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COINFECTION OF MALARIA AND INTESTINAL PARASITES AMONG SCHOOL CHILDREN IN AJAGBA, SOUTHWESTERN NIGERIA

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ABSTRACT

Concomitant parasitic infections in developing world is increasing. In this study, the extent of co-infections were investigated .Three hundred consenting individuals consisting of 136 males and 164 females participated in this study. Feacal specimens and venous blood were collected from the participants. The formol-ether concentration method were used to screen the feacal samples for helminths and protozoans, while Giemsa-stained blood smears was used for malaria parasite and packed cell volume (PCV) was determined by hematocrit. The prevalence of Malaria parasite , Hookworm , *Ascaris lumbricoides, Strongyloides stercoralis, Entamoeba histolytica* were 27.3%, 24.6%, 8.7%, 6.6%, 6.6% respectively. Females (55.0%) were generally more infected with all parasite than the males (45.1%) and it is statistically significance (p=0.000). Co-infection of parasites were observed as follows; Hookworm, *Ascaris lumbricodes* and Malaria parasite (2.7%), Hookworm, *Entamoeba histolytica* and Malaria parasite (0.7 *histolytica* %), Hookworm, *Ascaris lumbricodes*, Malaria Parasite and *Entamoeba histolytica* (1.3%), *Entamoeba* and Ascaris *lumbricodes* (0.7%), Hookworm and *Strongyloides stecoralis* (2.0%), *Ascaris lumbricodes* and hookworm (3.3%),Hookworm and Malaria Parasite (3.3%), *Ascaris lumbricodes* and Malaria (2.7%), *Entamoeba histolytica* and Malaria (2.0%), *Ascaris lumbricoides* and *Strongyloides stercoralis* (0.7%) and Ascaris , *Strongyloides stercoralis* and Malaria Parasite(0.7%).The overall Mean PCV of the population was 29.40±5.16 and it statistically significant(p = 0.029). These result showed the existence of polyparasitism in Ajagba community and it is a major public health problem hence there is need for improved environmental condition which includes clean water supplies, periodic deworming of children and action against deficiency in sanitary facilities, poor personal hygiene.

KEYWORDS: Multiple Parasitic Infections, School Children, Ajagba, Nigeria.

INTRODUCTION

Parasitic infection causes serious infection among children and elderly people, over two billion people worldwide are affected. Parasitic infections are among the major diseases of concern to public health throughout the world. This disease can affect child development, educational achievement, reproductive, health, social and development. Effort to control parasitic infection in developing countries are targeted at specific risk group e.g. children. Nevertheless the re-infection in endemic area is continuous.[1] Some of the parasite responsible are associated with severe morbidity often resulting in mortality, particularly in less developed tropical and subtropical countries.[2] Amoebiasis, Giardiasis, Ascariasis, Ancylostomiasis, Malaria are among the most common parasitic infections worldwide and are closely related to socio-economic status, poor sanitation, inadequate medical care and absence of safe drinking water supplies.[3,4] Infections are mostly common among children of tropics and developing countries where they are perpetuated by contamination of soil with human faeces and the use of untreated human faeces as fertilizer.[5] Malaria is a Mosquito-borne infectious disease of humans and other animals caused by parasitic protozoans (a type of single cell microorganism) of the *plasmodium* type and it is transmitted by female Anopheles mosquito. Malaria caused by this specie is the most dangerous form of malaria[6] with the highest rate

of complication and mortality. Malaria causes symptoms that typically include fever, fatigue, vomiting and headaches. In severe cases it can cause yellow skin, seizures, coma or death.[7] These symptoms usually begin ten to fifteen days after being bitten. A number of features account for their occurrence which includes a ubiquitous distribution, the durability of their eggs under a favourite of environmental condition, high numbers of eggs produce by a protozoan per day. Transmissions is enhanced by the fact individual can be asymptomatically infected and continue to shed eggs for years yet prior infection does not confer protective immunity.[8] Factors which increase the likelihood of ingesting material contaminated with faecal material play a role in the transmission of this intestinal parasite. Generally, situation involving close human–human contact and unhygienic conditions promote transmission.[9] Variability in human behaviour ,unskilled social practice and traditions can be an obstacle in controlling directly transmitted parasitic infection, Although disease control models have established that holistic approaches is the key effective and sustainable intervention against endemic diseases, especially those whose transmission is inexplicably linked to poverty, poor sanitation, low standard of living.[5]

