak_Investig tion_ Aketi_2016/links/587b4d0108aed3 826ae838d0/Monkeypox-Outbreak Investigation-Aketi-2016.pdf

64. World Health Organization. Regional Office for Africa. 2019. Weekly Bulletin on Outbreak and other Emergencies: Week 01: 29 Decem ber 2018–04 January 2019. Available from: https://apps.who.int/iris/ handle/10665/ 278 952

65. World Health Organization. Regional Office for Africa. 2020. Weekly Bulletin on Outbreak and other Emergencies: Week 01: 30 Dece mber 2019–05 January 2020. Available from: https://apps.who.int/iris/ handle/10665/ 3303 53

66. World Health Organization. Regional Office for Africa. 2020. Weekly Bulletin on Outbreak and other Emergencies: Week 41: 05–11 Octo ber 2020. Available from: https://apps. who.int/iris/handle/10665/ 336026

67. World Health Organization. Regional Office for Africa. 2018. Weekly Bulletin on Outbreak and other Emergencies: Week 31: 28 July—3 August 2018. Available from: https://apps. who.int/iris/handle/ 10665/273631

68. World Health Organization. Regional Office for Africa. 2020. Weekly Bulletin on Outbreak and other Emergencies: Week11:09–15 March 2020. Available from: https://apps.who. int/ iris/handle/10665/ 331451

69. The New Humanitarian. Congo: Monkeypox infects 60 in north [press release]. Reliefweb, 25 September 2007. Available from: https:// reliefweb.int/report/congo/congo monkeypox -infects-60-north

70. World Health Organization. Regional Office for Africa. 2019. Weekly Bulletin on Outbreak and other Emergencies: Week 21: 20–26 May 2019. Available from: https://apps.who.int/ iris /handle/10665/ 324950

71. World Health Organization. Regional Office for Africa, Health Emergencies Programme. 2017. Weekly Bulletin on Outbreaks and other Emergencies: Week 41: 7–13 October 2017. Available from: https:// apps.who. int/ iris/ han dle/10665/259263

72. Monkeypox confirmed in Liberia [press release]. April 11, 2018. Available from: http:// outbreaknewstoday.com/monkeypox confirmed-liberia-57035/

73. World Health Organization. Regional Office for Africa, Health Emergencies Programme. 2018. Weekly Bulletin on Outbreaks and other Emergencies: Week 1: 30 December 2017–5 January 2018. Available from: https://apps. who.int/iris/handle/10665/259809

74. Nigeria Centre for Disease Control. Nigeria monkeypox monthly situation report. Decem ber 2019. Available from: https:// reliefweb . int/sites/reliefweb.int/files/resources/5a1a98 0f21136842ba43f186b8d09e7.pdf

75. World Health Organization. Regional Office for Africa. 2021. Weekly Bulletin on Outbreak and other Emergencies: Week 4: 18–24 Janu ary 2021. Available from: https://apps. who. int/iris/handle/10665/ 338891

76. Monkeypox—United Kingdom of Great Brit ain and Northern Ireland (Nigeria) [press relea se]. 11 June 2021. Available from: https:// www.who.int/emergencies/disease-outbreak news/item/monkeypox—united-kingdom-of great-britain-and-northern-ireland-ex-nigeria

77. CDC Newsroom. CDC and Texas Confirm Monkeypox In U.S. Traveler [press release]. July 16, 2021. Available from: https:// www. cdc.gov/media/releases/2021/s0716-confirm monkeypox.html

78. World Health Organization. Monkeypox—United Kingdom of Great Britain and Northern Ireland [press release]. 8 July 2021. Available from: https://www.who. int/emergencies/disease-outbreak-news/item/ monkeypox—united-kingdom-of-great britai n-and-northern-ireland

79. Nigeria Centre for Disease Control. Monkeypox Situation Report in Nigeria. May 31, 2021. Available from: https://relief web. int/sites/reliefweb.int/files/resources/An%2 Update%20of%20Monkeypox% 20Outbreak%20in%20Nigeria_210521_21 df

80. Grant R, Nguyen LL, Breban R. Modelling human-to-human transmission of monkeypox. Bull World Health Organ. 2020; 98(9):638–640. https://doi.org/10.2471/ BLT. 19.242347 PMID: 33012864

81.Nguyen PY, Ajisegiri WS, Costantino V, Chughtai AA, MacIntyre CR. Reemergence of human monkeypox and declining population Immunity in the context of urbanization, Niger ia, 2017–2020. Emerg Infect Dis. 2021; 27(4).

