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Highlights

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VOLUME 13

ISSUE 5

VERSION 1.0



GLOBAL JOURNAL OF MEDICAL RESEARCH: F
DISEASES



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DISEASES

VOLUME 13 ISSUE 5 (VER. 1.0)

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Knowledge, Attitude, and Practice of the Community towards Malaria Prevention and Control Options in Anti-Malaria Association Intervention Zones of Amahara National Regional State, Ethiopia

By Zewdie Aderaw & Molla Gedefaw

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Abstract- Background: Malaria is one of the most important public health challenges in Ethiopia. Although the disease is endemic in many parts of the country, the knowledge, attitude and practice of the community about the disease prevention and control options is far from perfect, and misconceptions and malpractices are common.

Objective: To assess the level of knowledge, attitude and practices of the community towards malaria prevention and control options.

Methodology: A community based cross sectional study design was done in Antimaria Association intervention zones. A single population proportion sample size formula and design effect of two was used to determine sample size.

Keywords: malaria, knowledge, attitude, practice, insecticide treated net utilization.

GJMR-F Classification : ONLMC Code: QV 256, WC 750



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Knowledge, Attitude, and Practice of the Community towards Malaria Prevention and Control Options in Anti-Malaria Association Intervention Zones of Amahara National Regional State, Ethiopia

Zewdie Aderaw^α & Molla Gedefaw^σ

Abstract- Background: Malaria is one of the most important public health challenges in Ethiopia. Although the disease is endemic in many parts of the country, the knowledge, attitude and practice of the community about the disease prevention and control options is far from perfect, and misconceptions and malpractices are common.

Objective: To assess the level of knowledge, attitude and practices of the community towards malaria prevention and control options.

Methodology: A community based cross sectional study design was done in Antimaria Association intervention zones. A single population proportion sample size formula and design effect of two was used to determine sample size. A total of 864 participants were included in the study and proportional allocation was done among urban and rural residents. The data was collected by trained data collectors and supervisors using questionnaires and interviewing guidelines. The collected data was cleaned, coded and entered into SPSS version 16.0 for windows soft ware for analysis.

Result: This study revealed that 37.6% of the study participants mentioned fever as symptom of malaria. The acceptance rate of IRS as malaria control and prevention method is 5.37%. From the general population, 26.4% of the participants used ITN as malaria prevention and control method. Among febrile children in the last one year, 28.4% were taken to modern health care institutions for treatment. From the total study participants, 66.6%, 50.8%, 64.8% have a good knowledge on clinical manifestations, signs and symptoms, and prevention methods of malaria, respectively. Sixty nine percent of the respondents have positive attitude towards modern health care utilization for malaria treatment and 47% of them have good practice towards malaria prevention and control activities.

Conclusion and recommendation: knowledge, attitude and practice of the community towards malaria prevention and control options are still at low level. Therefore, the existing effort must be strengthened and continued to

improve the community knowledge, attitude and practice towards malaria prevention and control options.

Keywords: malaria, knowledge, attitude, practice, insecticide treated net utilization.

I. INTRODUCTION

Malaria affects the health and wealth of nations and individuals alike. In Africa today, malaria is understood to be both a disease of poverty and a cause of poverty. Annual economic growth in countries with high malaria transmission has historically been lower than in countries without malaria. Economists believe that malaria is responsible for a 'growth penalty' of up to 1.3% per year in some African countries. [1]. In Ethiopia, malaria is at the forefront among the health problems of the country. The actual number of malaria cases that occur annually throughout the country are estimated to be about 4-5 million. Due to climatic and geographic factors, the disease occurs in different parts of the country in epidemic form. About 75% of the total area of the country is estimated to be malarious wherein 68% of the total populations live, 40 million people, being at risk of infection [2].

Studies indicated that despite these bitter facts, communities are not well aware of the multi-dimensional challenges of malaria in our country. The knowledge of the community is far from perfect, and misconceptions are rampant [2]. There have been a considerable number of reports about knowledge, attitude, and practice relating to malaria and its control from different parts of Africa. These reports concluded that misconceptions concerning malaria still exist and that practices for the control of malaria have been unsatisfactory [3].

Ethiopian national malaria indicator survey of 2007 indicated that about 71% of the rural community and 80.5% of the urban community have heard about malaria. This survey also reported that about 87% of Amhahara national regional state respondents had heard about malaria. However, only 30.1% of the rural and 59.7 % of urban community knew that mosquito bite

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can transmit malaria. This survey indicated that 52.7% of pregnant women, 51.8% of children have slept under ITN in the previous day of the interview. Thus, understanding of the current knowledge of the community beliefs and practices with respect to the disease is required to obtain and maintain the community involvement in surveillance and control activities [3].

Many reports indicated, currently the burden of malaria is decreasing in the country with the government different prevention and control strategies. For the sustainability of the programs, capacity building must be done on the community knowledge, attitude and practice towards malaria prevention and control options. However, the attention of most researchers on the topic is reducing from time to time. Therefore, this study was designed to assess the current level of knowledge, attitude and practice of the community towards malaria prevention and control options. The output of this study is very important for the sustainability of the current malaria prevention and control options through assessing the current community knowledge, attitude and practice of malaria prevent and control options.

II. METHODS

a) Research Design

A community based descriptive cross sectional study design was used to assess knowledge, attitude and practice of the community towards malaria prevention and control options.

b) Study Area and Study Period

The study was conducted in five intervention zones of Anti-malaria Association intervention zones in Amahara National Regional State, namely: Awi, East Gojjam, West Gojjam and South Gondar zones. This study was conducted from January to March, 2011.

c) *Source Population:* All households in the intervention zones.

d) *Study Population :* people in the selected kebeles.

e) *Sampling Units :* selected Households for the quantitative study.

f) *Study units :* Individuals in the selected household

g) Sample Size Calculation and Sampling Technique

Sample size was calculated based on either the prevalence of knowledge, attitude and practice based on the following assumptions:

- 50% prevalence of knowledge, attitude and practice (p) to get maximum sample size
- 95% confidence level (CL)
- Maximum tolerable error of 5% (d)
- The sample size was then calculated using the usual single population proportion formula

Multistage stratified sampling technique was employed to get study subjects. First Anti Malaria Association intervention districts were listed in each zone. Based on size, one district from Awi zone, one district from south Gondar, two districts from west Gojjam and two districts from east Gojjam were selected randomly. Proportional allocation was done based on residence (urban vs rural) since there is an assumption that there are differences in Knowledge, attitude and Practice among the community towards malaria prevention and control options. From these districts using random sampling technique, three rural kebeles and one urban kebele from five intervention zones and four rural kebele from one district (since there is no urban kebele among the selected intervention kebeles) were selected randomly. From each urban kebele, 28 households and 38 households from each rural kebele were selected using systematic random sampling technique after sampling frame was obtained with the help of kebele leaders, district malaria focal person and local guiders.

h) Inclusion and Exclusion Criteria

People live at least 6 months in the selected kebeles were included in this study and people who live less than 6 months in the Keble and respondents in the households who cannot communicate due to impairment or sever sickness were excluded from this study.

i) Variables of the Study

Knowledge, attitude and practice of the community on malaria prevention and control options are outcome variables and socio demographic variables, and especial experience to malaria (once contracted malaria, lost family member due to malaria, training on malaria prevention and control, having especial access to information to malaria and other health related issues, leadership role in the kebele).

j) Operational Definition Of Selected Variables

Knowledgeable: A study participant whose score is equal to or more than means score of the total questions by the study participants.

Positive attitude: A study participant is said to have positive attitude towards malaria prevention and control options if his/her score towards the questions is equal to or more than the mean value.

Good Practice: An individual can be considered as having good practice when he/she practices with a score of equal to or more than the mean value.

k) Data Collection

Data was collected using structured questionnaire adapted from standardized questionnaires used by international organizations, national studies such as Demographic and Health survey and

published articles in peer reviewed journals. Data was collected by trained data collectors through face to face interview of the respondents. During data collection it has been tried to assess the socio demographic variables, special characteristics of respondents like once contracted malaria, lost family member due to malaria, training on malaria, having special access to information to malaria, participation in Community conversation, leadership role in the kebele, level of knowledge about mosquito behavior, signs and symptoms, treatment modalities and prevention mechanisms, attitude towards malaria prevention and treatment seeking and practices in malaria prevention of the community.

Knowledge assessment part of the questionnaire tries to measure causes of malaria, means of transmission, mosquito breeding site, biting time, signs and symptoms of malaria, signs and symptoms of severe malaria, susceptible groups to malaria, treatment modalities and prevention methods. Attitude assessment part of the questionnaire tries to assess attitude towards malaria prevention and treatment modality options. Similarly, the study tries to assess practices of the community towards malaria prevention.

l) Data Collection Procedure and Quality Issues

Data collection was carried out by diploma holder health professionals. Appropriate training was given to data collectors and supervisors by principal investigators. To ensure data quality, in addition to training of the research team, checklist was prepared starting from the sample selection to the end of interviewing the respondent. Similarly to get the maximum data quality, local guiders in each kebele and district malaria focal person assisted the data collection process. The collected data was checked on daily bases, and identified problems were corrected as soon as possible by supervisors. A mechanism was developed to bring letter of approval for collected data in the selected areas from the district and kebele administrators and communicate to monitor and witness that the data collectors collect data from the randomly selected households

m) Data Management and Analysis

Data was checked for completeness and consistency, and entered into SPSS version 16 by principal investigators. The data cleaned using frequency and analyzed using SPSS version 16 for windows. The result was presented using simple frequencies with percentages in appropriate tables to display the descriptive part of the result.

n) Ethical Consideration

Ethical clearance was sought from Amahara National Regional Health Bureau and permission letter was collected from the respective levels of administrative offices from all levels. Confidentiality and privacy of study participants were maintained. Participants were informed about their right not to participate, not to tell a certain information if they do not want to or even to withdraw without being denied from any possible benefit. Data was collected after obtaining verbal consent from each study participant.

