

Prevalence of Trachoma and Molecular detection of *Chlamydia trachomatis* Among children in Tabldiya village, Gadarif State-Sudan

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Abstract

Background: Trachoma is one of the main infectious causes of the blind Furthermore it brought on by visual spoiling with the bacterium *chlamydia trachomatis* (Ct). Trachoma is present all over the world but more prevalent in sub-Saharan Africa, the Middle East, Central and South America, Asia, Australia, and the Pacific Islands.

Objective: This study aimed to determine the prevalence of active trachoma and *Chlamydia trachomatis* among children in Tabldiya village -Gadarif State- Sudan.

Methodology: A population-based prevalence study was conducted in Tabldiya village, Alglabat eastern locality, Gadarif State during the period from Nov 2016 to Nov 2017.

A total of 900 children was surveyed, their ages range between 1 to 9 years old. The children eyes were examined for trachoma follicles and trachoma inflammatory intense (TF, and TI). Samples were collected on Swabs from children clinically diagnosed as active trachoma for the DNA analysis, collection were done from the tarsal conjunctival surface with a Dacron polyester swab and with UTM media, DNA was extracted and amplified by molecular technique with Touchdown protocol and primers for *C. trachomatis* outer membrane protein complex B (omcB).

Result: The overall result of 900 participants, 99 (11.0 %) were diagnosed as active trachoma, 4.0(3.96 %) of them had follicles in the upper tarsal (TF), 0 (00.0 %) had trachoma inflammatory intense (TI) and 95 (96%) had the two signs of trachoma follicles and trachoma inflammatory intense (TF and TI). 1.4% was the prevalence of *Chlamydia trachomatis* in whole children in the village and From the 99 child those who were clinically diagnosed as active trachoma patients were 13 (12.9%).

Conclusions : This study revealed that the clinical signs of active trachoma can persist even when ocular *Chlamydia trachomatis* was not detected ,this may be due to long lasting clinical sign or there may causative agent other than *Chlamydia Trachomatis*.

Keywords : Trachoma , *Chlamydia. Trachomatis*, PCR, Prevalence, Sudan

Introduction:

The World Health Organization (WHO) defines blindness as visual deficiency. Likewise, visual sharpness in the exceptional eye is about under 3/60 with accessible refractive correction, which predicts the inability to walk safely without help. The best published estimate suggests that 5.9 million people in the world had this paradigm due to trachoma, which makes it answerable for around 15% from claiming constantly on instances of blindness (Thylefors *et al.*, 1995). Furthermore, will the individuals recently blind, an assessed 600 million individuals live in territories from claiming Africa, the center East, What's more focal. Also the Pacific Islands, Australia, Asia, and South America the place trachoma will be endemic (Thylefors, 1998, Thylefors *et al.*, 1995). The correctness from claiming these estimates may be flawed, however (Solomon *et al.*, 2004). There is a pressing compelling reason for further investigation on the dissemination. Furthermore, predominance from claiming ailment. (Kuper *et al.*, 2003).

Trachoma, a neglected tropical disease, infection of the conjunctiva with specific serovars of *Chlamydia trachomatis*. The microorganism is spread by direct contact with eye and nasal discharges from infected person, by contact with fomites (inanimate objects that carry infectious

agents, such as towels or wash cloths), and by eye-seeking flies, contaminated by ocular or nasal discharge containing the bacterium *Chlamydia trachomatis*, particularly *Musca sorbens*, WHO *Epidemiol Rec*, (2017). The disease disproportionately influences people living in rural and resource poor settings, and kids would those essential transporters of infection with visual *C.trachomatis* and active disease (Lee *et al.*, 2014).

Active trachoma, characterized by an existence of follicles and inflammation in the upper tarsal conjunctiva, is characteristic of childhood infection. In cases of repeated infection, the upper tarsal conjunctiva may become extremely scarred and then the eyelashes transform inwards, scratching the eyeball and cause corneal opacity and blindness (Harding-Esch *et al.*, 2015).