Generally, symptoms signalling the presence of a parasite are related to the intensity of infection. Thus, a light parasitic infection is often asymptomatic whereas a mild to heavy infection can be associated with painful and severe symptoms. However, subtle damage and dysregulation can occur in the absence of any noticeable infection. Summary findings of two major studies of the literature found that in humans: The effects of parasite depend on the species, the mixture of species, and the duration of infection; The distribution of parasite among hosts is highly skewed such that a minority of individuals, almost entirely young, have moderate-to-heavy infections and are more likely to be clinically affected; The intensity of infections depends on the size and nutritional status of the host; Treating of infection can lead to improvements in growth and nutritional status, but the treatment alone does not treat any underlying nutritional deficits that have been caused or made worse by the predominant mechanisms by which parasite harm human hosts. Feeding on host tissues, including blood, leading to a loss of iron and protein. causing mal-digestion or mal-absorption of nutrients. Provoking inflammatory responses that may affect appetite and food intake or modify the metabolism and storage of key nutrients such as iron. Eliciting typical responses to infection, such as fever and increased metabolic rate. Eliciting immune responses to infection (which results in the diversion or use of nutrients and energy for purposes that would not have been necessary had parasite not been present). Although it is likely that impaired child development operates through the mechanisms outlined above, the causal links underlying stunted physical and intellectual development are still not well established. Polyparasitism, or co- infection with either multiple parasitic infection in the young is widespread.[10] There is evidence of Plasmodium (Malaria)-Helminth is associated with increase Anemia. Co-infection between the helminth themselves or with protozoa and the synergistic impact on the health and nutritional status of the host is not well understood and is understudied.[11] Similarly, there are few studies on the impairment of human or child development caused by chronic infection with parasite. In one study, children with *E.histolytica*-associated diarrhoea during the first 2 years of life were 2.93-times more likely to be malnourished and 4.69-times more likely to be.[12] Another study demonstrated that malnutrition and amoebic dysentery were associated with cognitive deficiencies.[13] However, it is conceivable, given the known consequences of parasitic infection generally such as malabsorption and malnutrition, that medium to high-intensity infections would have similar effects on a child. Since the presence of parasitic infection and the intensity of infection determine the risk for disease formation and their cognitive performance of children, in which any health impairment is substantially magnified in terms of individual patient performance status, especially in the rural developing world. It is therefore necessary to carry out research work on multiple parasitic infections among children in Ajagba community of Oyo so as to determine the prevalence and impact of multiple parasitic infections in Ajagba community in Oyo east local government, Oyo state, Nigeria.

MATERIALS AND METHODS

Study area and Population

The study area was Ajagba in Oyo East local government area of Oyo state Nigeria. Ajagba is about 5km from Kosobo, the Headquarter of Oyo East local government. Ajagba is located along Oyo/Iwo road, within rainforest zone, between latitude 161850.832 and 184759.488 and longitude 7^{0}58N, with the population of about 1,500 (National Population Commission, 1991). The area is rural and lack basic amenities such as standard hospital and adequate facilities for refuse and sewage disposal. There is a river which serves as a source of water for bathing other domestic activities. Members of the community are predominantly farmers.

Ethical consideration

Ethical Approval was taken from the local government. The parasitological survey was preceded by a pre-survey contact during which permission was obtained from the Director of Primary Health Centre of Local Government and the paramount ruler of the village. Informed consent was also obtained from all consenting individuals that participated in the study.

Methods of data collection

The study commenced after taken approval from the local government, duly signed informed consent was obtained from the parents of the children when they have understood the study and were willing to enrol. After

consenting, clinical and demographic data were obtained through questionnaire and interview from literate and illiterate parents respectively.

Sample Collection and Analysis

All subject samples were collected and fresh stool sample was obtained in a dry, clean, sterile, and well-sealed plastic container with a top cover, including a self-spoon to collect the sample, leak proof plastic container and there corresponding blood sample was collected in EDTA bottle. In addition, each container was labelled with names recorded. . A total of 300 stool samples were collected from children ranging from age 1 to 15 years.