III. RESULT OF THE STUDY

The study enrolled a total of 864 household representatives in six districts of Anti-Malaria Association intervention zones of the Amahara National Regional State. These 864 households comprised of 4626 people equaling an average family size of 5.46 individuals. Of these, 626 (13.5%) were under five children, and 95 (2.05%) were pregnant mothers.

a) Socio-Demographic Characteristics of Study Participants

Mean age of study participants was 37.84 years. Seven Hundred twenty two (84.2%) of the respondents were from rural kebeles, about 99% were orthodox Christians by religion, about 84% were Amahara by Ethnicity, nearly 85% were married, and 68% of the study participants were illiterate. However, 90% of the households had at least one individual, mostly children, who could at least read and write. Some 66% of the study participants were farmers by profession. Nearly 54% of the study participants were females. The table below displays socio-demographic characteristics of study participants.

Table 1 : Socio-demographic characteristics of study participants in Antimalaria Association intervention zones, Amahara national regional state, Ethiopia, March, 2011

<i>Variable</i>	<i>Variable category</i>	<i>Number</i>	<i>Percent</i>
Residence	Urban	133	15.6
	Rural	722	84.2
Sex	Male	403	46.6
	Female	461	53.6
Religion	Orthodox Christian	855	99.1
	Muslim	8	0.9
Ethnic group	Amahar	730	84.5
	Awi	131	15.2
	Tgrie	3	0.3
Marital status	Married	677	78.4

Educational status	Single	63	7.3
	Divorced	69	8
	Widowed	55	6.4
	Illiterate	587	67.9
	Only read and write	98	11.3
	Primary(1-4)	62	7.2
	Junior (5-8)	63	7.3
	Higher secondary (9-12)	45	5.2
Occupation	Above 12 th	8	0.9
	Farmer	571	66.1
	Housewife	157	18.2
	NGO employed	2	0.2
	Governmental employed	8	0.9
	Daily laborer	13	1.4
	Merchant	61	7.2
	Other*	52	6.0

*Carpenters, students, drivers, who have no work etc.

b) Sources of Information About Malaria

The study indicated that 540 (62.5%) of the respondents got information about malaria from different sources. Health organizations 430 (49.8%), religious institutions 270 (31.25%), radio 49(5.7%), Friends 44 (5.1%), Schools 39 (4.5%) and Television 12(1.4%) were major sources of information about malaria in the community. In health institutions, health extension workers were the dominant sources of information about malaria in the community.

Anopheles mosquitoes are responsible for the transmission of malaria. From these, 24.1% of urban residents and 21.5% of rural residents knew that malaria can be transmitted by Anopheles mosquitoes bite. Only 14(1.6%) of the respondents knew that malaria is caused by microscopic organisms (plasmodium species). Six hundred twenty seven (72.6%), 57 (6.6%), and 21(2.4%) of the study participants mentioned stagnant water, running water, and soil as breeding sites, respectively.

c) Knowledge on Mosquito Behavior, Causes and Means of Transmission of Malaria

The study indicated that only two hundred seventy nine (32.3%) of study participants knew that

Table 2 : Knowledge of the community about mosquito behavior, breeding site, causes and transmission of malaria among Antimalaria Association intervention zones, Amahara national regional state, Ethiopia march 2011

Variables		No (%)
Mosquito breeding site	Stagnant water	627 (72.6)
	Running water	57(6.6)
	Soil	21 (2.4)
Mosquito biting time	Day	94(10.9)
	Evening	242(28)
	Night	415(48)
	Any time	30(3.5)
	Do not know	212(24.5)
Means of transmission	Mosquito bite	279 (32.3)
	Microscopic organism	14(1.6)
Causes of malaria	Mosquito bite	187(21.6)
	Microscopic organism	6(0.7)

The study revealed that misconceptions about causes and transmissions of malaria are very common. The three most common misconceptions of study participants about causes of malaria were exposure to cold weather 219(25.3%), hunger 135(15.6%), drinking dirty water 127 (14.7 %). Also the mentioned eating corn cane 27(3.1%), sleeping with malaria patient 20(2.3%) and Evil spirit 10 (1.2%) as causes of malaria.

d) Recognition of Signs And Symptoms of Malaria by the Community

The study indicated that three commonly mentioned manifestations of malaria were feeling cold and rigor 557 (64.5), fever 325 (37.5%) and headache 317 (36.7%). From the total respondents, only 325 (37.6%) were identified fever as signs and symptoms of malaria.

Table 3 : Respondents Knowledge on signs and symptoms of malaria by district in Anti-malaria Associations' intervention areas, Amahara National Regional State, Ethiopia, March 2011

<i>Signs and symptoms of sever malaria</i>	<i>Residence</i>		<i>Total NO (%)</i>
	Rural	Urban	
Feeling cold and rigor	461 (63.85 %)	96 (72.2%)	557 (64.5)
Fever	271 (37.5%)	54 (40.6%)	325(37.6)
Headache	266 (36.8%)	50 (37.6%)	317(36.7)
Joint pain	152 (21.1%)	32 (24.1%)	184(20.6)
Vomiting	130 (18%)	14 (10.5%)	144(16.7)
Sweating	115 (15.9%)	24 (18.0%)	139(16.1)
Loss of appetite	86 (11.9%)	25 (18.8%)	111(13)
Thirsty	85 (11.8%)	20 (15.0 %)	105(12.2)
Nausea	66 (9.14 %)	14 (12.6 %)	82(9.5)
Bitterness	67 (9.3%)	4 (2.4%)	71(8.2)
Tiredness	42 (5.8%)	10 (7.5%)	52 (6)
Diarrhea	27 (3.7%)	4(2.4%)	31(3.6)
Other*	90 (12.5%)	19 (14.3%)	109(12.5)

*weight loss, back pain, stomachache, bloody urine, shaking etc

The study indicated that the loss of consciousness, seizure and vomiting were mentioned by 28.1%, 24.4%, and 16% of study participants as major manifestations of severe malaria.

Table 4 : Respondents Knowledge on Signs and symptoms of sever malaria by resident in Antimalaria Associations intervention zones, Amahara national regional state, Ethiopia, March 2011.

<i>Signs and symptoms of sever malaria</i>	<i>Residence</i>		<i>Total No (%)</i>
	Rural	Urban	
Loss of consciousness	194 (27.2)	30 (22.6)	244 (28.3)
Seizure/Convulsion	174 (24.8)	47 (35.3)	211(24.4)
Vomiting	118 (16.3)	17 (12.8)	138(16)
Unable to eat/drink	93 (12.6)	15 (11.3)	108(12.5)
High grade fever	80 (11.1)	12 (9)	92(10.6)
Dark red urine	36 (5)	9 (6.8)	46(5.3)
Restlessness	22 (3)	5 (3.8)	27(3.1)
Severe Anemia	23 (3.2)	4 (2.4)	27(3.1)
Do not know	218 (30.2)	25 (18.9)	243(28.1)
Others*	94 (13)	19 (14.3)	113(13.1)

* Neck stiffness, Crying all the time, weight loss, back pain, stomachache, bloody urine, shaking etc

e) *Perception of the Community towards Most at Risk Population Groups to Malaria* under five children and pregnant women as the most susceptible groups of the population in communities to malaria. Three Hundred twenty four (37.5%) and 213 (24.7%) of study participants were correctly identified

Table 5 : Respondents Knowledge on susceptibility for malaria by residence among Antimalaria Associations intervention zones, Amahara national regional state, Ethiopia, March 2011

<i>Susceptible group</i>	<i>Residence</i>		<i>Total No (%)</i>
	Rural	Urban	
Under fives	245 (33.9)	79 (59.4)	324 (37.5)
Pregnant women	147 (20.4)	13 (9.8)	213 (24.7)
Equal for all	155 (21.5)	23 (17.3)	171(19.8)
Elderly	51 (7.1)	23 (17.3)	120(13.9)
Adults	51 (7.1)	9 (6.8)	60(6.9)

HIV career	4 (0.6)	7 (5.3)	11 (1.3)
Never sick before	3 (0.4)	3 (2.3)	6(0.7)
Don't know	166 (23)	15 (11.3)	183(21.2)
Other *	36 (5)	8 (6)	44(5.2)

*malnourished, farmers, overloaded workers, people in cold areas

f) *Treatment Seeking Behavior of the Community to Malaria*

From the total respondents, 780 (90.8%) knew that malaria can be treated. Despite this, about 485 (61.1%) did not know any kind of antimalaria drug and only 171 (19.8%) and 109 (13.9%) mentioned coartem and Chloroquine as Anti malaria drugs, respectively.

