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ORIGINAL ARTICLE

Molecular Detection of *Chlamydia Trachomatis* Associated with Ocular Infection among Children in Gadarif State-Sudan

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ABSTRACT

Background: Trachoma is the leading cause of infectious blindness worldwide. Trachoma is endemic in parts of Africa, the middle east, and India. The disease is particularly problematic in particular Ethiopia and Sudan regions.

Objectives :To detect *Chlamydia trachomatis* among active trachoma children using molecular technique in - Gadarif State- Sudan

Methodology: A population-based prevalence study was conducted during the period from Nov 2016 to Nov 2017. A total of 318 children were surveyed; their ages range between 1 to 9 years old. The children's 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, and collection was 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). Data was collected by direct interviewing questionnaire; ethical approval was obtained from Ethical Research Committee -Al Neelain University

Result: Out of the total 318 children, 83(26.1%) children were positive for the *C trachomatis* omc B gene; Sequencing was performed for both strands of omc B genes, found that the circulating strain in Sudan Gdarif state is similar genetically to the classical one registered in NCBI

Conclusion: *Chlamydia trachomatis* is one of the causative agents of trachoma in Sudan, the circulating strain in Sudan Gdarif state is similar genetically to the classical one registered in NCBI

Keywords: *Chlamydia trachomatis*- omc B genes- PCR- Trachoma- Sudan

INTRODUCTION

Trachoma is an eye disease caused by ocular infection with *Chlamydia trachomatis*, which can result in blindness after cycles of repeated infections.[1]

The disease is easily transmitted through the transfer of ocular secretions infected with Chlamydia trachomatis to the eyes of an uninfected individual by flies, hands, towels, or sharing of other personal items [2]

The World Health Organization (WHO) has endorsed the SAFE strategy (Surgery for the correction of in-turned eyelashes, Antibiotics to treat infection, promotion of Facial hygiene, and environmental improvement to reduce transmission) to eliminate blindness due to trachoma by 2020 through While the S and A component have been widely implemented evidence, and specific targets are lacking for the F and E components, of which water, sanitation, and hygiene (WASH) are critical elements [3]

Sudan is part of this problem and many studies done in different parts of Sudan, a survey of 14 villages in Wadi Halfa (Northern State) revealed that prevalence of trachomatous inflammation follicular (TF) and/or trachomatous inflammation intense (TI) were 47% among children aged 1–10 years. While 4% of women aged over 40 years had trachomatous trichiasis (TT); confirming trachoma as a serious public health problem according to the WHO standards [1].

To complete the baseline trachoma map of Sudan, one study was performed for estimating the prevalence of trachoma and associated risk factors in the five Darfur States and Khartoum State. They found a high prevalence of trachoma in some areas; the highest prevalence of trachomatous inflammation – follicular (TF) in children was located in El Fashir district (18.7%) and the lowest in El Malha district (0.0%). Five districts (El Fashir, Zalinji, Azoom, Maleet, and El Koma) were in the three EUs that had TF prevalences above the 10% threshold at which the World Health Organization recommends mass treatment with azithromycin, but in general the prevalence throughout Darfur and Khartoum was Chlamydia[4].

This study aimed to detect *Chlamydia trachomatis* ompB gene, associated with ocular infection and blindness in Sudan- Gadarif State

METHODOLOGY

This study conducted at Alglabat Eastern Locality, Gadarif state (Alsaraf alahmar (Bawi East, Bawi West, Bawi South and Bawi Centre) and Saraf tabldia villages) during the period from Nov 2016 to April 2017.

Eye swabs (dracon swabs) collected from 318 Child aged between 1–9 years old with clinically active trachoma (Follicular trachomatous inflammation (TF) and Intense trachomatous inflammation (TI).

Chlamydial DNA was extracted from all samples using the G-spin Total DNA kit (iNtRON Biotechnology, Korea), then amplified using a conventional PCR machine (SENSOQUEST, USA). The 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) with

Concentration in final assay 900 nM and Amplicon size 106 pb (Pickett et al., 2005)

Amplification was performed in 30µl reaction volumes containing 17µl by add H2O, 1 µl of primer and 2µl of DNA template.

The protocol was optimized as follow : Denaturation 95° c for 30 sec, Annealing 59.9° c for 30 sec, Extension 72° c for 2 min The 106 bp fragment indicates the presence of C. trachomatis outer membrane protein complex B (omcB).The PCR product was visualized by gel electrophoresis.

Data were analyzed using SPSS 2016

RESULT

The overall results reveled thatout of 318 children enrolld 83 (26.1%) were positive for *C. trachomatis* omcB gene, the highest positive results percentages observed in Bawi center village (10.0%) and Bawi East village(5.7%) (Table 1) Figure (1) showed the distribution regarding residence.