Blood film Determination for Malaria Parasites Load

The blood smears were fixed stained with Giemsa and examined according to standard procedure. The slide was considered positive when malaria parasite were detected in the blood smear. A blood smear was considered negative when no parasite was detected after examination of 200 oil-immersion fields on thick smear. When malaria parasites were detected in the blood film, parasite density was determined by counting the number of parasite per 200 white blood cell (WBC) in thick smear and multiply by 40 to obtain an approximate parasite count of 8000/ul of blood, considered as a representative standard value allowing calculation of parasite count to identify plasmodium species.

Procedure for Thick and Thin Blood Film

A drop of blood was placed on clean grease free slide and it was spread about 2mm thickness and allowed to air dry.1:30 dilution of giemsa stain was poured on it and allowed to stain for 15mins.It was rinsed after 15mins and allowed to air dry .The slides were then viewed using x100 objective (Oil immersion microscopy).

A drop of blood was placed very close to the edge of a clean grease free slide. A spreader was used to make a thin film by placing it at an angle of 45, the smear was air dried, stained and was rinsed. The slides were viewed using x100 objective (Oil immersion microscopy).

Diagnosis of Intestinal parasite

Macroscopic examination was done as immediately the stool specimens were received, the consistency that is formed, loose or watery were checked. A drop of saline and iodine was placed on different slides and a small amount of faeces were placed on microscope slide using applicator stick, and mixed and covered with cover slips. The samples were examined microscopically at X10 and X40 power magnifications. Concentration of samples was carried out using formol ether concentration method.[14] Using applicator stick, estimated 1g (or 2ml if it's a fluid specimen) of each participants specimen were emulsified in 4ml of 10% formol water contained in a screw cap bottles. Further, 3ml of 10% v/v formol water was added and the bottles were capped, and mix very well by shaking. The emulsified faeces were sieved and the sieved suspensions were collected in new bottles. The suspensions were then transferred into centrifuge tubes, and 3ml of diethyl ether was added. The tubes were covered and mixed for 1minute. Using tissue wrapped around the top of the tubes, the covers were loosed to reduce the pressure that has developed inside the tubes. The tubes were then centrifuged immediately at1000g (3000rpm) for 1 minute.

After centrifugation, the faecal debris were loosed from the side of the tubes using applicator sticks, the tubes were then completely inverted to discard the ether, faecal debris and formol water with the sediments still in the tubes. The sediments were examined microscopically using10x objective and 40x objective for the saline preparation. For Packed Cell Volume (PCV), Microhematocrit capillary tubes filled with blood and sealed at one end were centrifuged in a microheamatocrit rotor for 5mins at 10,000g. Statistical analysis was carried out using SPSS version 15 software. A significant level of 0.05 was used for test.

RESULTS

From 300 participant, (136 males and 164 females) with the Mean Packed Cell Volume (PCV) was 29.40±5.16. A.*lumbricoides* + Hookworm (3.3%) and Hookworm + Malaria parasite (3.3%) was the most prevalent co-infection in the study area, followed by A.*lumbricoides*+Malaria parasite+Hookworm (2.7%), A.*lumbricoides* + Malaria parasite (2.7%),Hookworm+ *Strongyloides stercoralis* (2.0%), *E.histolytica*+Malaria parasite(2.0%), Hookworm+*A.lumbricoides*+Malaria parasite +*E. histolytica* (1.3%), Hookworm + E.histolytica + Malaria parasite (0.7%), *E. Histolytica* + *A.lumbricoides* (0.7%), *Strongyloides stercoralis* + Malaria parasite (0.7%) (Table1). The prevalence Malaria parasite (27.3%) was the highest among the parasites found from the collected samples, followed by Hookworm (24.7%) then A.*lumbricoides* (21.3%), the least prevalence was E.*histolytica* (6.6%) and *Strongyloides stercoralis*(6.6%) as shown in Table 2. Also according to age related prevalence, the total number examined in age group <5,6-10 and 11-15 were 106, 134 and 60 giving the number infected as 56 (52.8%), 92 (68.7%) and 34 (56.7%).Also among the infected subjects in age 6-10years had the highest infection (50.6%) followed by children in age group 1-5 with 30.8% and the least infection of 18.7% in age group 11-15.The difference in the rate of infection with respect to age was statistically significant $p<0.05$ (Table 3). In Table 4, the prevalence rate of infection among the infected children in the community by sex as shown in table 4 revealed that out of 136 males examined 82 were infected with the prevalence of 60.3% while in 164 females examined only 100 were infected with prevalence of 61.0%. The overall prevalence of infection according to sex is 60.7%.The difference was statistically significant ($p<0.05$). Table 5 shows the mean PCV of infected and non infected subjects, the mean PCV of non-infected is greater than the PCV of infected subject