A total of 626 under five children were present in 864 households studied. Of these, 176 (28.11%) were reported to have an acute febrile illness within the last 6 months before data collection. Of these 50 (28.4%) of them were taken to any health institution for seeking advice and treatment within 24 hrs of starting the fever and the rest did not taken to health institutions within 24 hours of starting fever. For this the following factors were mentioned as possible reasons: the illness was mild (28.3%), financial constraint (21.7%), work overload of care takers (19.6%), and distance of health care institutions (12.8%).

Of 176 under five children, 126 were claimed to be taken to health institutions and (64.28%) were taken to government health centers, 33(21.19%) were taken to health posts, and (23.8%) were taken to private hospitals.

The study revealed that children were taken to health institutions with an average of 2.72 days after the occurrence of febrile illness. According to the respondents, they got drugs for their children (45.1%), 27.8% 20.1% from governmental health centers, health posts and from private hospitals or clinics and from other sources like drug store, pharmacy, shop and market places, respectively. However, they also admitted the wide spread utilization of traditional or local remedies of malaria. These local remedies of malaria included herbal medicine, herbal medicine with butter or honey, goat blood, animal fat especially tails of sheep locally known us "lat" with alcohol.

Table 6 : Level of negative attitude (mind set/position) towards malaria prevention and treatment in Antimalaria Association intervention areas, Amahara national regional state, Ethiopia, 2011. (n=864)

Opinion	Total
Traditional methods such as killing hen or sheep prevents malaria better than bed nets and draining water ponds (Agree)	168(19.5%)
There is no any effective means to prevent malaria, but God (Agree)	343(39.7%)
Best way to prevent malaria is to go to wizards follow their instruction (Agree)	36(4.2%)
The best way to treat malaria is to remain at home ,drink holly water and pray (Agree)	316(36.6%)
The available modern drugs can not treat malaria, they rather worsen the disease (Agree)	112(13.0%)
Locally available tradition remedies of malaria obtained from Wizards can treat malaria better than the modern drugs prescribed by health professionals (Agree)	47(5.5%)
Modern drugs for the treatment of malaria should be taken only when traditional remedies fail (Agree)	130(15.0%)
Malaria is a punishment for sinner and taking any modern drugs can only double their Sin (Agree)	118(13.7%)

g) *ITN Possession and Utilization by the Community*

From the total respondents, 61.29 % reported that malaria is a preventable disease using bed nets. Further inquiries revealed that of the total 864 study participants, 770 (89.1%) of them claimed that they currently owned bed net (any kind) in the households. Of these, 652/770 (84.67%) of the households possessed functional bed nets.

About twenty six percent of the general population uses ITN in the previous date of the survey. Of the total 626 under five children in the study households, 447 (71.4%) of them were reported that they have been slept under bed net a day before the

interview took place. Moreover, of the total 95 pregnant women, 56 (56.95%) of them were reported to have been slept under bed net a day before data collection.

From the total 4626 people in the study area, 61.4% of the study population have been slept under insecticide treated net a day before the interview took place.

The study also revealed that traditional methods which are not yet proven scientifically were applied to prevent malaria in the community. Nearly 40% of study participants admitted that there are some traditional activities practiced to prevent malaria. These include: eating garlic with green paper, drink juices of

haregerasa, polishing the floor with tenjut, drinking endode juice, never eat vegetables etc.

h) Insecticide Residual Spraying (IRS) And Utilization In The Community

Researchers further assessed the level of knowledge and utilization of indoor residual spray. From the total respondents, only 5.37% believe that malaria can be prevented using IRS. Study participants were also asked whether indoor chemical spray was performed for their house, and whether their house was plastered after indoor chemical spray. Of the total study participants, 369(42.7%) remembered that indoor chemical spray was carried out within 24 months (mean 2.68 months) before data collection, while 104(12.03%) of study participants remembered that their house was plastered or painted after indoor chemical spray within the last 24 months.

i) KAP of the Community Towards Malaria

The levels of knowledge and attitude of study participants regarding to causes, transmission, and prevention of malaria indicated that 66.3%, 50.8%, and 64.8% of the study participants had been regarded as knowledgeable about causes & transmission, clinical manifestations, and prevention of malaria, respectively while 78.1%, 69%, and 47.1% of the study participants were considered as having positive attitude towards malaria prevention, treatment, and good malaria prevention practices, respectively.

IV. DISCUSSION

The study indicated that health and religious institutions are major sources of information on malaria prevention and control activities. This study indicated that 32.3% knew that mosquitoes are responsible for malaria transmission. This finding is somewhat consistent with KAP study done in Tripal community of Baigachaek area which revealed that 37.6 % of them knew that mosquito bite transmits malaria. [4]

However, this study subject's knowledge about the means of transmission of malaria is lower than studies done in Ethiopia. For instance KAP surveys done in butajira Ethiopia and three towns of Western Ethiopia revealed that 48% and 43.7% have knowledge about the transmission of malaria, respectively [5,6]. This disparity may be due to the difference in study setting which was done in towns for the first study and data collection period was in malariosus period for later studies, respectively. It is believed that people in towns may have good access to information and in malarias period there will be more practices about malaria prevention and control activities. This condition may increase the communities' knowledge about malaria prevention and control activities.

On the other side, this study result is higher than the result of Ethiopia national malaria indicator

survey of Amahara national regional state which indicated that 26.7% of the respondents have knowledge on mosquito bite as causes of malaria [7]. This difference is due to the fact that there is a study period difference between the two studies.

This study indicated that only 14 (1.6%) were knew that malaria is caused by microscopic organisms. Similarly, a qualitative study done in Amahara and Oromia national regional states supported this study result [8]. Also a study done in North West Tanzania indicated that only 6% of the respondents have mentioned plasmodium organisms as a cause of malaria [4]. This low knowledge level of the community may be related with malaria prevention activity gives emphasizes on mosquito related problems rather than the parasite. In addition to the above findings, the study showed that exposure to cold weather (25%), Hunger (15.5%) and drinking dirty water (14.7%) are common misconceptions about the causes and transmission of malaria. This finding is consistence with other similar studies done in different parts of Ethiopia [7,9].

Regarding to the biting time of mosquito, majority (48%) of the respondents mentioned night as a biting time. This study result is lower than studies done in Assosa and Butajira which indicated that 95% and 73.2% of the respondents knew that mosquito bites human beings during night time [5,6]. This variation may be due to differences in study settings.

The result of this study indicated that study participants mentioned stagnant water as breeding sites. The study indicated that 627 (72.6%) of the respondents mentioned stagnant water as a breeding site for malaria. This finding agrees with the findings of a study done by Wagari and a qualitative study done in Amahara and Oromia regional states indicated that mosquitoes are mainly believed to breed in stagnant water (71%) [8].

This study indicated that 37.6% of the respondents mentioned fever as signs and symptoms of uncomplicated malaria. This study figure is lower than Amahara regional state data as reported by Ethiopian national malaria indicator survey of 2007 which indicated that 50.2% recognizing fever as signs of malaria [7]. This difference may be related to differences in study period in which ours is done during none malarias season and the later one is in malarias season. This finding was supported by a study done in butajira district southern Ethiopia reported fever, headache and shivering and chills as signs and symptoms of uncomplicated malaria [6]. Also a study done in Swaziland, mid 2007 indicated that symptoms such as headache, high temperature/fever and chills were the three most frequently mentioned signs and symptoms of malaria [10].

Also the present study revealed that unconsciousness (28.3%), seizure/convulsion (24.4%) and vomiting (16%) were most frequently mentioned signs and



symptoms of severe malaria. This finding was supported by a study done in Myanmar which reported unconsciousness and convulsion are most frequently mentioned signs and symptoms of malaria. [11]

Regarding to malaria prevention options, 86.2% of the respondents reported that malaria is a preventable disease which is consistent with a study done by Wagari in Ethiopia indicated 85.7% and a study in Swaziland indicated 78 % of the respondents knew that malaria is preventable disease [6,12]. But this study result is somewhat higher than a study done in Nepal indicated only 50.3% knew that malaria is a preventable disease [10].

Eight hundred thirty one (96.2%) of the respondents reported that they had heard about mosquito net and 52.8% of them believe ITN can prevent malaria. This study figure is higher than Amahara national regional state data which indicated that only 38.5% have heard about ITN [7].

This study indicated that 26.4% of the general population and 71.4 % of underfive children was slept under ITN in the previous night of the survey. This study figure is higher than Ethiopian MIS 2007 of Amahara national regional state indicated only 51.8% were slept under ITN [7]. This is due to the fact that there is study year difference between the two surveys. Despite the study year differences in this study and MIS of 2007 Amahara national regional state data there is no difference on pregnant women ITN utilization which indicated 56.9% and 52.7% respectively [7].

The study revealed that only 5.37% of the study participants had accepted indoor residual spray as malaria prevention method. This study indicated that the acceptance rate is lower, 42.7% of the participants house had been sprayed with insecticide residual. This figure was higher than the report of Ethiopian MIS 2007 which indicated that 14.2% of the country and 18.7% of Amahara regional state residents' houses were sprayed with chemicals [7].

Seven hundred eight (90.8%) knew that malaria is a treatable disease. This result agrees with a study in Nepal indicated that among the total respondents, 86.4% were knew that malaria is treatable [10]. Despite this, the study finding indicated that about 485 (61.1%) did not know any kind of antimalaria drug, 171 (19.8%) and 109 (13.9%) mentioned coartem and Chloroquine as anti malaria drugs respectively.

