



## Urinary Schistosomiasis and Its Potential for Cancer and Hepato-Renal Function Alterations among the Residents of Asejire Dam, South-Western Nigeria

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### Abstract

Urinary schistosomiasis is of public health significance in sub-Saharan Africa with its prevalence being linked to carcinogenesis. However, there is a paucity of data to support this relationship. This study explored the prevalence and intensity of *Schistosoma haematobium* infections and its associations with cancer and hepatorenal function alterations in villages along the Asejire dam in the State of Osun, Nigeria. Urine samples from 152 consented participants (aged 18–75 years) from four communities were collected and examined for *Schistosoma haematobium* using the microscopy method. The blood serum of randomly selected infected and non-infected individuals (10 per group) was screened for tumor, inflammatory and hepatorenal function biomarkers. From the results, only one out of the four communities recorded cases of schistosomiasis with a prevalence of 26.5% (22/83) and overall infection prevalence was 14.47% (22/152). There was no statistical difference ( $p > 0.05$ ) in kidney, liver antioxidants, and lipid peroxidation markers in the infected and non-infected participants. The concentrations of antioxidants, inflammatory, and tumor markers were higher in *Schistosoma* infected individuals as compared with non-infected individuals. This justifies the need to monitor schistosomiasis-infected individuals for tumor, inflammatory and hepato-renal function biomarkers before they develop into cancer, liver, and kidney failure.

**Keywords:** Urinary Schistosomiasis, tumor, and inflammatory markers, antioxidants, hepato-renal function, Asejire dam Nigeria.

### Introduction

Schistosomiasis (bilharziasis) is of great public health importance mostly in sub-Saharan Africa (Shokeir 2004, WHO 2022). Schistosomiasis is found in around 75 nations worldwide and distresses over 200 million individuals with approximately 600 million individuals at risk of the infections (Wallace 1979, Mostafa et al. 1999). The brunt of the disease is mostly borne in rural and agricultural communities, according to WHO (2015) and Adeleke (2017) who has shown the prevalence of the disease in urban areas

(WHO 2015, Adeleke 2017). *Schistosoma haematobium* is a urogenital system inhabiting parasites responsible for urinary schistosomiasis. The disease is caused by infections of host cells by these parasitic blood flukes that belong to the genus *Schistosoma*, family Schistosomatidae, order Digenea, class Trematoda, and phylum Platyhelminthes (Zaghloul et al. 2020). Previous investigations have depicted the possible relationship of the disease with bladder malignancy, though, the data available are few to make a concrete

conclusion (Gouda et al. 2007, Parkin 2008). Studies have reported that *Schistosoma haematobium* causes bladder malignancy through indirect means; adult worms release eggs that cause chronic inflammation which subsequently releases growth factors and biochemical substances possessing carcinogenic effects (Efares et al. 2022). Scientific evidence has also shown that the eggs laid in the urinary bladder usually lead to fibrosis which may serve as a fulcrum for the human cancer-causing nature (Fried et al. 2011). Lesions induced by entrapped eggs result in inflammation of the bladder as well as organ deformities, with untreated cases ultimately resulting in cancer (Santos et al. 2021). Zhong et al. (2022) also reported that eggs entrapped in the liver caused hepatic damages due to host immune response (Zhong et al. 2022). Dam construction projects for agricultural and socio-economic development have been associated with widespread of schistosomiasis in various parts of Africa (Ofoezie et al. 1991, Sam-Wobo et al. 2011). Previous researchers have linked the outbreaks of the disease around the dams to poor or little consideration of public implications during the design and implementation of the dams (Oladejo and Ofoezie 2006, Sam-Wobo et al. 2011).

In Southwestern Nigeria, studies have reported a wide spread of urinary schistosomiasis around Oyan dam (Ofoezie et al. 1991, Akinwale et al. 2010, Sam-Wobo et al. 2011) and Erinle dam (Oladejo and Ofoezie 2006, Hassan et al. 2014, Surakat et al. 2020). However, there is a paucity of information on the prevalence of schistosomiasis around the Asejire dam. This present study was therefore carried out to document the prevalence of urinary schistosomiasis and its implications in cancer and hepato-renal function alterations among the residents of Asejire Dam, Southwestern Nigeria.