82. Petersen E, Kantele A, Koopmans M, Asogun D, Yinka-Ogunleye A, Ihekweazu C, et al. Hu man monkeypox: Epidemiologic and clinical characteristics, diagnosis, and prevention. In fect Dis Clin North Am. 2019; 33(4) : 1027 – 1043. https://doi.org/10.1016/j.idc. 2019. 03. 001 PMID: 30981594

83. Simpson K, Heymann D, Brown CS, Edmunds WJ, Elsgaard J, Fine P, et al. Human monkeypox—After 40 years, an unintended consequence of smallpox eradication. Vaccine. 2020; 38(33):5077–5081.https: //doi. org/ 10.1016/j.vaccine.2020.04.062 PMID: 32417140

84. Reynolds MG, Carroll DS, Karem KL. Factors affecting the likelihood of monkey pox's emergence and spread in the post-small pox era. Curr Opin Virol. 2012; 2(3):335–343. https://doi.org/10.1016/j.coviro.2012.02.004 PMID: 22709519

85. Kugelman JR, Johnston SC, Mulembakani PM, Kisalu N, Lee MS, Koroleva G, et al. Genomic variability of monkeypox virus am ong humans, Democratic Republic of the Congo. Emerg Infect Dis. 2014; 20 (2): 232 – 239. https://doi.org/10.3201/eid2002.130118 PMID: 24457084

86. Centers for Diseae Control and Prevention. History of smallpox. Available from: https: // www.cdc.gov/ smallpox/history/history.html

87. Whitehouse ER, Bonwitt J, Hughes CM, Lushima RS, Likafi T, Nguete B, et al. Clinical and epidemiologic findings from enhanced mo nkeypox surveillance in Tshuapa Province, Democratic Republic of the Congo during 20 11–2015. J Infect Dis. 2021; 223 (11) : 18 70–1878. https://doi.org/ 10.1093/ infdis/ jiab 133 PMID: 33728469

Does Rhesus C Antigen Provide a Protective Function against HIV Infection?

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ABSTRACT

Background and Objectives: Red blood cell antigens have been reported to be involved in the epidemiology of various diseases. Other stud-ies have suggested that the Rhesus C antigen has a protective effect against HIV infection. However, there is limited information to support these findings. Therefore, this study sought to investigate whether there was an association between Rhesus C antigen and HIV infection. Materials and Method: A clinical and laboratory based analytical cross-sectional study was carried out on 165 HIV positive samples from the Centre of Excellency at the Parirenyatwa Group of Hospitals and 165 HIV negative samples from National Blood Service Zimbabwe. Rhesus blood grouping for the 5 principal Rhesus antigens (D, C, E, c and e) was done using reagents supplied by Lorne Laboratories. Results: A total of 330 HIV positive and negative samples were used for the study. One hundred and sixty-five (50%) of them were from HIV negative regular blood donors at the National Blood Service Zimbabwe and the other 165 (50%) were from HIV positive patients enrolled at the Centre of Excellency Clinic of the Parirenyatwa Group of Hospitals. Ninety-four (57%) and 71(43%) of the patients were males and fe-males respectively. Forty-four (27%) and 46 (28%) of the HIV positive patients were in the 31-40- and 41-50-year age ranges respectively. Only 2 (1%) of the patients were over the age of 70 years. The study showed no association between Rhesus C antigen and HIV infection (p>0.05). E and C antigens were the least frequent Rhesus phenotypes in both HIV negative and HIV positive samples. The total number of probable genotypes containing the C gene was also very low at 57(17%). There were 33 (20%) of HIV negative donor samples with Rhesus C antigen, compared to 21 (13%) of HIV positive patient samples. Conclusions: The study was unable to establish the link between the Rhesus C antigens with protection against HIV infection. Since Rhesus C antigen is of low frequency in the Black population, very few patients with this antigen were identified with HIV infection.