This study indicated that there are people who has wrong attitude towards malaria prevention and treatment where 39.7% of the study participants responded wrongly to the item "There is no any effective means to prevent malaria, but God". Moreover, 36.6% of the study participants agreed with the statement "The best way to treat malaria is to remain at home, drink holy water and pray". This study indicated that 37.5% of the study participants recognized fever as signs of malaria. However, this figure is slightly higher than MIS of 2007

report of Amahara national regional state report indicated 25.2 % had febrile illness [7].

The study indicated that 28.11% of underfive children had febrile illness in the last last six months of the survey. Of these, only 28.4% had been taken to health institutions for treatment of illness within 24hrs of starting febrile illness. This study result is higher than the report of MIS 2007 of Amahara national regional state that indicated only 15% of the respondents had sought a prompt treatment for their febrile child [7].

V. CONCLUSION AND RECOMMENDATION

a) Conclusion

- Health and religious institutions are the main sources of information about malaria control and prevention activities in the study community.
- Even though there are improvements on the knowledge of the community about the causes, means of transmission, and clinical manifestations compared to MIS of 2007 in Amahara regional state, it was lower than other studies conducted previously in the country.
- There are common misconceptions on causes, means of transmission, clinical manifestations and prevention practices in the community.
- In this study the ability of the community to recognize fever as signs and symptoms of malaria is lower than Malaria Indicator Survey 2007 of Amahara regional state.
- The study indicated that underfive children, pregnant women and HIV carriers were identified by the community as at most risky population groups which is in parallel with the national priority on malaria prevention and control.
- More than half of the respondents did not know any kind of antimalaria drugs that can be currently used in this study.
- This study indicated that there is an improvement on believes that ITN can prevent malaria as compared to MIS 2007 in Amahara regional state.
- The study indicated that lower number of people was slept under ITN in the previous date of the survey as compared to Malaria Indicator Survey 2007 result in Amahara regional state.
- The acceptance rate of the community to IRS had showed improvements from the previous studies in the country as well as MIS of 2007 in Amahara regional state.
- There is an improvement on recognizing Febrile illness as symptom of malaria from the result Malaria Indicator Survey 2007 result in Amahara regional state.
- There is an improvement on treatment seeking for underfive children within 24 hrs starting febrile illness compared with MIS of 2007 in Amahara regional state.

- Knowledge, attitude and practice of the community towards malaria prevention and control options.

b) *Recommendation*

Based above conclusion we recommend the following points

- To achieve the intended plan of the intervention program after three years, it is recommended to focus on common misconceptions about malaria causes, means of transmission and clinical manifestations through community involvement activities as a main strategy.
- The ability to recognize febrile illness as symptom of malaria and treatment seeking behavior of the community for febrile children within 24 hrs are low. Therefore special attention must be give to mothers /care takers/ of underfive children. This can be done by integrating with ANC, PNC and immunization programs.
- Even though there are improvements on the importance of ITN on malaria prevention and control, ITN utilization rate is still very low. Among many reasons for this, the community uses ITN for other purposes. Therefore, efforts are needed to increase utilization of ITN in the community through community conversation and education. In addition, AMA must put efforts on the regional government to develop rules and regulations on proper utilization of ITN in the community.
- IRS has a negative effect on bees and this may reduce the acceptance rate of IRS. Therefore inter sect oral collaboration is essential between the agricultural sector and AMA to reduce the effect of chemical on bees and to scale up IRS acceptance rate in the community.

VI. ACKNOWLEDGEMENT

We are great full to Bahir Dar University, and Debre Markose University for giving permission to do this research. We use this opportunity to express its deep gratitude to Antimalaria Association with Global Fund, Ministry Of Health, and Amahar Regional Health Bureau for their generous financial support.

At last but not least we want to appreciate the efforts of researchers, supervisors, data collectors and district health office malaria focal persons for their efforts to complete this research.

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GLOBAL JOURNAL OF MEDICAL RESEARCH
DISEASES

Volume 13 Issue 5 Version 1.0 Year 2013

Type: Double Blind Peer Reviewed International Research Journal

Publisher: Global Journals Inc. (USA)

Online ISSN: 2249-4618 & Print ISSN : 0975-5888

Preliminary Investigation on Anti Typhoid Properties of Acacia Nilotica Leaf Extract

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Keywords: *typhoid fever, anti typhoid, acacia nilotica, salmonella typhi and Id.*

GJMR-F Classification : *NLMC Code: WC 500*



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Preliminary Investigation on Anti Typhoid Properties of *Acacia Nilotica* Leaf Extract

Sarkiyayi S.^α & Abdulrasheed K.^σ

Abstract- Anti typhoid properties of methanol leaves extract of *Acacia nilotica* was investigated. The phytochemical screenings for detection of the presence of bioactive constituents were carried out. Among other parameters investigated include LD₅₀ of the leaves extract, inoculation of mice with *Salmonella typhi* parasites treating them with leave extract of *Acacia nilotica* followed by widal tests. The phytochemical screening of the methanol leave extract of *Acacia nilotica* revealed the presence of alkaloid, anthraquinone, carbohydrates, cardiac glycosides, flavonoid, saponin, tannins and terpens. The leaves extract of *Acacia nilotica* has possess some acute toxicity effect on animals (mice) at a dose of (LD₅₀) 288.5mg/kg. The plant extract produced inhibitory activities against *Salmonella typhi*. It is interesting to note the widal test titer valve was found to be 1\40 for the treated group while the untreated group had titer value of 1\160, suggesting that the plant leaves extract was effective as anti typhoid agent against *salmonella typhi* on mice infected with typhoid parasites. The extract demonstrated high activity against *Salmonella typhi*, bacterial typhoid causing agent.

Keywords: typhoid fever, anti typhoid, acacia nilotica, salmonella typhi and ld.

1. INTRODUCTION

The emergence and spread of *Salmonella typhi* resistance to many commonly used antibiotics (Ampicillin, Chloromphenicol, Amoxicillin is now a subject of international concern. The problem has become endemic in many developing countries, causing enormous childhood morbidity and high cost of treatment (Leume, 1999). Multidrug resistant *Salmonella* species are being increasingly reported from the developed world. There is therefore, the need for efficient and safe vaccine which can be used as a preventive public health tool (Leume, 1999). Thus the resistance of *Salmonella typhi* to these antibiotics couples with the high cost of treatment have prompted the present study of local herbs for

The treatment of typhoid fever. *Acacia* leaf is a plant used in Northern part of Nigeria and many have claimed to have gotten remarkable improvement in their condition after taking the preparation from the herbs (Aussie, 2006). The medicinal plant is used in various ways but commonly is boiled in water and then allowed

to cool before drinking. The patient may inhale the steam after which he or she drinks the herbal preparations.

Typhoid fever is an infectious disease cause by bacterial of salmonella group-*Salmonella typhi* and *Salmonella para typhi* A, B or C. The organism is Gram negative, flagellated, non encapsulated, non sporulating and facultative anaerobic bacillus. The strain differs from other salmonellas in that it does not produce gas from glucose and from little or no water. It has only one phase antigen and posses a capsular antigen called vi (Cook, 1988). *Salmonella typhi* causes typhoid (enteric) fever, the bacterial pass from the small intestine into the blood through Lymphatic system. The reticulum-endothelial system becomes infected as well as the gallbladder and kidneys. From the gallbladder, the organism invades the intestine causing inflammation and ulceration (Cook, 1988).

Typhoid fever is characterized clinically by continual high fever of 40^oc and headache and the incubation period is normally two weeks, in the second or third week the organism becomes disseminated in the body and can be isolated from urine and feces. According to Cook (1988), Ivanoff et al, (1997) symptoms of infection includes fever with low pulse rate, headache, enlargement of the spleen and mental confusion. A rash (rose spots) may be seen on light colored skin. Epitasis intestinal hemorrhage and perforation may also occur. In uncompleted (Asymptomatic) typhoid, the total white cell count is low with a relative Lymphocytosis and there may also be anemia. The condition is an immune complex disorder of the kidney and is characterized by fever, edema and marked albuminuria. It also causes osteomyellitis (Inflammation of the bone marrow), especially in children with sickle cell disease and thalassaremia, typhoid nodules can be found in the bone marrow. Inflammation of the joints (Typhoid Arthritis) may also occur. *Salmonella para typhi* A and B causes paratyphoid (Enteric Fever) and the diseases are generally mild with *Salmonella paratyphi* A and B being less invasive than *Salmonella typhi*. These are usually characterized with diarrhea and vomiting and enteric intestinal treat may be inflamed especially in *Salmonella paratyphi* B infection.

In tropical and other developing countries paratyphoid is more commonly used by *Salmonella paratyphi* A than *Salmonella paratyphi* B (WHO). Before the early 20th century, typhoid fever was a common

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disease that occurred in large epidemics everywhere. In countries where modern method of sanitation and sewage disposal is only rarely encountered but in parts of the world lacking good sanitary facilities, it continuous to represent a serious health problem.