and the P-value 0.029 which is statistically significant. Table 6 shows the frequency of the multiple infections among the subjects. From the result it was deduced that children with single parasitic infection are of greater percentage than children with multiple infections. Figure 4.1 shows the frequency of subject examined by age range, in which 6-10 years old had the highest number (92) followed by 1-5 years old (56) and age 11-15 years (34) for parasitic infection. Figure 2 represents the PCV of all examined individual. Figure 3 shows the frequency of the subject with single parasite (121), Double (45), three (12) and four (4). Figure 4 represents the frequency of infected and non infected subjects examined.

Table 1: Distribution pattern of multiple parasites among the study area

Parasites	Frequency=300	Percentage
Hookworm+A.l+M.P	8	2.7
Hookworm+E.h+M.P	2	0.7
Hookworm+A.l+M.P+E.h	4	1.3
E.h+A.l	2	0.7
Hookworm +S.s	6	2.0
A.l +Hookworm	10	3.3
Hookworm+M.P	10	3.3
A.l+M.P	8	2.7
E.h +M.P	6	2.0
A.l+S.s	2	0.7
A.l+S.s+M.P	2	0.7

Keys: M.P= Malaria parasite, S.s=*Strongyloides stercoralis*, A.l=*Ascaris lumbricoides*, E.h=*Entamoeba histolytica.*

Table 2: Distribution pattern of the single parasite among the study area

Parasites	Frequency=300	Percentage (%)
Hookworm	74	24.7
E.h	20	6.7
M.P	82	27.3
A.l	64	21.3
S.S	20	6.7
No parasite	118	39.3

Keys: M.P= Malaria parasite, S.s=*Strongyloides stercoralis*, E.h=*Entamoeba histolytica.*

Table 3: Age related prevalence of parasite infection among subjects

Age(years)	No examined	No infected	Percentage (%)	p-value
< 5	106	56	52.8	*0.000
6-10	134	92	68.7	
11-15	60	34	56.7	
Total	300	182	60.7	

*p-value <0.05 considered statistically significant.

Table 4: Gender related prevalence of parasites infected among subjects

Gender	No examined	No infected	Percentage (%)	P-value
Male	136	82	60.3	*0.000
Female	164	100	61.0	
Total	300	182	60.7	

*p-value <0.05 considered statistically significant.

Table 5: Mean Pcv Of Infected And Non Infected Subject

Variable	No	Mean±SD	T-value	P-Value
Infected subjects	182	28.90±5.18		
			2.19	*0.029
Non infected subjects	118	30.25±5.05		
Total	300	29.40±5.16		

*p-value <0.05 considered statistically significant.

Table 7: Frequency of multiple infections among the children

	FREQUENCY	Percent
No parasite	118	39.3
Single	121	40.3
Double	45	15.0
Three	12	4.0
Four	4	1.3
Total	300	100.0

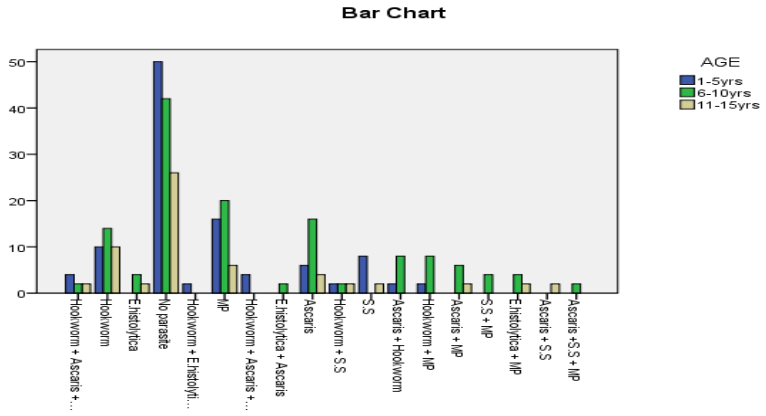

Figure 1: Showing the co-infection in relation to age.