The WHO and its partner targeting the year 2020 for the global elimination of trachoma as a public health problem. As the first step to achieving this goal, mapping will be necessary should assess the endemicity for trachoma and figure out the necessity for intercessions (Bio *et al.*, 2017).

The (WHO) designed a strategy called SAFE strategy (Surgical procedure for trichiasis, Anti bacteria for active infection, Facial cleanliness to prevent Infection spread and environmental development to

increase access to water and sanitation) for trachoma elimination. Mass Drug Administration (MDA) of the antibiotic azithromycin goals to eliminate infection from societies such that trachoma ceases marked as public health problem (Last *et al.*, 2017).

In 2012, the (WHO) considered that 21 million persons had active trachoma (trachomatous inflammation – follicular, TF and/or trachomatous inflammation – intense, TI) (Thylefors *et al.*, 1987), and more than 7.0 million individuals had in-turned eyelashes (trachomatous trichiasis) that could scratching corneal and blindness WHO rec (2017).

Sub-Saharan Africa bears those biggest load about disease, for more than 80% of the situations from claiming trachoma WHO rec (2017).

The Sudanese Trachoma mapping was completed in the northern states except for the Darfur States. TF frequency was more than 10% in three districts and in range of 5% and 9% in 11 districts in different states . TT frequency among adults was more than 1% in 20 districts which included the three districts with TF frequency (Hassan *et al.*, 2011).The overall number of individual had TT in the community was estimated to be 31,072 (Hassan *et al.*, 2011).

Trachoma grading: away broadly utilized within research, Group assessment, Also

system following toward both restorative collaborator and ophthalmologists. The system obliges the analyst should survey a distinct to those vicinity or absence of each of five signs (Solomon *et al.*, 2004).

Methods:

Ethical approval: This study is in accordance with the declaration of NHREC, Sudan Federal Ministry of Health, and Central Institutional review board, Al-Neelain University.

Oral informed consent was took from all study applicants and their guardians. During survey, all infected children on the study village were treated with a single height-based dose of oral azithromycin in accordance with WHO and national protocols, and Opticyclin ointment for the children had ocular infection other than trachoma.

Study area and Study design:

A population - based prevalence study was conducted in Tabldiya village, Alglabat eastern locality, Gadarif State during the period from Nov 2016 to Nov 2017. According to trachoma map in 2010 surveys of trachoma prevalence in the northern states of Sudan TF prevalence was above 10% in three districts ,Gadarif state was some of endemic district (Thylefors *et al.*, 1987) .

Trachoma clinical diagnosis : A total of 900 children in the age between 1 to 9 years old were clinically examined which

are represent all eligible children residents on the day of the survey in Tabldiya village . Examination for the signs of trachoma was done by ophthalmic medical assistants trained in using the WHO simplified grading system(Thylefors *et al.*, 1987). A reliability study was conducted using a set of standardized photographs.

Each eye was examined for trachoma follicles and trachoma inflammatory intense (TF, and TI). Both eyes were examined and findings for the worst affected eye recorded. Signs had to be clearly visible by the investigator in accordance with the WHO simplified grading system in order to be considered present. The investigator used alcohol or finger cleaning between examinations. People with indications for dynamic trachoma (TF and/or TI) were offered free treatment with antibiotics according to WHO and national guidelines.

Sample Collection: Conjunctival swab samples collected from children clinically diagnosed as active trachoma (TF and TI) for DNA analysis collection were done from tarsal conjunctival surface by Dacron polyester swab with UTM media (Fisher Scientific, UK) sampling was standardized as four horizontal passes across the conjunctiva, with a one-quarter turn between each pass. After collection, swab will be returned to transporting media and stored until used . Sample divided into two

parts communities covered by mass drug administrations (MDA) with azithromycin and communities not participate in MDAs program. The patients with conjunctivitis or any other eye infections were excluded.

DNA extraction: DNA was extracted from all samples using the G-spin Total DNA kit(iNtRON Biotechnology, Korea) according to the manufacturer's instructions.