## **Materials and Methods**

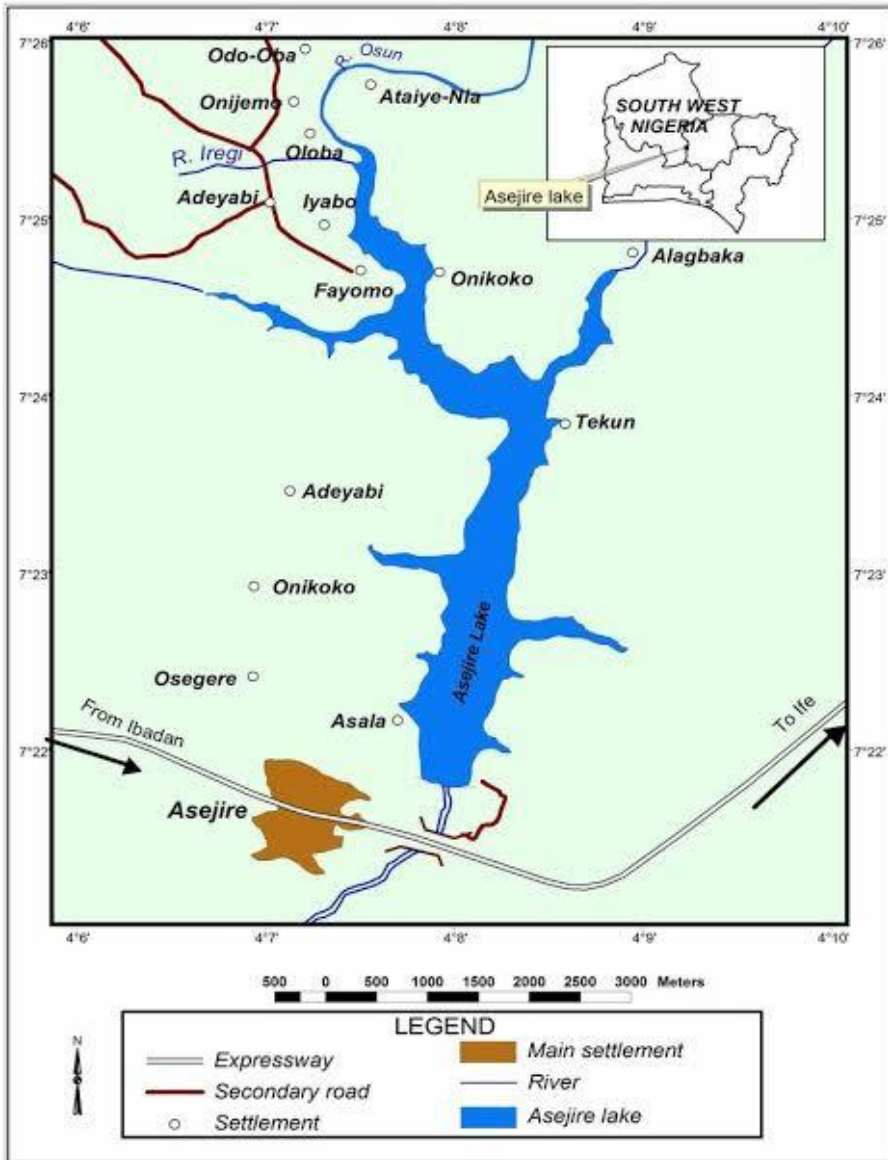
### **Study area and population**

The study was carried out in four communities, namely Asejire, Arogunyo, Baale Ayo, and Ogun-Mogo which are all located around the Asejire dam between July and October 2019. The four communities are located in the Isokan Local Government area of Osun State and lie between longitude 54° E and latitude 60° N (Figure 1). The majority of the dwellers are fishermen and farmers. The vegetation of the study area is typically rainforest. Several water bodies intersect in the study area, some of which include ponds, streams, and dams water bodies which serve as the major sources of water supply to the residents for their daily activities. These communities do not have access to some basic amenities such as pipe-borne water, or a waste disposal system. The lack of access to a reliable waste disposal system results in their indiscriminate disposal of human wastes into the dam which may predispose them to schistosomiasis in the study area.

### **Study design and participants**

Consented individuals both men and women were recruited in four selected communities located in Isokan Local Government around Asejire Dam.