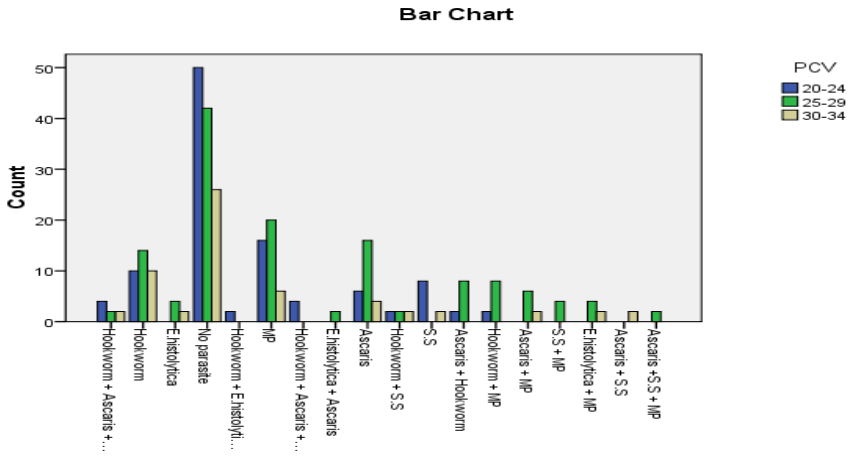

Figure.2: Showing the association between PCV and infection.

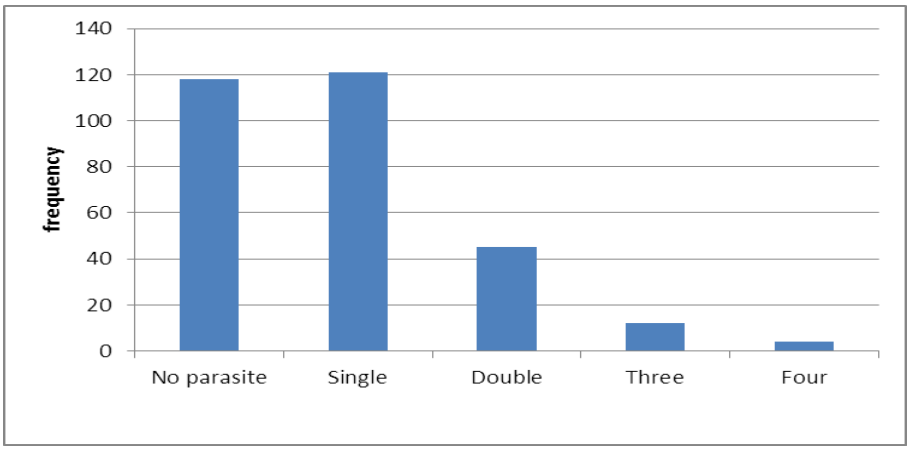

Figure 3: Showing the level of co-infection.

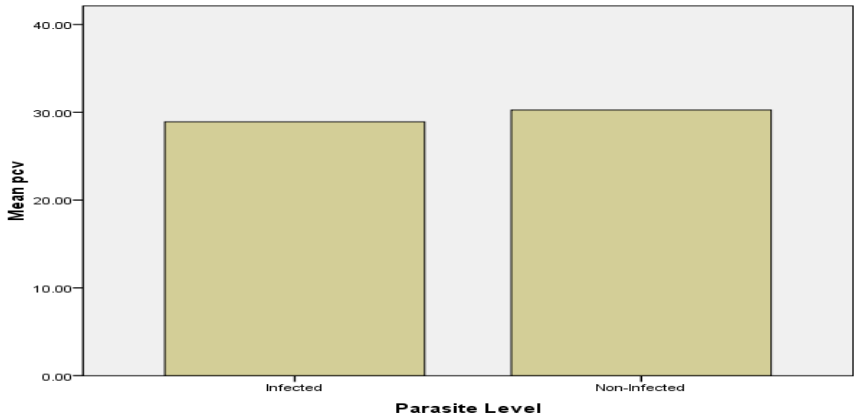

Figure 4: Showing infection in relation to mean PCV.