There are two main diagnostic laboratory test for typhoid. These are the specimen cultured in which the organism is isolated from blood, bone marrow, urine or stool of the patient and the serological method, which is based on agglutination test with antisera from "O" and "H" antigen. The two main serological test are rapid slide (widal) test. The serological method were used to give fast results (Within an hour) in contrast to the culture method, which takes weeks . The widal test is based on the fact that the serum agglutination rise sharply during the second and third week of salmonella infection. High or rising titre of "O"antigen (1:160) suggest active infection. However, the result of serological test for *salmonella* infection must be interpreted cautiously because cross reaction antibodies can give a false positive result. Typhoid fever is now an endemic disease in our community.

Many medicinal plants are used to treat many health problems including internal and external forms depending on the region where people inhabit. Proven medical plant are used in the treatment of diseases either alone or in combination with other plants, they are used as anti-infections agent, anti-malaria, anti-hormonal and nerves remedy. Several laboratories have reported the effectiveness of common indigenous herbs against gram-negative and gram-positive micro-organism. In clinical research, the extract of *Acacia* has been used in the treatment of inflammation in the respiratory ailment. It is also helpful for cough, sore throat, eye wash, diarrhea and dysentery.

This study, therefore intends to scientifically assess the effectiveness of *Acacia leaf* in the treatment of typhoid fever using experimental animals and also to substantiate the claims by the traditional medicine practitioners that *Acacia* leave extract is used for the treatment of patient caught with typhoid fever.

II. MATERIALS AND METHODS

a) Materials

Fresh leaves of *Acacia nilotica* were collected from Kaduna Polytechnic Main Campus Tudun Wada Kaduna. Experimental animal were obtained from animal house in the department of pharmaceutical science, Ahmadu Bello University Zaria. They were kept in clean cage and fed on chow diets and water for 2

weeks in order to be acclimatizing to room temperature before being exposed to the plant extract.

b) Preparation of Extract

The sample collected was air dried at room temperature, the dried leaves were then pounded to powder form using mortar and pestle. It was then properly store until required. Methanol extraction was carried out in a soxhlet apparatus .Preliminary phytochemical screening Extract obtained were screened for their phytochemical constituents using standard quantitative procedures (Harbane, 1973, Trease and Evans 1989, and Safowora, 1993). Alkaloid, anthraquinones, saponins, flavanoids, tannins, steroids ,cardiac glycosides, carbohydrate (reducing sugar), and terpenes

c) Acute toxicity test

Pilot study was carried out using Lorke's (1983) method to determine the LD₅₀ Value of the methanol extract of *Acacia* leaves. Pilot study was carried out using Lork's method to determine the LD₅₀ value of the methanol extract of *Acacia* leaves. The pilot study was carried to determine the maximum dose that could not produce death and the minimum dose that could be lethal, the range of toxicity orally Lorke's method of determination of LD₅₀ is carried out in two stages (Lorke, 1983).

d) First stage of Lorke'test

Three groups I, II and III of albino-mice for each of the extract used weighed separately and placed cages. The groups were given 10mg/kg, 100mg/kg and 1000mg/kg of doses of the extract respectively. The mice were observed for 24hours and all symptoms of intoxication and number of dead mice were recorded.

e) Second stage of Lorke's test

Based on the result obtained from the first stage of pilot studies, dose were chosen from the extracts and further experiment were carried out as detailed below Four groups I, II, III and IV of one mice each was weighed and placed in separate cage, the mice were given the extracts at doses of 140mg/kg, 225m/kg, 370mg/kg and 600mg/kg respectively. For the methanol extracts they were then observed closely for 24hours for signs and symptoms of intoxication and death (Lorke 1983).

First stage of Lorke's test

Group	Doses in mg/kg	Initial number of mice	Survived	Mortality
A	1000mg/kg	3	0	100
B	100mg/kg	3	3	0
C	10mg/kg	3	3	0

Second stage of Lorke's test

Group	Doses in mg/kg	Initial number of mice	Survived	Mortality
A	140mg/kg	1	1	0
B	225mg/kg	1	1	0
C	370mg/kg	1	0	100
D	600mg/kg	1	0	100

LD₅₀ =

LD₅₀ =

 $225 \times 370 = 288.5 \text{mg/kg}$

LD₅₀ = 288.5mg/kg.

f) Parasite inoculation

Salmonella typhi obtained from 44 Army Hospital was maintained by serum passing in albino mice. Nine mice were distributed into 3 groups with three mice in each group. These were kept in separate apartments. The mice in each group I, II and III were injected intravenously with typhoid fever bacterial causative organism (Salmonella typhi). Group I and II were however placed on oral treatment with 125mg/kg and 150mg/kg of the extract for 3days, while group III was served as control. After which a widal test was carried out on them to confirm if they were infected with typhoid fever.

g) Widal test

2 drops of serum to be tested is place on a while tile, the antigen suspension was shaken and 1 drop of the antigen was added. It was then mix over an area of 3cm, rock gently and examine for agglutination after 1 minute

h) Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) of the methanol Extract was determined by method described by Akinpelu and Kolawole (2004).

III. RESULTS AND DISCUSSION

The result of anti-typhoid properties of *Acacia* is presented as follows.

Table1 : Phytochemical composition of methanol extract of *Acacia nilotica* leaves

Phytochemicals	Methanol extract
Alkaloids	+
Anthraquinones (Free state)	+
Anthraquinonea (Combined States)	+
Cardiac Glycooide	+
Flavanoids	+
Saponins	+
Steroids	-
Tannins	+
Terpenoids	+
Key: + = Present, -- = Absent	

a) *Phytochemical composition of the extract*

The phytochemical screening of the methanol extract revealed the presence of Anthraquinone, Terpenoids, Saqorin, Tannin, Alkaloid, Flavanoid and Cardiac glycoside (Table 1)

Acute toxicity test

The LD₅₀ of the plant extract was 288.5mg/kg as calculated using Lorke's method.

b) *Widal Test*

Significant titer value for widal test range from 1/160 and above while non-significant valve range from 1/40 and below. The widal test titer valve was found to be 1\40 which is not significant for the treated groups Suggesting that the leaves extract has some anti typhoid properties.

Table 2 : Results for Widal Test

	Three days inhibitory effect of plant extract on Salmonella typhi growth		
	Day I	Day II	Day III
Group I (125mg/kg of <i>Acacia leaf</i>)	H= $\frac{1}{40}$,O= $\frac{1}{40}$	H= $\frac{1}{20}$,O = $\frac{1}{40}$	H= $\frac{1}{20}$,O= $\frac{1}{20}$
Group II (150mg/kg of <i>Acacia leaf</i>)	H= $\frac{1}{40}$,O= $\frac{1}{20}$	H= $\frac{1}{40}$,O= $\frac{1}{40}$	H= $\frac{1}{20}$, O = $\frac{1}{40}$
Group III(no extract given)Control	H= $\frac{1}{160}$,O= $\frac{1}{160}$	-	-

Titer of 1/160 and above are significant ; The O and H are alphabets used to represent the salmonella antigen (somatic) and (flagella) antigen respectively

c) *Minimum Inhibitory Concentration*

Our findings revealed that methanol extract of *Acacia n.* plant material has some antimicrobial activity. The minimum inhibitory concentration of the plant was at 2mg/ml suggesting that the leaves methanol extract of *Acacia n.* possess some anti typhoid properties.

IV. DISCUSSION

According to the centre for disease control, approximately 5% of people who effectedwith typhoid continues to carry the disease after they recover. The world Health Organization (WHO) identified typhoid as a serious public health problem. Its incidence is highest in children and young between 5 and 19 years old (WHO ,2008). According Ivanoff et al (1997) , symptoms of infection include fever with low pulse rate, headache, enlargement of the spleen and mental confusion.

From the analysis carried out, it was observed that the methanol extract of *Acacia nilotica* have some phytochemicals like Alkaloids, Anthraquine, Glycoside, Flavanoids and Tannins . The presence of these compounds promises it potential application in the treatment of microbial ailment. While the acute toxicity test showed that the LD₅₀ of *Acacia nilotica* was 288.5mg/kg. Also the group of the experimental animal (mice) treated with the methanol extract of *Acacia nilotica* after infection with *Salmonella typhi*, showed total

clearance and 100% protection from *Salmonella typhi*. A similar studies was conducted by Vivek *et al* (2010) which showed that aqueous extract of fruits of *Citrus sinensis* confer anti typhoid activity against *Salmonella typhi* . The results in table 2 revealed that the mice in group I, and II that were placed on oral treatment with 125mg/kg and 150mg/kg of the extract respectively had recovered, suggesting that *Acacia nilotica* has some anti typhoid activity.While the mice in group 3 had died. Similarly, Sarkiyayi *et al.*, (2011) reported that aqueous methanol leaves extract of *Albazia ferruginea* plant was effective as anti typhoid agent against *Salmonella typhi* on mice infected with typhoid parasites. Furthermore, our findings revealed that methanol extract of *Acacia n.* plant material has some antimicrobial activity with a minimum inhibitory concentration of 2mg/ml, suggesting that the leaves methanol extract of *Acacia n.* Possess some anti typhoid properties. In a related development, the ethanol extract of ginger at 0.8g/ml concentration produced higher inhibition zone diameter than the garlic extract. The inhibitory property of ginger against *S. typhi*, *E. coli* and *B. subtilis* has been demonstrated by Azu and Onyeagba (2007). Ayogu and Amadi (2009) reported that raw *Allium sativum* and ethanolic extracts of *Allium sativum* and *Zingiber officinale* have inhibitory activity against the test organism.