Gel electrophoresis of *C. trachomatis* omcB gene PCR product was demonstrated in figure (2).

Also Sequencing was performed for both strands of omcB genes by Macrogen Company (Seoul, Korea) sample shown in figure (3)

Nucleotides sequences of merged strands omcB *C .Trachomatis* genes was searched for similarity BLAST in (http: //blast.ncbi.nlm.nih.gov/Blast.cgi). Then multiple sequence alignment of nucleotides and translated proteins was done by Bioedit software, and it matched the classical *C .trachomatis* strain TW-5 OmcB (omcB) gene example shown in figure (4)

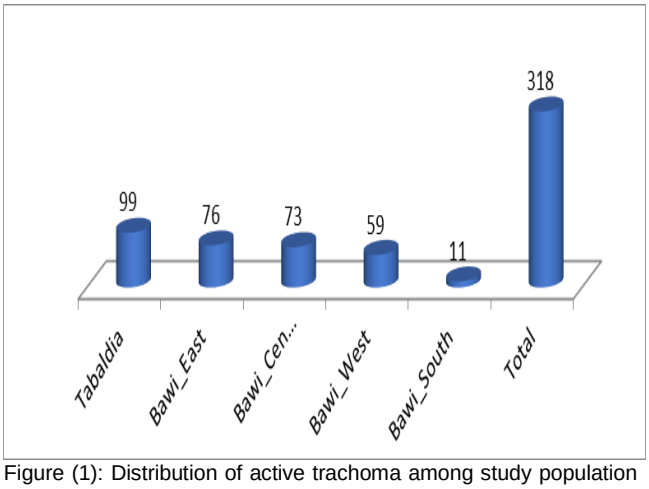

Figure (1): Distribution of active trachoma among study population regarding their residence (n =318)

Table 1: Prevalence of *Chlamydia trachomatis* among active trachoma according to residence

	Active trachoma	Positive pcr for *Chlamydia trachomatis*	%
Tabaldia	99	13	13.1%
Bawi_East	76	18	23.6%
Bawi_Center	73	32	43.8%
Bawi_West	59	14	23.7%
Bawi_South	11	6	54.5%
Total	318	83	26.1%

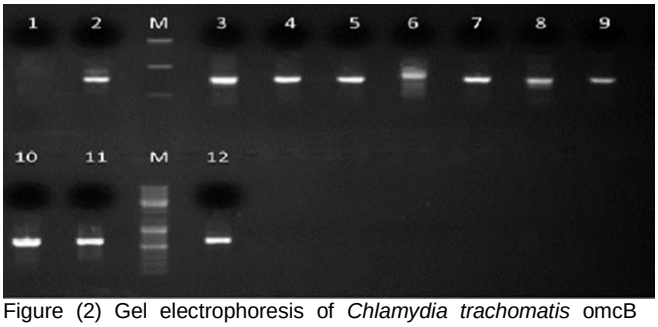

Figure (2) Gel electrophoresis of *Chlamydia trachomatis* omcB gene PCR product (106 bp). Key. M: Marker (100bp). Lane 1: negative control, Lane 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 are positive samples.

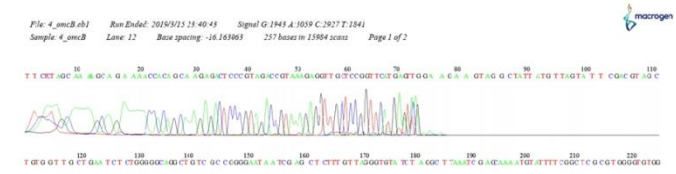

Figure 3: Sequencing were performed for both strands of omcB genes by Macrogen Company (Seoul, Korea)

>EU247644.1:176-232 *Chlamydia trachomatis* strain TW-5 OmcB (omcB) gene, complete cds
AAAACCACAGCAAAGAGACTCCCGTAGACCGTAAAGAGGTTG
CTCCGGTTCATGAGT
Query: 4_omcB Query ID: lcl|Query_51051 Length: 257
>Chlamydia trachomatis strain TW-5 OmcB (omcB) gene, complete cds
Sequence ID: EU247644.1 Length: 1644
>Chlamydia trachomatis strain Apache2 OmcB (omcB) gene, complete cds
Sequence ID: EU247645.1 Length: 1644
Range 1: 176 to 232
Score:96.0 bits(105), Expect:2e-15,
Identities:56/57(98%), Gaps:1/57(1%), Strand: Plus/Plus
Query 18
AAAACCACAGCAAGAGACTCCCGTAGACCGTAAAGAGGTTGC
TCCGGTTCATGAGT 73
||||||||||||
||||||||||||||||||||||||||||||||||||||||||
Sbjct 176
AAAACCACAGCAAAGAGACTCCCGTAGACCGTAAAGAGGTTG
CTCCGGTTCATGAGT 232

Figure (4): NCBI matching

DISCUSSION

Trachoma, caused by the bacterium *Chlamydia trachomatis*, is the most common infectious cause of blindness and the leading cause of preventable blindness worldwide [5]

The present study detected *Chlamydia trachomatis* ocular infection bacteria among trachoma patients aged between 1—9 years using the molecular technique.