PCR amplification with omcB gene primers :

Chlamydial DNA from clinical samples was amplified using a conventional PCR machine (SENSOQUEST, USA). Touchdown protocol was performed using Maxime PCR Pre Mix kit (iNtRON, Biotechnology, Korea) , and primers for *C. trachomatis* outer membrane protein complex B (omcB) (F/ GAC ACC AAA GCG AAA GAC) (R/ACT CAT GAA CCG GAG CAA CCT) (Pickett *et al.*, 2005) .

Amplification was performed in 20µl reaction volumes containing 17µl by add H₂O,1µl of primer and 2µl of DNA template .The 106 bp fragment indicates the presence of *C.trachomatis* outer membrane protein complex B(omcB).

PCR Products Detection: All PCR products were subjected to electrophoresis on agarose gel along with a 100 bp DNA marker.

An amounts of 5µL of each PCR mixture separated in 1.5% agarose gel, then stained with 3µl Lethidium bromide and viewed under gel documentation system with a 100 bp DNA marker. A result was considered positive when a band of the size 106 bp was visible in the gel. Standard procedures for reducing contamination were strictly followed.

Results:

In total, 900 eligible children were examined in this survey over the Tabldiya village. the children of age between 1 to 9 years old participate, 99 (11.0%) of them had sign of active trachoma divided as follow 4.0 (3.96 %) of them had follicles (TF) in the upper tarsal, 0 (00.0 %) had trachoma inflammatory intense (TI) and 95

(96%) had the two signs of trachoma follicles and trachoma inflammatory intense (TF and TI) (table 1). Chlamydia trachomatis was found in 13 child by molecular technique (figure 2). 46 (46.5%) of those examined were females, and 53 (53.5%) of those examined were males (figure 3). Figure 4 show the distribution of the disease over the age of the children 1.44% is the prevalence of *Chlamydia trachomatis* in whole children in the village (table 2) and From the 99 child those who were clinically diagnosed as active trachoma patients is 13 (13.13%) (figure 5).