Key words: Association, HIV infection, protection, Rhesus C antigen

INTRODUCTION

The Rhesus blood group is one of the most important blood group systems in transfusion medicine practice because of its involvement in some transfusion related reactions. Apart from A and B antigens of the ABO blood group, Rhesus antigens are the next most immunogenic. There are currently more than 40 Rhesus antigens, with D, C, c, E and e being the principal antigens which are dealt with in routine Blood Transfusion Practice.1

Some antigens such as Kidd and Duffy are transmembrane glyco-proteins which are known to aid in the transportation of substances across the cell membrane.1 Although a lot has been said about blood group antigens, their exact function is still not clearly understood. Several studies are now associating these antigens with certain disease states.2, 3 Rhesus antigens are an integral part of the red blood cell membrane. Individuals with no Rhesus antigens (Rhnull) have been found to develop a haemolytic anaemia known as Hereditary Stomatocytosis.4 The structure of Rhesus antigens has been reported to be similar to that of ammonia transporter /methylamine permease (Amt/Mep). The Amt/Mep proteins are also found in yeast, bacteria and simple plants. The Amt/Mep proteins are responsible for ammo-nia transportation. This is believed to be one of the functions of the Rhesus antigens in human beings. Therefore, there is more to blood group antigens than challenges in blood transfusion alone.5

Since Rhesus antigens have been linked to possible transmembrane transportation of microorganisms, studies have also associated some of these antigens with human immunodeficiency virus (HIV) infec-tion. One of such studies linked the red blood cell antigens with pos-sible protection against HIV infection6. Some blood group antigens are known to act as receptors or ligands for bacteria, parasites and viruses and many research studies have linked red blood cell anti-gens with HIV epidemiology.7

Some studies have indicated the significance of red blood cells in the pathogenesis of HIV. Red blood cells are thought to enhance the viral infectivity by binding free viruses and viral immune complexes, thereby cross-infecting HIV-susceptible cells. The Duffy antigen receptor for chemokines (DARC) has been reported as a binding site for HIV-1 on red blood cells. This binding increases infectivity of susceptible immune cells. Another study linked Rhesus C, Lub and P1 antigens with protection against HIV infection.8, 9, 10, 11

As Zimbabwe is in one of global HIV endemic regions, and based on the above findings, this study was found appropriate to establish the role of Rhesus C antigen in HIV infection in the country. The objective of this study was to determine the frequencies of Rhesus C antigen in HIV positive and negative samples and determining its role in HIV infection.

MATERIALS AND METHODS

A clinical and laboratory based cross-sectional study was carried out on samples obtained from the Parirenyatwa Centre of Excellency (CoE) and the National Blood Services Zimbabwe (NBSZ), from February 2019 to April 2019. Ethical approval to conduct the study was granted by the Joint Research Ethical Committee of the Parirenyatwa

Group of Hospitals and the then College of Health Sciences (JREC/372/18).

Permissions to access patient data, collect blood samples and perform laboratory tests were granted by the Clinical Director of Parirenyatwa Group of Hospitals (PGH), the Director of the Centre of Excellency (CoE) and the Chief Medical Laboratory Scientist, respectively. Access to stored donor blood samples at National Blood Service Zimbabwe (NBSZ) was granted by the Planning, Information and Research Manager at NBSZ.

The study was carried out in the Safety, Health, Environment and Quality (SHEQ) laboratory at the NBSZ. Blood samples from HIV negative blood donors at NBSZ and HIV positive patients from CoE were used.

To further maintain strict confidentiality during the study, collected data was used for research purposes only. All the results were saved on memory storage devices and password protected computer that was only accessible to the researchers.

The inclusion criteria were samples from HIV negative regular blood donors and those from HIV positive CoE-enrolled patients. The exclusion criteria were samples from HIV negative and first-time blood donors.

A calculated sample size of 303 was determined using the Dobson's Formula. To maintain confidentiality, HIV positive samples were assigned new study identification numbers, and HIV negative donor samples retained their original donor identification numbers in addi-tion to new study numbers.

Rhesus phenotypes were determined on patient and donor red blood cells using commercial antibodies to the five principal Rhesus anti-gens (D, C, c, E and e). The phenotyping technique was based on agglutination for positive reactions as outlined in the Lorne Labora-tories Limited manufacturer's instructions. Positive and negative controls for C antigen were also done at the same time to validate the test results.