V. CONCLUSION

From the analysis carried out, it was observed that the methanol extract of *Acacia nilotica* showed anti-typhoid activities against the species of organism (*Salmonella typhi*) used for this study. As such, the anti-typhoid activities of the extract promises it potential application in the treatment of typhoid fever.

VI. RECOMMENDATION

Since the presence of some phytochemical compounds (such as alkaloids, tannins, saponin, flavanoid and so on) were detected, further work is recommended in order to identify the bioactive components of the plant extract for the purpose of drug development.

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GLOBAL JOURNAL OF MEDICAL RESEARCH
DISEASES

Volume 13 Issue 5 Version 1.0 Year 2013

Type: Double Blind Peer Reviewed International Research Journal

Publisher: Global Journals Inc. (USA)

Online ISSN: 2249-4618 & Print ISSN : 0975-5888

HIV Test Uptake and Sexual Risk Behaviour Assessment among Patients with Pulmonary Tuberculosis in A Resource-Limited Setting

Busari, Olusegun Adesola, Fasakin, Kolawole Asimiyu, Adeola, Olatoun Adefunke, Adebara, Idowu Oluseyi, Adewara, Olumide Emmanuel, Ayodele, Lawrence Majekodunmi, & Busari, Olusogo Ebenezer,

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Abstract- HIV testing among patients with tuberculosis is critical to preventing missed diagnosis of HIV, improving morbidity and mortality and ensuring continuum of care for HIV-TB co-infected patients. The objective was to determine the HIV test uptake and assess sexual risk behaviour among patients with pulmonary tuberculosis attending DOTS clinic in a tertiary hospital in Nigeria. The study also determined the HIV sero-prevalence and evaluated the HIV-TB co-infection pattern among these patients.

Keywords: *HIV test uptake, routine hiv counseling and testing, pulmonary tuberculosis,dots, nigeria.*

GJMR-F Classification : *NLMC Code: WC 503, WF 200*



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HIV Test Uptake and Sexual Risk Behaviour Assessment among Patients with Pulmonary Tuberculosis in A Resource-Limited Setting

HIV Test Uptake and Sexual Risk Behaviour Assessment in Pulmonary Tuberculosis

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Abstract- HIV testing among patients with tuberculosis is critical to preventing missed diagnosis of HIV, improving morbidity and mortality and ensuring continuum of care for HIV-TB co-infected patients. The objective was to determine the HIV test uptake and assess sexual risk behaviour among patients with pulmonary tuberculosis attending DOTS clinic in a tertiary hospital in Nigeria. The study also determined the HIV sero-prevalence and evaluated the HIV-TB co-infection pattern among these patients. Routine HIV counseling and testing was offered to consecutive patients with pulmonary tuberculosis attending DOTS clinic between January 2008 and December 2010. Those who accepted to be tested and also consented to the study were interviewed using a pre-tested questionnaire. A total of 301 patients with pulmonary tuberculosis were seen in the DOTS clinic between January 2008 and December 2010. Two hundred and fifty two (83.74%) were tested for HIV and 19.84% of those tested were positive. Smear positive and smear negative patients were responsible for 2.78% and 17.06% of HIV prevalence respectively. 38.8%, 21.7% and 0% missed HIV test in 2008, 2009 and 2010 respectively. Only 30% of the patients used condom with sexual partners and 25% reported having multiple sexual partners. The study showed that HIV test uptake among patients with pulmonary tuberculosis was high and progressively increased from 2008 to 2010. It also revealed an HIV sero-prevalence of 19.8%. There is need to improve on the integration of HIV-TB services so that more patients with tuberculosis will access HIV counseling and testing and for effective continuum of care to reduce morbidity and mortality among those co-infected.

Keywords: HIV test uptake, routine hiv counseling and testing, pulmonary tuberculosis, dots, nigeria.

I. INTRODUCTION

Human immunodeficiency virus (HIV) is a potent risk factor for tuberculosis (TB) infection. By producing a progressive decline in cell-mediated

immunity, HIV alters the pathogenesis of tuberculosis, greatly increasing the risk of developing disease in co-infected individuals and World Health Organization (WHO) estimated that one third of the world's population was infected with TB and new infection occurred at the rate of one per second and majority co-existed with HIV (2). Tuberculosis has been found to be the leading cause of morbidity and mortality in HIV infected African populations (3-6). Globally, TB is also the most common opportunistic infection affecting HIV-seropositive individuals (6). Routine HIV counseling and testing (RHCT) is therefore recommended for persons treated for TB in settings of generalized HIV epidemics (7). This has the benefit of early diagnosis of infection thereby preventing morbidity and mortality and sustained transmission through initiating prophylaxis and timely antiretroviral treatment (8, 9). HIV test uptake among patients with TB varies widely between 12% and 98% depending on facility settings, availability of trained personnel, patient categorization and testing approach (9, 10). HIV prevalence among patients with TB in sub Saharan Africa varied from 20-60% between 1995 and 2005 (10). In settings where well trained personnel at DOTS clinic carried out RHCT, HIV test uptake was higher than where the voluntary counseling and testing (VCT) approach was used (10). Wanyenze *et al* (11) reported HIV test uptake rate of 70-90% where RHT was adopted; and 12-62% was found where VCT was used (12). There is limited data on HIV test uptake rate among patients with pulmonary tuberculosis (PTB) in Nigeria. The objective was to determine the HIV test uptake and assess sexual risk behaviour among patients with pulmonary tuberculosis attending DOTS clinic in a tertiary hospital in Nigeria. The study also determined the HIV sero-prevalence and evaluated the HIV-TB co-infection pattern among these patients.

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II. METHODS

a) Study Design

Descriptive cross-sectional Study Setting

The study was done at the DOTS clinic of the Federal Medical Centre (FMC), Ido-Ekiti, south-west Nigeria from January 2008 to December 2010. The FMC is a tertiary health facility located in a sub-urban town of an estimated population of 107,000 people but serving five contiguous states in south-west Nigeria.

i. Ethical Considerations

The study protocol was approved by the Ethics and Research Committee of the hospital. The consent of the patients was sought and obtained.

III. RECRUITMENT

Consecutive newly diagnosed PTB patients attending DOTS clinic were offered RHT according to the existing WHO guidelines (1, 2, 13). The pre-test counseling was carried out by trained Personnel; and adult patients who gave informed consent or minors whose parents or guardians gave consent on their behalf were tested with rapid HIV testing techniques and received their results immediately. Post-test counseling was done for all the patients tested. Those who were tested positive were subsequently enrolled into HIV treatment and care services. Those excluded from the study included patients who had been tested HIV positive before TB diagnosis was made and those who did not give consent. Pre-tested questionnaire was used for data collection. The questionnaire included socio-demographic factors such as age, sex, marital and educational status. It also contained questions on sexual risk behaviour.

IV. RAPID HIV TESTING

In sub-Saharan Africa, rapid testing for human immunodeficiency virus (HIV) is the most efficient and sometimes the only feasible way to quickly provide information about HIV status among adults and children ≥ 18 months of age (14). HIV rapid tests are relatively cheap, easy to use, fast to perform and accurate and reliable by applying a quality system approach recommended by the WHO (14). Rapid HIV testing was done using two distinct rapid assays. Whole blood from capillary puncture was used and the tests were performed based on the WHO rapid HIV testing guidelines which involved using two distinct rapid assays according to a serial testing algorithm (14). The first testing used Determine™ assay (Abbot Laboratories, Wiesbaden, Germany) and the second testing was by Unigold™ assay (Trinity Biotech, Ireland).

For discordant results, the third testing was done using Stat-Pak™ (Chembio, Medford, US) as the tie breaker. Concordant positive and negative results from the first and second testings were considered as positive and negative results respectively. For quality control, discordant results were first repeated by a senior research counselor and tester to ascertain true inconclusive results and finally tested with the tie-breaker. Final test results were considered positive or negative on the basis of the tie-breaker result and corresponding similar result from one of previous test assays (1, 14).

V. PTB DIAGNOSIS

PTB diagnosis was made on the basis of clinical manifestations, radiological features and the result of sputum smear microscopy for acid fast bacilli (AFB). Patients who tested positive for sputum smear microscopy for AFB were diagnosed as sputum smear positive PTB. Those with negative sputum smear microscopy for AFB were diagnosed with both clinical and radiologic findings as sputum smear negative PTB (15, 16). The chest radiograph is a very important diagnostic modality for PTB. Upper lobe infiltrates and cavities are the typical findings in reactivation TB, whereas intrathoracic lymphadenopathy and lower lobe disease are seen in primary TB. In early HIV infection, the radiographic pattern tends to be one of reactivation disease with upper lobe infiltrates with or without cavities while in HIV infection with greater degree of immunological suppression, a pattern of primary disease with intrathoracic lymphadenopathy and lower lobe infiltrates is seen.

VI. DATA ANALYSIS

Statistical analysis was done using SPSS™ 18.0. Descriptive analysis of the data was done. The HIV test uptake and HIV prevalence rates were expressed in percentages, with 95% confidence intervals (95% CI). Some percentages were compared using chi square test and p value < 0.05 was taken as significance.