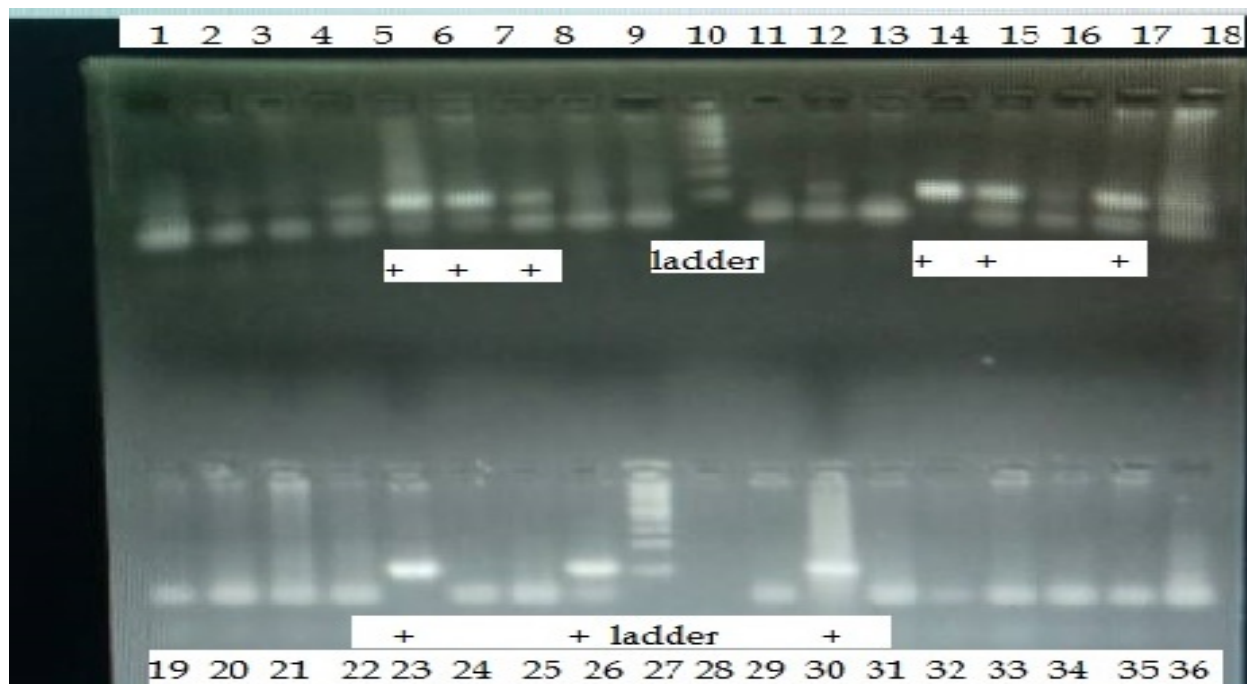


Figure 1 : PCR result of *Chlamydia trachomatis omcB* gene

Table 1: The frequency of Trachoma Follicular (TF),Trachoma Inflammatory Intense (TI) and TF+TI

Trachoma grade	Frequency	Percent
Trachoma Follicular (TF)	4.00	4.00 %
Trachoma Inflammatory Intense(TI)	0.00	0.00 %
TF+TI	95.00	96.0 %
Total	99.00	100.0%