DISCUSSION

Polyparasitism causes a significant and additive problem against the host and it is a major public health problem particularly in the sub-Sahara Africa. Understanding the epidemiology and impact of these infections within the community is essential in the design and implementation of control strategies by policy maker. Result of this study indicates a prevalence of 20.1% of polyparasitism in the study area. This means only 60 out of 300 subjects examined were infected, which is similar to a study undertaken by[15] in which a prevalence of 31.8% was recorded. This prevalence were considered higher compared to prevalence rate of previous studies undertaken by[16] which is 8%. Other studies associated high prevalence to poor environmental and personal hygiene, shortage of good drinking water supply and indiscriminate defecation. The water they drink i.e. stream water attribute to the transmission of infectious disease. One of the most prevalent of case of polyparasitism in this study involved *Ascaris lumbricoides* and Hookworm (3.3%); Hookworm and Malaria parasite (3.3%). According to[17] parasitic infection like malaria is directly related to environmental situation observed in an area which promotes the transmission of malaria especially during rainy season and,[18] had observed that malaria is holo-endemic with year round transmission peak. The prevailing water in the community might also be responsible for high involvement of malaria in the polyparasitic infection cases encountered but other factors may include ignorance, poor sanitation and poverty. The prevalence was observed to be higher in females than in male in the study area which is statistically significant. This difference appears to confirm the possible roles of peculiar female activities that enhance relative contact with the infective stages of the parasites.

Multiple parasitic infection or co-infection of Malaria, hookworm, *Ascaris lumbricoides* were prevalent among the participant in the study area. Similar types of co-infection of malaria and intestinal helminths have been reported in North –western Tanzania[19] and other countries of sub-Sahara Africa[20] and this maybe as a result of endemicity level of parasites in the study area. The age group 6-10 years had more infection than other age groups. This is a reflection of the exposure patterns in view of the fact they are more active, adventurous and mindless of hygienic habits. Therefore, the infection rates in the less motile age 1-5 are due to reduced exposure to the infective stages of parasite. However, age group 11-15 may be a little bit conscious of them and listen to instruction of washing hands before eating. This could be attributed to low prevalence of those in this age group compared to others. Anaemia is one of the most widespread and common health condition afflicting individuals living in the tropics and in Africa. It contributes to 23% of nutrition-related associated with impairments in physical growth, cognition and school performance.[21] Although the aetiology of anaemia is complex and multi-factorial in origin, parasitic infection including Malaria parasite and hookworm have reportedly played a role in contributing to anaemia in endemic area.[22] The low PCV observed in this study, in subject with co-infection may be attributed to blood an iron loss which was augmented by feeding habit of people in the studied area since they feed on fresh vegetables and fruits which prevent them from anaemic despite the fact that they were infected. It is therefore not uncommon that a considerable proportion of the population is polyparasitized with one or more parasite. Multiple parasitic infections is known to have a substantial impact on the physical health, social and intellectual development of children.[23]

CONCLUSION

In conclusion, the study clearly revealed that polyparasitism exists in Ajagba community and also emphasized that the epidemiological factors that enhances susceptibility of individuals to multiple parasitic infection are still in existence in this community .There are many several effective controls for improving or eradicating the menace of polyparasitism, such as counselling mothers at the clinic, health inspectors setting standard hygiene, promoting better hygiene practices in school and villages and mass chemotherapy.

RECOMMENDATION

In view of the devastating impact of polyparasitism on the physical and mental conditions of individuals, the following are therefore recommended to combat it. Health education on the dangers of being co-infected with parasites, mode of transmission of multiple parasites and prevention should be intensified particularly in primary schools. Prevention of infestation can be attained by the provision of latrines in such communities. Efforts should also be made to ensure that they are used and maintained. Provision of adequate quantity of clean water to wash the hands after defecation, before preparing and eating food, and for washing soil contaminated fruits and vegetables are some of the other preventive measures. Reduction in community worm load can be achieved by mass chemotherapy with antihelminthics after a screening survey and education of the populace. Insecticide treated mosquito bed nets should be subsidised by the government of the day. This will go a long way in reducing the rate of malaria transmission. Finally, efforts should also be geared towards making our hospitals open and functional all the time. This is by putting an end to incessant strikes in our health institutions and public places in the third world. Thus, allowing for prompt diagnosis and treatment of diseases.

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