- i. *Inclusion criteria:* Individuals (men and women) who resided in these villages and those that do business within the communities.
- ii. *Exclusion criteria:* Underaged children, women in menstruation, and women on post-natal bleeding were exempted.
- iii. *Sample size determination:* The sample size was determined using the statistical formula proposed by Wayne (1987),  $N = pq(Z)^2/e^2$  with prevalence of 50% since there is no documented prevalence of schistosomiasis in the study area. Thus, the proposed estimated sample size was 385, but only 152 participants consented to the study.



**Figure 1:** The study area. All the other three communities used in this study were small communities located around the main community (Asejire).

**Ethical approval**

Ethical approval was obtained from the Osun State Ministry of Health Research Ethical Committee with Reference Number: OSHREC/PRS/569T/156. The participants of the study were pre-informed about the study through community mobilization and contact with community heads. Written informed

consent was appended by each participant at the beginning of the study.

**Collection of urine samples**

Of the 385 expected participants (as calculated by the sample size), only 152 (40%) participants consented to the study. The 30 ml sterile collection bottles (black) were distributed to the participants to collect

the urine samples between 9:00 hr to 11:00 hr. The urine samples were placed inside dark containers and quickly transported to the laboratory for analysis.

### Parasitological analysis

The detection of *Schistosoma* ova in the urine samples was done using the microscopy technique. About 10 ml of each well-labeled urine sample was transferred into a centrifuge tube and spun at 5000 rpm for five minutes. The supernatant obtained after centrifuging was decanted. A drop of the deposit was placed on the microscope slide and one drop of Iodine's iodine was added and covered with a cover slip. The slides were viewed under a binocular microscope for *Schistosoma haematobium* eggs.

### Blood collection for biochemical analysis

Blood samples were collected from all consented individuals. The blood samples were collected into sterile bottles and labeled accordingly for further analysis. In the process of further analysis, ten schistosomiasis-infected and ten non-infected participants of the sample age group were selected randomly. Random selection was obtained from the schistosomiasis-infected participant, using the result from the urine microscopy test, by selecting the ones with a higher number of *Schistosoma haematobium* eggs.

### Biochemical analysis

- i. *Estimation of lipid peroxidation and total antioxidant capacity*: Enzymes-Linked Immunosorbent Assay (ELISA) method was used to determine the concentration of serum human Malondialdehyde (MDA; marker of lipid peroxidation), while serum total antioxidants capacity (TAC) was assessed using fortress diagnostics kits (USA).
- ii. *Quantification of glutathione peroxidase (GPx) activity*: The protocol of Paglia and Valentine (1967) was used in quantifying GPx, the assay protocol is based on the measurement of the disappearance of NADPH at 35 °C and expressed as

unit/ml of the blood (Paglia and Valentine 1967)

- iii. *Estimation of superoxide dismutase (SOD) activity*: SOD activity in the sample was estimated based on WST-8 method by ELISA kits. The principle is based on the colorimetric action of WST-8. Xanthine oxidase (XO) oxidizes xanthine to form a superoxide anion, this, in turn, quenches WST-8 to form purple formazan that is water-soluble. When SOD quenches the superoxide anion, the overall colorimetric reaction is inhibited. The inhibition levels are measures to specify the SOD activities in the samples.
- iv. *Determination of catalase (CAT) activity*: CAT activity is measured using ELISA kits based on the ability of catalase to degrade hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Excess H<sub>2</sub>O<sub>2</sub> complexes with ammonium molybdate yield a light yellow complex that has an absorbance of 450 nm. The absorption is used to calculate excess H<sub>2</sub>O<sub>2</sub> concentration that indirectly specifies CAT activity.
- v. *Determination of serum inflammatory and tumour markers*: The concentration of tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-6 (IL-6), tumour growth factor- $\beta$  (TGF- $\beta$ ), and total protein (p53) in the serum were assessed using the ELISA method as described by the manufacturer.
- vi. *Determination of renal and hepatic function marker*: Creatinine and urea were assessed using Randox kits (England) following the producer's instructions and absorbances (nm) were read employing UNISPEC SM7504UV spectrophotometer (UNISCOPE, England). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were measured using Biorexdiagnostic kits, while bilirubin was detected using Randox® kits (England). Alkaline phosphatase (ALP) was determined using Teco diagnostic kit following the manufacturer's instructions and the absorbances (nm) were read using UNISPEC SM7504UV spectrophotometer (UNISCOPE, England).