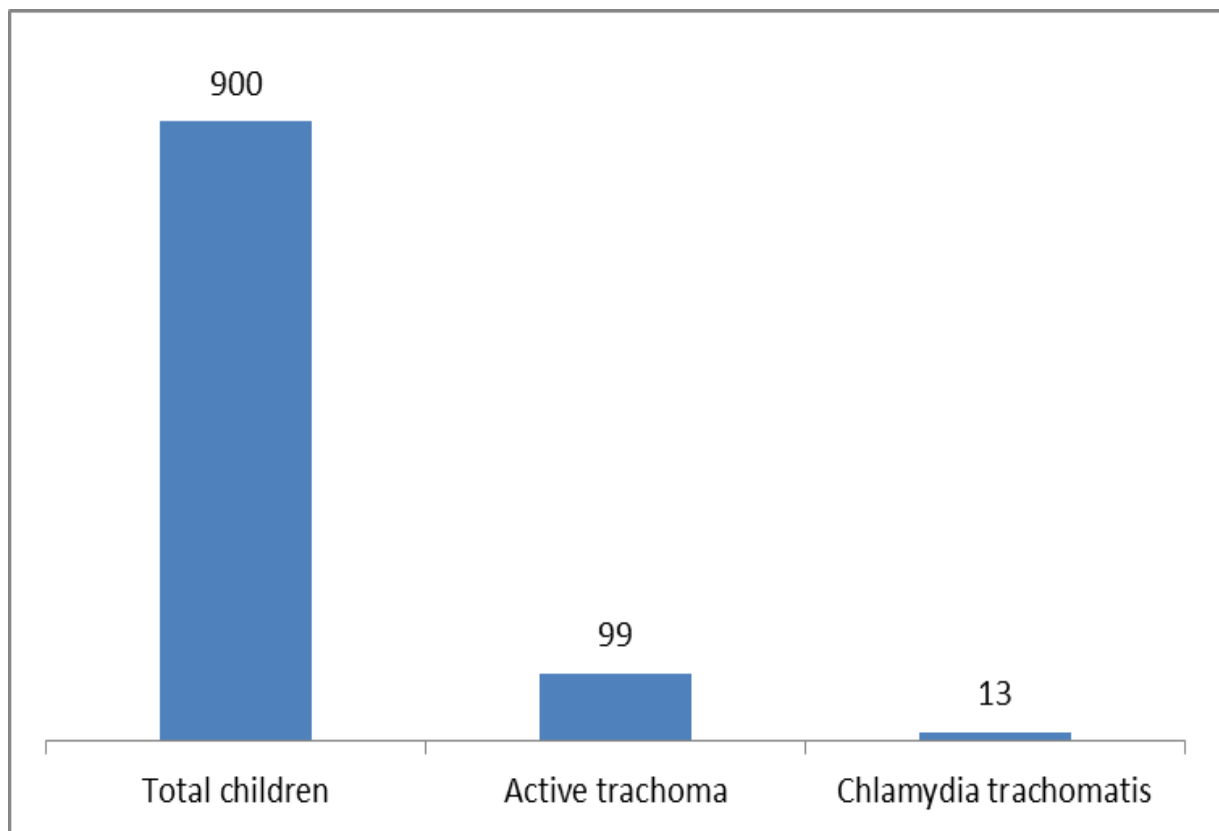


Figure 2 : The prevalence of active Trachoma and *Chlamydia trachomatis* among children of age 1–9 years old in Tabldiya village