The results revealed (83/318) are positive for *C. trachomatis*, and the prevalence rate of *C .trachomatis* among active trachoma children was (26.1%). When compared with other study,it found to be higher than that reported in some neighboring and African countries like Coˆte d'Ivoire was 10.3%, Guinea 4.3%, Malawi 6.4%, Nigeria 3.0%, Lao People's Democratic Republic 1.0% [6], Niger ranged between 4.6% to 7.1% [7] and Casamance

region of Senegal was 2.5% (95% Confidence Interval (CI) 1.8–3.6) [8]

There were not many studies that examine the Trachoma in Sudan at the genetic level (PCR test and gene sequencing).

Other research is done in Sudan examine and diagnose trachoma clinical symptoms and trachoma grading in population-based cross-sectional surveys; a previous study was conducted in 88 localities (districts) in 12 northern states of Sudan between 2006 and 2010. Trachoma grading was done using the WHO simplified grading system. Key prevalence indicators were trachomatous inflammation-follicular (T.F.) in children aged 1-9 years and trachomatous trichiasis (T.T.) in adults aged 15 years and above.[1]

Another study was done in Darfur in 2016, investigating the prevalence of Trachoma in Darfur state. The highest prevalence of trachomatous inflammation - follicular (T.F.) in children was found in El Fashir district (18.7%), and the lowest in El Malha district (0.0%)[4]

Another study was conducted between July and September 2009. Agreed with this study, Seven villages in Northern Bahr-el-Ghazal State south Sudan and 13 villages in Unity State were surveyed; an average of 50 children (age 1-9 years) and 44 women (Age 15 years and above) was examined per village. Samples for analysis using the APTIMA Combo-2 nucleic acid amplification test (NAAT) were collected from participants with active trachoma in eight villages in Unity State. In Northern Bahr-el-Ghazal State, only three children with active trachoma (trachomatous inflammation follicular (T.F.) and/or trachomatous inflammation intense (T.I.)) and two women with trichiasis (T.T.) were found, in two of the seven villages surveyed. In Unity State, Trachoma was endemic in all thirteen villages surveyed; the proportion of children with active trachoma ranged from 33% to 75% between villages, while T.F. in children ranged from 16% to 44%.

4% to 51% of examined women showed signs of T.T. Samples from active trachoma cases tested using the NAAT was positive for *Chlamydia trachomatis* infection for 46.6% of children and 19.0% of women. Trachoma presents a major problem to public health Unity State, while the disease is of low priority in Northern-Bahr-el-Ghazal State [9].

Our study results agreed with aresults of study done in Eye Clinic of G. d'Annunzio University of Chieti, Italy from 2006-2008 the study enrolled 171 patients affected by occasional mild, moderate or severe conjunctivitis in a three-arm prospective open study, using traditional analysis such as Immune Fluorescent Assay and EnzymeLinked Fluorescent Assay (IFA and ELFA) and molecular analysis with Polymerase Chain Reaction (PCR) procedure for Ct DNA research they found patients, negative to IFA and ELFA, were positive to Ct-DNA analysis. These data indicate a higher rate of Ct infection in patients with severe or moderate chronic conjunctivitis, resistant to usual therapies even after eradication of common germs, thus showing the advantage of introducing this molecular technique of analysis in mild to severe chronic or recurrent conjunctivitis [10]. there advantage of introducing this molecular technique of analysis in mild to severe chronic or recurrent conjunctivitis [10] that it will give picture and help in elimination of *C. trachomatis* the causative agent of trachoma and search for other causative agent for trachoma

Also study done by Butcher et al in Solomon Island 2017 by using the molecular technique who indicate Follicular conjunctivitis meeting criteria for the active trachoma is highly prevalent in the Solomon Islands, but the prevalence of *C. trachomatis* infection is curiously low 3.9% . Although *C. trachomatis* was the only pathogen to associate with T.F. in their study, the prevalence was much lower than may be expected of a population with this level of active Trachoma [3] but this study showed the importance of using molecular technique to detect *C .trachomatis*.

In this study, Sequencing was carried out from the PCR product. DNA sequencing was performed for 47 PCR products of *C. Trachomatis* positive for omcB gene.

This study detected The *C. trachomatis* in this area of Sudan 56/57 identical, so it considers to be very similar to the (http: //blast.ncbi.nlm.nih.gov) no mutation detected, and all the result of the test showing matching nucleotide according to what found in the NCBI so no change in the genes.

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