**Data analysis**

The difference in prevalence obtained in the communities was analyzed using chi-square, while the difference in biochemical parameters between infected and non-infected participants was subjected to paired t-test to determine the level of significance at a 95% confidence interval. All analyses were done using SPSS version 17.0.

**Results**

**Prevalence of urinary schistosomiasis in selected communities around Asejire Dam**

The prevalence of the infections in the four communities around the Asejire dam is

presented in Table 1. Out of 152 participants examined, 22 (14.4%) were positive for urinary schistosomiasis. The prevalence of the infections was only observed at Baale-Ayo of which 22 (26.50%) out of 83 participants examined were positive for schistosomiasis. Zero prevalence (0%) was observed at Asejire, Arogunyo, and Ogunmogbo. The males were more infected (15/69 = 0.217) as compared with females (7/83 = 0.084), though the difference was not statistically significant ( $p > 0.05$ ).

**Table 1:** Prevalence of urinary schistosomiasis in selected communities around Asejire Dam, Osun State, Nigeria

<i>Communities</i>	<i>Number of people examined</i>	<i>Number of positive</i>	<i>Percentage positive</i>
Asejire	42	0	0%
Arogunyo	20	0	0%
Ogunmogbo	6	0	0%
Baale- Ayo	83	22	26.50%
<b>Total</b>	152	22	14.47%

The prevalence by age group showed that the age group 18–33 (26.76%) had the highest number of infected participants followed by 34–49 (7.50%), 50–65(0%), and 65 (0%)

above, respectively (Table 2). The difference in prevalence was statistically significant ( $p < 0.05$ ).

**Table 2:** Prevalence of urinary schistosomiasis among age groups

<i>Selected communities around Asejire Dam, Osun State, Nigeria</i>							
<i>Age (Years)</i>	<i>Arogunyo</i>	<i>Asejire</i>	<i>Ogunmogbo</i>	<i>Baale- Ayo</i>	<i>Total</i>	<i>No. Infected</i>	<i>No. Infected (%)</i>
18–33	0	4	0	67	71	22	26.76
34–49	8	13	5	14	40	0	7.50
50–65	0	26	1	0	27	0	0
65 above	12	0	0	2	14	0	0
<b>Total</b>	20	43	6	83	152	22	34.26

**Antioxidant status**

Table 3 showed that the activity of SOD and concentrations of GSH in the serum of infected (Positive) participants was higher when compared with non-infected (Negative) participants. The mean activity of CAT was lower in serum of schistosomiasis infected

(Positive) participants than non-infected (Negative) participants. The differences in the mean activities and concentrations of these antioxidant enzymes and molecules in infected and non-infected participants were not significant ( $p > 0.05$ ).

**Table 3:** Antioxidant status of schistosomiasis infected and non-infected participants at Asejire Dam, Southwestern Nigeria

<i>Biomarkers</i>	<i>Infection status</i>	<i>Enzyme activity (M ± SEM)</i>	<i>P-value</i>
SOD	Positive	250.62 ± 96.16	P = 0.88
	Negative	224.95 ± 109.47	
GPx	Positive	502.82 ± 231.34	P = 0.26
	Negative	244.81 ± 107.15	
CAT	Positive	93.70 ± 8.60	P = 0.63
	Negative	101.74 ± 10.60	
TAC	Positive	0.63 ± 0.09	P = 0.26
	Negative	0.85 ± 0.16	

The results are presented as mean ± standard error of mean (M ± SEM). Values are not significant at p = 0.05.

**Lipid peroxidation**

Table 4 revealed that the mean concentration of malondialdehyde (a marker of lipid peroxidation) in the serum of infected (Positive) participants was higher when

compared with non-infected (Negative) participants (by 23.08%). However, this increase is not statistically significant at p < 0.05.