Data was captured and analyzed using the Microsoft Excel and IBM SPSS version 19 software, respectively. Descriptive statistics were used to analyze both categorical and continuous variables.

Comparison of proportions of blood group antigens between HIV positive and negative samples were conducted using Pearson's chi-square test ($\chi 2$), and the results were considered significant at $p < 0.05$.

RESULTS

A total of 330 HIV positive and negative samples were used for the study. One hundred and sixty-five (50%) of them were from HIV negative regular blood donors at the National Blood Service Zimba-bwe and the other 165 (50%) were from HIV positive patients en-rolled at the Centre of Excellency Clinic at the Parirenyatwa Group of Hospitals.

Ninety-four (57%) and 71(43%) of the patients were males and fe-males respectively. Most of the HIV positive patients, (44 (27%)) and 46 (28%), were in the 31-40- and 41-50-year age ranges respec-tively. Only 2 (1%) of them were over 70 years old (Table 1).

Table 1: Demographic Characteristics of HIV positive patient Samples (n= 165)

Characteristic	n	%
Participant Gender : (N=165)		
Male	94	57
Female	71	43
TOTAL	**165**	**100**
Patients Age Group: (N=165)		
0 – 10 years	8	5
11 – 20 years	22	13
21 – 30 years	17	10
31 – 40 years	44	27
41 – 50 years	46	28
51 – 60 years	20	12
61 – 70 years	6	4
71 – 80 years	2	1
TOTAL	**165**	**100**

The study showed no association between Rhesus C antigen and HIV infection ($p>0.05$) (Table 2). The C and E antigens were the least frequent Rhesus phenotypes in both HIV negative and positive sam-ples (Tables 2 & 3). There were 33 (20%) of HIV negative donor samples with Rhesus C antigen compared to 21 (13%) of HIV posi-tive patient samples. The total number of probable genotypes con-taining C gene were also very low at 57(17%) (Figure 1).

Table 2: Distribution of Rhesus antigens in HIV Negative and Positive blood samples.

Principal Rhesus antigens	HIV-negative (n=165) n (%)	HIV-positive (n=165) n (%)	p value
D	157 (95.2)	158 (95.8)	0.792
C	34 (20.6)	27 (16.4)	0.321
c	161 (97.6)	163 (98.8)	0.410
E	26 (15.8)	31 (18.8)	0.467
e	162 (98.2)	164 (99.4)	0.314

Table 3: Rhesus phenotypes in HIV Negative and Positive blood samples

Phenotypes	HIV-Negative (n=165) n (%)	HIV-Positive (n=165) n (%)
D, c, e	115 (70)	116 (70)
D, c, E, e	14 (8)	25 (15)
D, C, c, e	19 (12)	18 (11)
D,C, c, E, e	9 (5)	3 (2)
D, C, e	5 (3)	0 (0)
D, c, E	3 (2)	3(2)

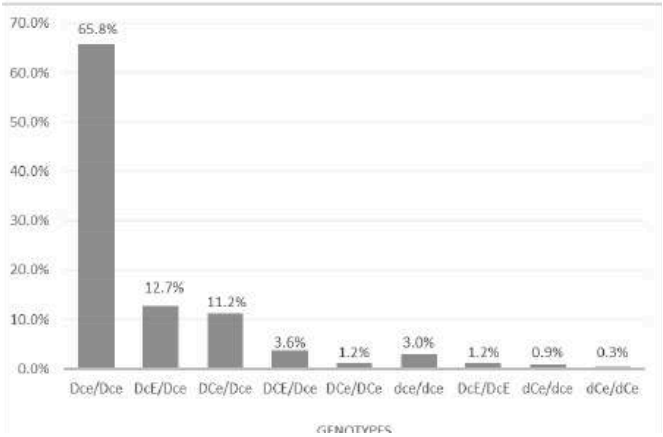

Figure 1: Frequencies of most probable Genotypes in study samples (n=330).

DISCUSSION

Although the calculated sample size was 303, the total number of participant samples exceeded this. Therefore, the results were not affected by the sample size. An equal number of HIV negative donor samples and HIV positive patient samples were used to justify the outcome of the study results.