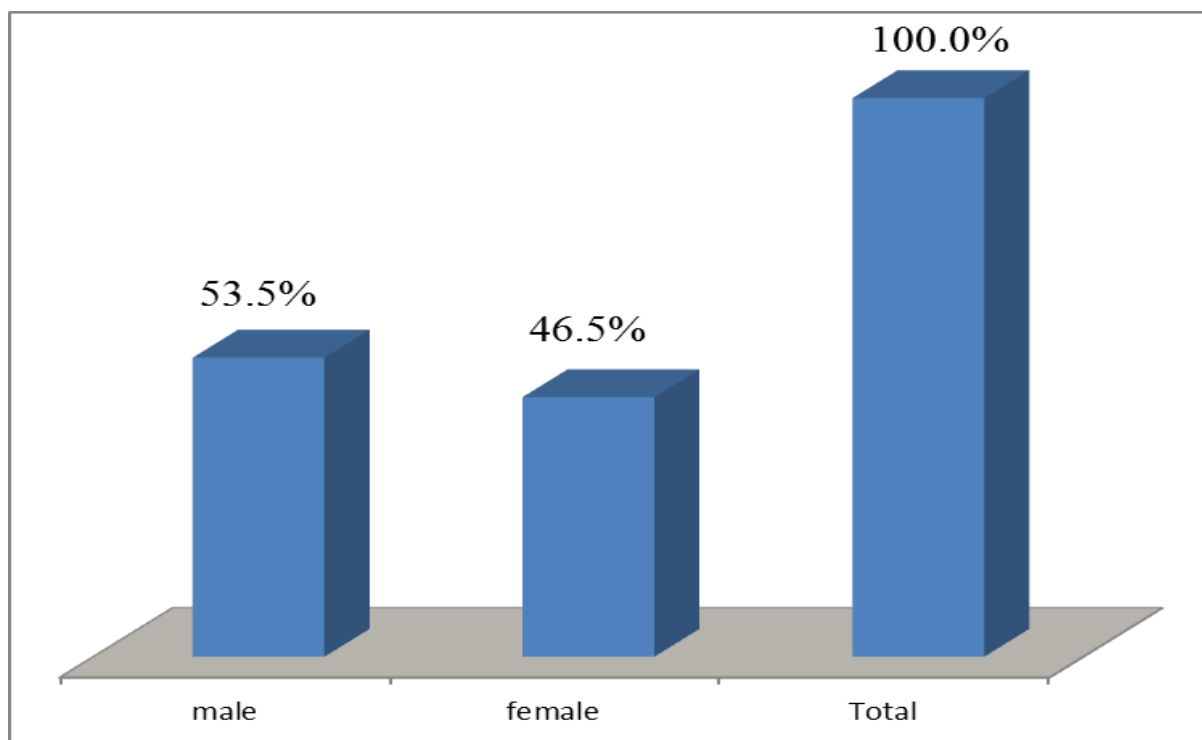


Figure 3 : The frequency of Trachoma in gender

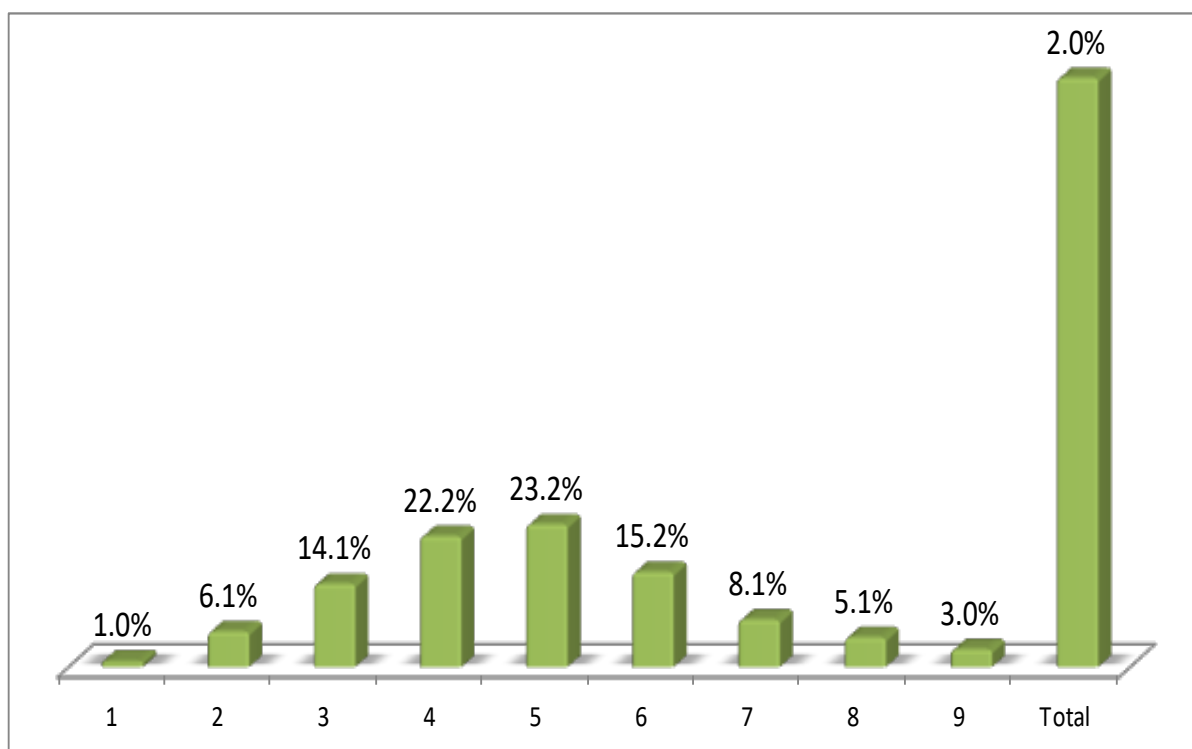


Figure 4 : The prevalence of Trachoma among children of age 1–9 years old in Tabldiya village

Table 2: The percentage of active Trachoma and *Chlamydia trachomatis* among children of age 1–9 years old in Tabldiya village