**Table 4:** Lipid peroxidation level in the serum schistosomiasis infected and non-infected participants at Asejire Dam, Southwestern Nigeria

<i>Group</i>	<i>MDA (µmol/l)</i>
Positive	4.42 ± 0.086
Negative	3.40 ± 0.69

Values are expressed as mean ± standard error mean (n = 10). Values are not significant at p = 0.05. MDA = Malondialdehyde.

**Tumor and inflammatory biomarkers**

The concentrations of TGF-β, P-53, TNF-α, and IL-6 were higher in schistosomiasis-

infected individuals than in the control (uninfected) but the differences were not statistically significant (p > 0.05) (Table 5).

**Table 5:** Tumor and inflammatory biomarkers in schistosomiasis infected and non-infected participants at Asejire Dam, Southwestern Nigeria

<i>Biomarkers</i>	<i>Infection status</i>	<i>Concentration (M ± SEM)</i>	<i>P- value</i>
TGF-β	Positive	0.67 ± 0.13	P = 0.39
	Negative	0.43 ± 0.19	
P-53	Positive	4.42 ± 1.42	P = 0.29
	Negative	2.33 ± 0.86	
TNF-α	Positive	217.45 ± 19.20	P = 0.13
	Negative	155.57 ± 36.85	
IL-6	Positive	53.62 ± 20.66	P = 0.78
	Negative	47.17 ± 14.66	

Values are expressed as mean ± standard error mean (n = 10). Values are not significant at p = 0.05.

**Liver and kidney function test**

The results in Table 6 revealed that the concentrations of aspartate aminotransferase (AST), total bilirubin, and conjugate bilirubin were lower in infected participants when

compared with non-infected participants. However, the differences between the concentrations of the two groups were not significant (p > 0.05). The analysis of alanine aminotransferase (ALT) and alkaline

phosphatase (ALP) levels showed that the infected participants had the mean ALT and ALP levels higher than that of the non-infected participants, but there was no statistical difference ( $p > 0.05$ ) between the positive and negative groups. The urea concentrations were lower in infected

participants compared to non-infected participants, but the reverse was the case for creatinine. The differences in the concentrations of the two parameters determined were not statistically significant ( $p > 0.05$ ).

**Table 6:** Markers of the hepatic functions of schistosomiasis infected and non-infected participants at Asejire Dam, Southwestern Nigeria

Groups	TAC (mmol/l)	AST (U/L)	ALT (U/L)	ALP (IU/L)	TB ( $\mu$ mol/l)	CB ( $\mu$ mol/l)
Positive	0.63 $\pm$ 0.09	14.84 $\pm$ 1.45	18.42 $\pm$ 1.45	28.60 $\pm$ 4.65	6.50 $\pm$ 0.98	4.92 $\pm$ 1.32
Negative	0.85 $\pm$ 0.16	17.43 $\pm$ 1.73	16.01 $\pm$ 1.59	23.30 $\pm$ 4.29	8.15 $\pm$ 1.34	5.41 $\pm$ 0.88

Values are expressed as mean  $\pm$  standard error mean ( $n = 10$ ). Values are not significant at  $p = 0.05$ . AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; ALP = Alkaline phosphatase; TB = Total bilirubin; CB = Conjugate bilirubin.

### Discussion

Dams and artificial lakes have been linked with the transmission of urinary schistosomiasis (Ofoezie et al. 1991). The results of the present study showed that only one out of the four communities selected around Asejire dam had cases of schistosomiasis. This is contrary to most of the reports on the communities around dams in Southwestern Nigeria. Ofoezie et al. (1991), Akinwale et al. (2010), Sam-Woboet al. (2011) and Hassan et al. (2014) reported positive cases of schistosomiasis in all communities around Oyan, Eko-Einde, and Erinle dams, albeit, with variations. It was observed that the closer the communities to the dam, the high prevalence of schistosomiasis. Baale Ayo and Asejire are the two closest communities to the dam, even though, Asejire has boreholes, schools, a community health center, and presence of other basic amenities as compared with Baale Ayo which some of these amenities are lacking. On the other hand, Arogunyo and Ogun-Mogbo are some kilometers to the dam downstream and could have limited their frequency to the dam and man-water contact. The summation of these factors could have accounted for the zero prevalence in other communities except Baale Ayo which recorded 26.5% prevalence. On the other hand, other variables such as occupation and favorable environmental conditions have also

been observed to play roles in the variations of prevalence of schistosomiasis in various communities (Lee et al. 2019). To what extent could these factors influence the results obtained in the current study would be a subject of further studies.