There were more male than female patients with HIV infection. Some studies have shown that women have lower risk of HIV/AIDS than men.12 However, previous studies have shown that Zimbabwe-an females were at higher risk of HIV infection than males, and women in Sub-Saharan Africa carried a disproportionally high bur-den of HIV/AIDS.13, 14 The majority of HIV positive patients were in the 31-50 years age range. This could be a direct result of increased sexual activities associated with the age group, as indicated in a previous study in the country.15 Only two HIV positive patients lived beyond 70 years. This could be due to strict antiretroviral (ARV) treatment adherence. There has been a remarkable improve-ment in life expectancy of people living with HIV across the globe1.6

The study showed no association between Rhesus C antigen and HIV infection, although some studies have indicated an association of blood group antigens with HIV epidemiology.6 In the general population, the frequencies of Rhesus C and E antigens are very low. Therefore, the apparent association of Rhesus C antigen with HIV negativity had more to do with the antigen's low prevalence in the general population, especially in Blacks.1 Rhesus C and E anti-gens are antithetical to Rhesus c and e respectively. That is, Rhesus C and c antigens are always present together or one of them may be present, and there is never a situation where both are absent. The same applies to Rhesus E and e antigens. Since Rhesus c and e are high frequency antigens, Rhesus C and E are respectively very low frequency antigens in the Black population.1, 17, 18 In a similar study on the role of Rhesus D antigen, Rhesus D positive people appeared to be more susceptible to HIV infection than their Rhesus D nega-tive counterparts. It was argued that more than 95% of Black people have the D antigen. Therefore, it was expected to have more HIV positive people in Rhesus D positive than Rhesus D negative indi-viduals.19

The exact role of Rhesus antigens remains largely unknown, except for the removal of ammonia from the red blood cells by the Rhesus associated antigen (RhAG) and the maintenance of cell membrane. Another possible function of Rhesus antigens is transportation of carbon dioxide (CO2) and oxygen (O2).4, 20 It has also been argued that Rhesus antigens, as non-glycosylated transmembrane proteins, may prevent any interaction of the antigens with HIV virus.18, 21

The probable genotypes containing C gene were very low. This finding is consistent with frequencies of C gene in the Black popula-tion as published in many literature sources. However, in a recent study, the frequency of Rhesus C antigen in an Indian population was found to very high while that of Rhesus c antigen was low.21, 22, 23 Therefore, it appears the distribution of Rhesus blood group antigens vary according to ethnic groups.

Limitations: The study excluded other antigens, P1 and Lub that were also thought to be associated with possible protection against HIV infection.

CONCLUSION

The study was unable to establish the link between Rhesus C antigen with protection against HIV infection. Since Rhesus C antigen is of low frequency in the Black population, very few HIV positive pa-tients with this antigen were identified. The study also showed de-creased frequencies of Rhesus C gene in created probable genotypes in both Rhesus positive patient and HIV negative donor samples in the study.

RECOMMENDATIONS

The role of red blood cell antigens is still a topical issue which re-quires continuous research work. Therefore, more research should be carried out in this area in different ethnical population groups.

REFERENCES

1. Walker RH, Hoppe PA, Judd WJ, Polesky HF, Ness P, Rolih SD et al., 1990. ABO, H and P Blood Groups and Structurally related antigens and Other Blood Groups. Technical Manual; (10th Edition): 173-247.

2. Batool Z, Durrani SH, Tariq S, 2017. Association Of ABO and Rh Blood Group Types with Hepatitis B, Hepatitis C, HIV and Syphilis Infection: A Five Year' Experience In Healthy Blood Donors in a Tertiary Care Hospital. Journal of Ayub Medical College Abbottabad JAMC; 29(1):90–92.

3. Mandisodza A, Chakachaka K, Maramba A, Mangezi W, Chikwasha V, Zuze M, 2016. Changes in ABO blood group frequencies in mental health patients in Zimbabwe. Journal of Applied Science in Southern Africa; 22 (2):1-8.

4. Westhoff CM, 2007. The Structure and Function of the Rh antigen Complex. Seminars in Hematolology; 44(1):42–50.

5. Marini AM, Matassi G, Raynal V, André B, Cartron JP, Chérif-Zahar B, 2000. The human Rhesus-associated RhAG protein and a kidney homologue promote ammonium transport in yeast. Nature Genetics; 26(3):341.