Total children	Active trachoma	Chlamydia trachomatis
900	11.00%	1.44%

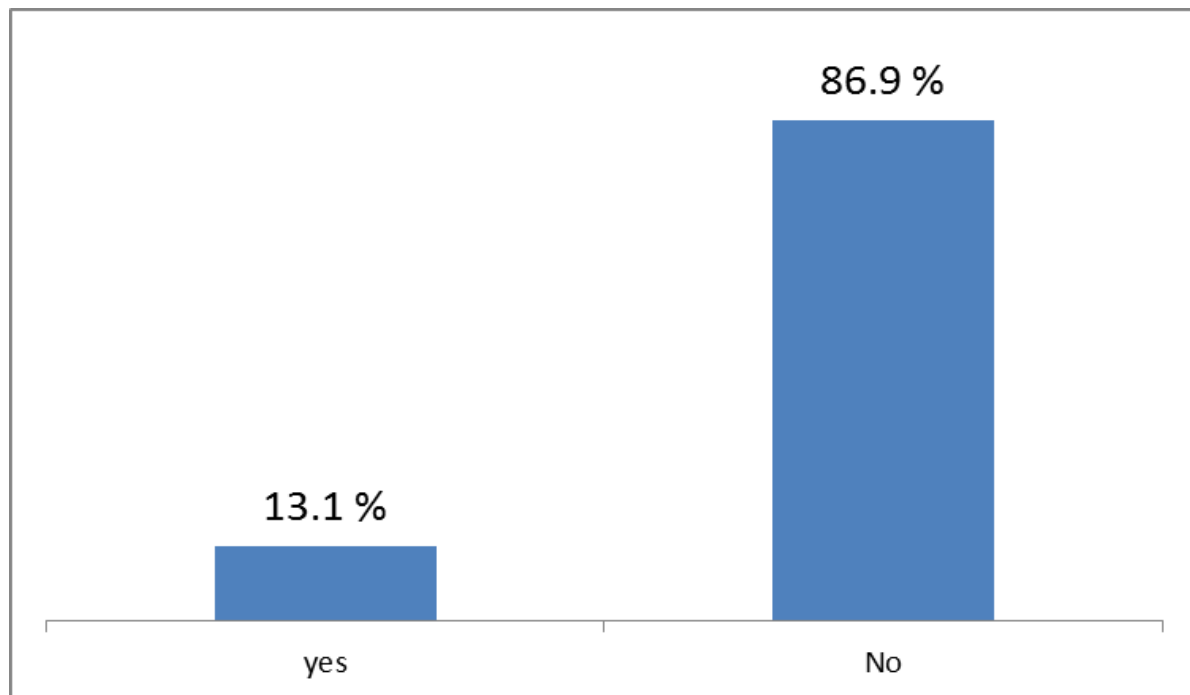


Figure 5 : The percentage of *Chlamydia trachomatis* among active Trachoma in children of age 1–9 years old in Tabldiya village

Discussion:

The WHO led Global Alliance for the Elimination of Trachoma (GET2020) aims to elimination the disease as a public health problem in the world by 2020. The key trachoma control strategy is SAFE strategy endorsed by WHO: “surgery” for patients with advanced disease, “antibiotics” azithromycin or tetracycline eye ointment for active trachoma, “facial” cleanliness

and “environmental” improvement on water supply and sanitation(Stocks et al., 2014, Wu et al., 2018).

Information for trachoma predominance In region level will be especially paramount done arranging the national trachoma control system so as on accomplish those objective of eliminating trachoma Similarly as a blinding malady by quite a while 2020. Region level mapping from

claiming trachoma predominance might have been conveyed out in the northern States of Sudan 2006- 2010 Knowledge of trachoma prevalence at district level is particularly important in planning the national trachoma control program in order to achieve the goal of eliminating trachoma as a blinding disease by year 2020. District level mapping of trachoma prevalence was carried out in the Northern States of Sudan 2006- 2010(Hassan et al., 2011).

Total of children in the village were 900 children ages between 1 – 9 years old , all of them were clinically examined for ocular trachoma by ophthalmic medical assistants trained in using the WHO simplified grading system .The prevalence of trachoma in Tabldiya village was 11.0% ,the previous study of mapping trachoma in Northern State of Sudan presented the prevalence of trachoma in Gadarif state which our study village is located was between 0.9 and 19.8(Hassan et al., 2011).

The results of these population-based impact study demonstrated that The prevalence of active trachoma between children ages 1 to 9 years was more than 10% trachoma remains one of the major public health problem in this areas of Sudan which agree with data from, South Sudan (Edwards et al., 2012), countries in Pacific island (Mathew et al., 2009) , India (Khanduja et al., 2012) and Ethiopia (Ejigu et al., 2013) Trachoma is referred to as an

epidemic. The rate of active trachoma ranged from 25.2%–71.0% .

The prevalence of *Chlamydia trachomatis* in whole children in the village was 1.44% and From the 99 child those who were clinically diagnosed as active trachoma patients 13 (13.1%), this may be due to long lasting clinical sign of trachoma or there may causative agent other than *Clamydia. Trachomatis* ,

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