VII. RESULTS

A total of 301 PTB patients participated in the study. One hundred and sixty (53.2%) were male compared with 141 (46.8%) female ($p > 0.05$) (Table 1). The median age of the patients was 35 years. Seventy (22.8%) had no formal education while 74 (24.6%) had primary school as the highest level of education. There were more males than females with post-secondary degree (62.1% versus 37.9%, $p < 0.05$). Only 93 (30.8%) of the patients were married (Table 1). Sexual risk behaviour assessment showed that 89 (29.6%) reported previous HIV testing; 75 (24.9%) had sexual intercourse with two or more partners in the last 3 months; and only

99 (32.9%) reported using condom in the last sexual intercourse.

Of the 301 patients, 252 (83.7%) consented to HIV testing. The highest HIV test uptake rate (91.7%) was found among age the group 15-24 years while the smallest uptake rate (69.7%) was in the 45-54 year age group (Table 2). The HIV sero-prevalence among PTB patients tested was 19.8%. The highest prevalence was found in the 35-44 year age group. Sputum smear

negative PTB patients were more likely to have HIV than sputum smear positive patients (17.1% versus 2.7%, $p = 0.001$). Table 3 shows a progressive increase in HIV test uptake among PTB patients from 2008 to 2010. It also depicts a progressive reduction in HIV-TB co-infection during the same period of time. There is an inverse relationship between HIV test uptake and HIV-TB co-infection.

Table 1 : Socio-demographic characteristics and sexual risk behaviour assessment among PTB patients

Characteristics	Age (years)	Frequency (%)	P value
<15	17 (5.6)		
15-24	36 (12.0)		
25-34	70 (23.3)		
35-44	57 (18.9)		
45-54	33 (11.0)		
55-64	29 (9.6)		
≥65	59 (19.6)		
Sex		>0.05	
Male	160 (53.2)		
Female	141 (46.8)		
<i>Marital Status</i>			
Single	50 (16.6)		
Married	93 (30.8)		
Separated/Divorced	15 (5.0)		
Widowed	23 (7.6)		
<i>Education</i>		>0.05	
None	70 (22.8)		
Primary	74 (24.6)		
Secondary	120 (39.9)		
Post-secondary	37 (12.3)		
<i>Previous HIV Testing</i>			
Yes	89 (29.6)		
No	212 (70.4)		
<i>Condom Use (in the last sex)</i>			
Yes	99 (32.9)		
No	202 (67.1)		
<i>No of Sexual Partners (in the last 3 months)</i>			
Nil	25 (8.3)		
1	201 (66.8)		
≥2	75 (24.9)		

Table 2 : HIV test uptake rate and HIV sero-prevalence among PTB patients

Age group (yrs)	No (%) of PTB patients	No (%) of PTB patients tested for HIV	No (%) of PTB patients positive for HIV
<15	17	12 (70.6)	4 (33.3)
15-24	36	33 (91.7)	6 (18.2)
25-34	70	58 (82.9)	9 (15.5)
35-44	57	51 (89.5)	19 (37.3)
45-54	33	23 (69.7)	5 (21.7)
55-64	29	24 (82.4)	2 (8.3)
≥65	59	51 (86.4)	5 (9.8)

Table 3 : Trend of HIV test uptake rate among PTB patients from 2008 to 2010

Year	2008	2009	2010
No of PTB patients	80	83	138
No (%) of PTB patients tested for HIV	49 (61.3)	65 (78.3)	138 (100)
No (%) of PTB patients not tested for HIV	31 (38.7)	18 (21.7)	-
Prevalence of HIV among PTB patients	32.7%	23.1%	13.8%

VIII. DISCUSSION

The global understanding of the strong synergy between HIV and TB and the need to scale up ART programme and link HIV infected partners to treatment and care underscores the importance of this study (18). Tuberculosis remains the most important opportunistic infection in HIV patients and the leading cause of increased morbidity and mortality (19). Claudia et al (20) demonstrated that RHCT method for TB patients is indispensable in HIV endemic region as recommended by the World Health Organization (WHO) and remains a critical strategy to detect individuals with undiagnosed HIV infection (1,2). Our study showed an increasing HIV test uptake rate from 69.7% in 2008 to 91.7% in 2010 with an average of 83.7%. An HIV test uptake rate of 83.7% among PTB patients is high and this finding is in concordance with previous studies in sub Saharan Africa which reported between 12% and 98% (4, 5, 9, 10). Irrespective of the age group, this HIV uptake rate was high. Our study also revealed that 18.9% of PTB patients were co-infected with HIV. This prevalence rate is nearly four times the threshold (5%) at which the WHO recommends intensified intervention to address HIV-TB co-infection, including HIV counseling and testing for all TB patients (1, 3, 21). In our study, HIV prevalence was significantly higher in sputum smear negative than in smear positive patients. In HIV-infected patients, clinical features of PTB reflect different levels of immunological suppression. Earlier in the course of HIV disease, tuberculosis is more likely to present as classical reactivation-type disease, whereas patients with advanced immunological suppression are more likely to present with findings consistent with primary TB (22). Our study also showed that HIV prevalence was higher among age groups 35-44 and 45-54 years than the young adults aged 15-34 years. This finding corroborates the report of a previous study by Long and Boffa (23) which showed that HIV co-infection was significantly higher in middle-aged than in young adult TB patients in sub Saharan Africa. The HIV prevalence of 33.3% in patients with PTB aged < 15 years was the highest in our study. This prevalence is about eight times the national prevalence of 4.4% in Nigeria (24). Some of these children were born to mothers who had

HIV-TB co-infection. This finding supports the emphasis that household members of TB patients should be encouraged to screen for HIV as they tend to have a greatly elevated HIV sero-prevalence in comparison to the general population (25-27). In the study, less than 30% of the patients reported using condom in the last sex and about 25% had more two or more sexual partners in the last three months. These findings reflect a high level of sexual risk behaviour among PTB patients and this may, in part, be responsible for high HIV prevalence among them.

Finally, our study also showed that there was an inverse relationship between HIV uptake rate and HIV seroprevalence over the years of the study from 2008 to 2010. As the HIV uptake rate increased over the years and with the progressive reduction in missed HIV diagnosis, the sero-prevalence of HIV among PTB patients declined. This finding could be attributed to improvement in the clinical setting such as improved infrastructure and more efficient physical integration of TB/HIV services over this period which facilitated increased patients' attendance, reduced loss of patients to follow up and improved communication among healthcare workers and between healthcare workers and patients (28). There was also continued capacity building training for healthcare workers involved in counseling and testing and in HIV and TB management; more robust monitoring and evaluation, and scale up of community involvement and mobilization.

IX. CONCLUSION

The study showed a high HIV test uptake among PTB patients and a progressive increase from 2008 to 2010. It also revealed an HIV sero-prevalence of about 20% using RHCT approach. The PTB patients also reported high risk behaviour. There is need to expand community-based education programme emphasizing HIV-TB co-infection pattern and providing increased access to DOTS clinic based HIV testing. There should also be increase in efforts to more effectively integrate TB/HIV services so that all patients with TB would be screened for HIV and vice versa. Finally, all PTB patients with or without HIV should receive HIV risk reduction counseling.

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GLOBAL JOURNAL OF MEDICAL RESEARCH
DISEASES

Volume 13 Issue 5 Version 1.0 Year 2013

Type: Double Blind Peer Reviewed International Research Journal

Publisher: Global Journals Inc. (USA)

Online ISSN: 2249-4618 & Print ISSN : 0975-5888

Connexin 43 and Ewing Sarcoma: Stay Tuned

By Marilyn M. Bui, David L. Becker & Damon Reed

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Abstract- To the editor: In our study¹ published in *Sarcoma* in 2011, we found that Connexin 43 (Cx43) was frequently (78%) expressed in the 36-Ewing sarcoma (ES) patient tissue microarray specimens. Most interestingly, a higher level of Cx43 overexpression was correlated with adverse clinical outcome and shorter survival regardless of tumor stage, location, tumor size and clinical management. Positive score of Cx43 was significantly correlated with reduced overall survival ($p=0.02$). The average positive Cx43 scores for patients alive and dead at 3 years was 46.08 and 96.98 ($p=0.004$) at 5 years was 46.06 and 96.43 ($p=0.02$) respectively. Recently, a study published in *Biochimica et Biophysica Acta*² demonstrated that expression level of Cx43 was repressed by EWS-FLI1, Cx43 gene expression was associated with the gap junction intercellular communication changes and Cx43 inhibits ES growth via modulation of cell proliferation via p27. Surprisingly, ES overexpression of Cx43 reduced tumor growth and was associated with better survival. Although these two studies show different prognostic values regarding to Cx43 and ES, both confirm that Cx43 has a potential role in ES tumorigenesis and prognosis.