Given the 26.5% prevalence observed only in the Baale-Ayo community, the prevalence was far low compared to other reports on schistosomiasis prevalence around the dams. Emejulu et al. (1994), Sam-Wobo et al. (2011), and Hassan et al. (2014) reported over 60% prevalence of urinary schistosomiasis in Agulu (in Anaocha, Anambra State, Nigeria), Oyan (in Abeokuta, Ogun State, Nigeria), and Eko-Ende (in Otin, Osun State, Nigeria) dams. The World Health Organization (WHO) stipulated that a prevalence of 25% by microscopy technique is considered to be moderate, while a value above 25% is regarded as endemic (WHO 1985). The value obtained in Baale Ayo is slightly above 25% and the community could be endemic to schistosomiasis. Even though more males than females were positive for schistosomiasis in Baale Ayo, the non-significant variations probably signified that both sexes have comparable man-water contact activities in the area. This result agrees with the findings of Deribe et al. (2011), Lee et al. 2015, Afifi et al. (2016) and Sulieman et al. (2017). The age-distribution pattern observed in the infected individuals is

expected water contact for swimming, fishing, and recreational activities (Hassan et al. 2014).

Reactive oxygen is usually an outcome generated under disease conditions. This may increase the lipid peroxidation of membranes polyunsaturated fatty acids (PUFA) culminating in the formation of lipid peroxides like malondialdehyde (MDA) and hydroxyl radicals which can destroy some biological tissues and cells (Ostalowska et al. 2006). Therefore, the removal of free radicals relies on the absolute regulations of the antioxidant defense system (Valdez et al. 2000). The higher values obtained in antioxidants markers in infected individuals as compared to non-infected were expected and in tandem with the observations of Ostalowska et al. (2006). However, the non-significant differences in the values of antioxidants and lipid peroxidation biomarkers in infected and non-infected individuals may be due to the low intensity of the eggs of the parasite in infected persons. One to three eggs per ml were observed in the present study, which might not be strong enough to create tense and unhealthy conditions to prompt an extreme rise in antioxidants biomarkers.

Chronic glomerular and hepatic fibrosis has been associated with hepato-renal failure in individuals infected with schistosomiasis (Da Silva et al. 2013, Olveda et al. 2014). Moreover, malignancy and inflammation of the urinary bladder orchestrated by a high deposit of eggs of *S. hematobium* have been reported in the literature (Barsoum 2013). Elevated levels of inflammatory cytokines such as TNF- $\alpha$  and IL-6 have been closely linked to carcinogenesis (Guerville et al. 2022). Inflammation promotes mutations in DNA via the generation of reactive oxygen species by these inflammatory cytokines (Guerville et al. 2022). The elevated values of hepato-renal markers and SOD, GSH, TGF- $\beta$ , P-53, TNF- $\alpha$ , and IL-6 in *S. hematobium* infected individuals may signify a probable association between *S. hematobium* and hepato-renal damage and tumor/inflammation of the bladder. Andrade et al. (2017) posited that the egg intensity in the urine of infected

individuals is related to the disease progression and morbidity. Since the low intensity of eggs was recorded in the present study, this could have also accounted for a statistically non-significant difference in the values of the biomarkers in the infected and non-infected individuals screened in this study.

## Conclusion

The results of this study have shown that only one (Baale Ayo) out of four communities surveyed is endemic for urinary schistosomiasis around Asejire dam, Southwestern Nigeria. The results also showed elevated values of the hepato-renal, tumor, inflammatory, and lipid peroxidation biomarkers with imbalances in concentrations of antioxidants among schistosomiasis-infected individuals as compared with non-infected individuals which justifies the need for close monitoring to avoid progression to hepato-renal failure and bladder tumorigenesis. Although not entirely significant, the results of this study still point to the need for immediate treatment of infected individuals in Baale Ayo. Health education on the etiology of the disease and the provision of basic social amenities such as motorized boreholes, toilets, and the clinic will also abate contact with the infection/re-infection.

**Conflict of interest:** The authors declared that they have no conflict of interest.

**Authors' contributions:** All authors contributed equally to this work.

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