6. Motswaledi MS, Kasvosve I, Oguntibeju, 2013. The Role of Red Blood Cells in Enhancing or Preventing HIV Infection and other Diseases [Internet]. BioMedi-cal Research International. 2013 [cited 2018 Sep 13]. Available from: https:// www.hindawi.com /journals /bmri/2013/758682/

7. Abdulazeez A, Alo E, Rebecca S, 2008. Carriage rate of Human Immunodefi-ciency Virus (HIV) infection among different ABO and Rhesus blood groups in Adamawa state, Nigeria. BioMedical Research International; 19 (1):41–4.

8. Hafeez-ud-din, Siddiqui TS, Lahrasab W, Sharif MA, 2012. Prevalence of Hepa-titis B and C in healthy adult males of paramilitary personnel in Punjab. Journal of Ayub Medical College Abbottabad; 24(3- 4):138–40.

9. Lund N, Olsson ML, Ramkumar S, Sakac D, Yahalom V, Levene C, et al., 2009. The human Pk histo-blood group antigen provides protection against HIV-1 infection. Blood Reviews; 113(20):4980–91.

10. Bamisaye O, Akanni O, Akinbo D, 2017. Association between Blood Group Antigens, CD4 Cell Count and Haemoglobin Electrophoretic Pattern in HIV Infection. International Journal Life-Sciences Research; 3(1):1300–4.

11. Motswaledi MS, Kasvosve I, Oguntibeju OO, 2016. Blood Group Antigens C, Lub and P1 may have a role in HIV infection in Africans. Blackard J, editor. PLOS ONE; 11(2):e0149883.

12. Jarrin I, Geskus R, Bhaskaran K, Prins M, Perez-Hoyos S, Muga R, et al., 2008. Gender Differences in HIV Progression to AIDS and Death in Industrialized Countries: Slower Progression following HIV Serocon version in Women. Ameri-can Journal of Epidemiology; 168 (5):532–540. https://doi.org/10.1093/aje/kwn179

13. ZIMBABWE-Factsheet.FIN.pdf [Internet]. [cited 2018 Oct 22]. Available from: https: // phia. icap. columbia. edu/wp-content/uploads/2016/11/ZIMBABWE-Factsheet.FIN_.pdf

14. Magadi MA, 2011. Understanding the gender disparity in HIV infection across countries in sub-Saharan Africa: evidence from the Demographic and Health Surveys. Sociol Health Illn; 33(4): 522–539. doi: 10.1111/j.1467-9566.2010.01304.x

15. Parirenyatwa DP et al., 2004. Age and Sex Distribution; The HIV and AIDS Epidemic in Zimbabwe: 1-56.

16. Katz IT, Maughan-Brown B, 2017. Improved life expectancy of people living with HIV: who is left behind?; Open Access; https://doi.org/10.1016/S2352-3018(17)30086-3

17. Bethesda DL, 2005. Blood Groups and Red Cell Antigens [Internet]: The Rh blood group. https:// www.ncbi.nlm.nih.gov/books/NBK2269/

18. Sonneborn H, Voak D, 1997. A review of 50 years of the Rh blood group system. Biotest Bulletin; 5 (4):389-552.

19. Mandisodza A, Siduna M, Musekiwa Z, Mvere D, Mapako T, 2010. The role of Rhesus D antigen in HIV Infection: Is it a possible receptor for the virus?. Journal of Applied Sciences in Southern Africa (JASSA); (Special Issue):43-47.

20. Conroy M , Bullough PA, MerrickM, Avent ND, 2005. Modeling the human rhesus proteins: implications for structure and function. BJH; https:// onlinelibrary. wiley.com/doi/pdf/10.1111/j.1365-2141.2005.05786.x

21. Lund N, Olsson ML, Ramkumar S, Sakac D, Yahalom V, Levene C, et al., 2009. The human Pk histo-blood group antigen provides protection against HIV-1 infection. Blood Reviews; 113(20):4980–91.

22. Flegel W, 2007. The genetics of Rhesus blood group system. Blood Transfusion; 5(2):50-57. doi: 10. 2450 /2007.0011-07.

23. Makroo RN, Bhatia A, Gupta R, Jessy P, 2013. Prevalence of Rh, Duffy, Kell, Kidd & MNSs blood group antigens in the Indian blood donor population. Indian Journal of Medical Research; 137 (3): 521-526.