GJMR-F Classification : NLMC Code: QZ 345



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Connexin 43 and Ewing Sarcoma: Stay Tuned

Marilyn M. Bui ^α, David L. Becker ^σ & Damon Reed ^ρ

I. INTRODUCTION

To the editor: In our study¹ published in *Sarcoma* in 2011, we found that Connexin 43 (Cx43) was frequently (78%) expressed in the 36-Ewing sarcoma (ES) patient tissue microarray specimens. Most interestingly, a higher level of Cx43 overexpression was correlated with adverse clinical outcome and shorter survival regardless of tumor stage, location, tumor size and clinical management. Positive score of Cx43 was significantly correlated with reduced overall survival ($p=0.02$). The average positive Cx43 scores for patients alive and dead at 3 years was 46.08 and 96.98 ($p=0.004$) at 5 years was 46.06 and 96.43 ($p=0.02$) respectively. Recently, a study published in *Biochimica et Biophysica Acta*² demonstrated that expression level of Cx43 was repressed by EWS-FLI1, Cx43 gene expression was associated with the gap junction intercellular communication changes and Cx43 inhibits ES growth via modulation of cell proliferation via p27. Surprisingly, ES overexpression of Cx43 reduced tumor growth and was associated with better survival. Although these two studies show different prognostic values regarding to Cx43 and ES, both confirm that Cx43 has a potential role in ES tumorigenesis and prognosis. While there is a larger effort to study the association of Cx43 with other cancers such as carcinoma, melanoma and hematopoietic malignancy, the need for further studies of Cx43 and ES cannot be overemphasized. ES is the second most common bone tumor in children and adolescents and also can arise in the soft tissue. If the tumor is not metastatic, with surgery, chemotherapy and radiation therapy, patients have a 75% chance of 5-year survival. However, there is only 20% 5-year survival for metastatic ES. Drs. Becker and Mendoza-Naranjo have preliminary data showing that Cx43 is largely up-regulated in a panel of metastatic/chemoresistant ES compared to primary ES cell lines which would support the clinical association we found in our work. In addition, the silencing of Cx43

reduces tumor growth and survival in metastatic ES cells. More effort should be invested to investigate the biology of Cx43 and ES, especially for metastatic ES. The ultimate hope is to discover potential novel targeted therapy that can improve the outcome of this devastating disease. We are looking forward to more exciting results in this field. We are hopeful and stay tuned.

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GLOBAL JOURNAL OF MEDICAL RESEARCH
DISEASES

Volume 13 Issue 5 Version 1.0 Year 2013

Type: Double Blind Peer Reviewed International Research Journal

Publisher: Global Journals Inc. (USA)

Online ISSN: 2249-4618 & Print ISSN : 0975-5888

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By Tesfaye Setegn, Abulie Takele, Nagasa Dida & Begna Tulu

Madawalabu University, Ethiopia

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GJMR-F Classification : NLMC Code: WC140



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Correlates of Risk Perception to Hiv Infection, Abstinence and Condom use among Madawalabu University Students, Southeast Ethiopia: Using Health Belief Model (HBM)

Tesfaye Setegn^α, Abulie Takele^α, Nagasa Dida^σ & Begna Tulu^ρ

Abstract- Background : People living in sub-Saharan African countries have been more vulnerable for HIV infection. Youths and adolescents including university students are among the risk group to acquire HIV infection due their risk behaviors. Many vulnerable young adults do not yet recognize their susceptibility, seriousness of the HIV infection, and are not motivated to alter their risky behavior. Therefore, this study is designed to assess HIV infection risk perception and abstinence and condom use among regular Madawalabu University students.

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Result : Forty two percent of the respondents were sexually active and 60.6% of them had used condom in their last sexual intercourse. Forty five percent (45.4%) of the respondents have low risk perception HIV towards infection. Perceived self-efficacy and abstinence for sexual intercourse were statistically significant (OR=0.38[CI (95.0%):0.24– 0.59]) and perceived benefits of HIV infection risk prevention and control method utilization showed significant association with perceived behavioral control among sexually active students (OR=0.46[CI (95.0%):0.27 – 0.83]).

Conclusion : Nearly half of study participants have low risk perception to HIV infection. Perceived behavioral controls (abstinence for sexual intercourse and condom use) were statistically associated with perceived barriers and benefits of HIV infection risk prevention and control measures utilization. Perceived self-efficacy is the important predictor of perceived behavioral control utilization. Therefore, university based HIV risk reduction intervention should be geared towards the identified factors.

I. BACKGROUND

Millions of young people around the world face a high risk of HIV infection and other negative sexual and reproductive health outcomes as a

result of behaviors that they adopt, or are forced to adopt. Those who have sex with someone who is or is likely to be HIV-infected are at risk of acquiring HIV if they do not use a condom. Using condoms consistently reduces the risk of HIV infection among exposed groups. But the young people who most need such protection often have difficulty of accessing appropriate services and adopting behaviors that protect them from HIV infection. The behaviors that put them at risk are usually heavily stigmatized and take place secretly, often illegally [1,2].

Two-thirds of the world's total number of people living with HIV resides in Sub-Saharan Africa of which 60% of all AIDS cases and majority of the new infections are seen among youth (15-24 years) population both nationally and internationally. Since, majority of tertiary level (university) students belong to this age group and they have been among the high risk groups to HIV infection [2-6].

Despite this fact, many young adults undermine their level of risk and susceptibility for HIV infection. Youths do not perceive the seriousness of the pandemic and are not motivated to alter their risky behavior. Experimental behaviors, the need to get social and peers approval and sense of non-vulnerability have been enumerated as reasons for youths to stay with their risky behaviors. People need to have recommended level of HIV risk perception and preventive activities. Because, the actual risk perception matters the decision making process to stay risk free. Individuals or groups who do not understand that they are at high risk of HIV infection would take less protective measures. On the contrary, those who perceived that they are at risk for HIV infection were more likely to comply with HIV infection prevention actions. [7, 8, 9, 10].

Ethiopia is one of the most heavily affected countries by HIV epidemic. Young people are at the center of the HIV/ AIDS epidemic. An estimated 10 million young people aged 15-24 years are living with HIV/AIDS and more than 6000 contract the virus every day. The college environment offers great chance

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for HIV high-risk behaviors, including unsafe sex and multiple sexual partnerships. [11,12,13].

A study conducted in Addis Ababa indicated that (23.6%) of youth participants did not perceive that they are at risk of HIV infection, (43.3 %) claimed to have low risk, (6.7%) medium and only (2.4%) perceived that they have high chance of acquiring the virus [14].

Realizing the socio-economic and development impacts of HIV/AIDS, the government of Ethiopia committed itself to strengthen prevention and control activities for many years through designing a road map for accelerated access to HIV prevention, treatment and care (2007-2010), plan of action for universal access to HIV prevention, treatment, care and support, strategic plan for intensified multi-sectoral response and instituting workplace and school-based IEC/BCC activities (20, 21). Special intervention strategies are in place to ensure equal access for different subpopulation groups including youths. Creating awareness to increase risk perception among youth through information and communication is an important strategy. But university youths evidenced while practicing risky behaviors which probably indicate that interventions might have been done without adequate research on perceived behavioral control of university youths [8]. This suggest that there is a need for more sustained effort and designing targeted and innovative approaches to increase risk perception, especially among in school youths (20).

Therefore, this study has assessed factors associated with perceived behavioral control among Madawalabu University students using Health Believe Model (HBM).

II. HEALTH BELIEF MODEL (HBM)

The Health Belief Model (HBM) was developed in the 1950's to explain the public's failure to participate in screening programs to detect tuberculosis in many setups. But few individuals actually took advantage of these opportunities. The HBM was the resulting theory that helped explain this lack of participation in preventive behaviors. The public's reaction to the TB health crisis during the 1950's is alarming in its resemblance to the HIV/AIDS epidemic today. Individuals who were at risk for TB were able to explain lack of participation in prevention via perceptions about the disease and personal susceptibility, time constraints, finances, fear of the procedure, or other barriers to the behavior. Today, there are many opportunities for individuals to participate in HIV preventive behaviors, specifically abstinence and condom use.

Consequently, the creed of HBM is well suited to assess HIV infection preventive. The HBM theorizes that in order for a behavior change to occur, three factors regarding health related action must be present. An individual must feel threatened by his/her current

behavior, believe that a specific change in behavior will be beneficial by resulting in a valued outcome at an acceptable cost, and must feel that she or he is competent to implement the recommended change. These components are particularly salient when dealing with HIV infection. Specifically, a person must feel that there is a realistic, not just statistical, probability of contracting HIV infection as a result of his or her current behavior.

Thus, the HBM takes an individual's past experience and characteristics into account as a pre-existing component of the model. An individual's perceptions of a specific disease are founded in an individual's background and allow for assessment of issues salient to an individual (Fig 1).

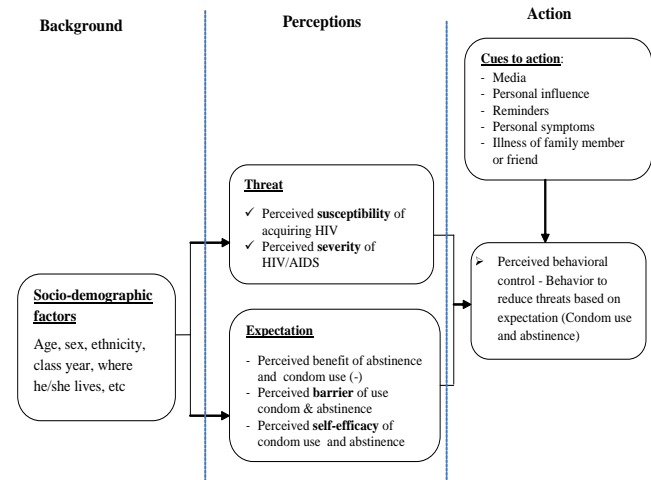


Figure 1. Schematic representation of the components of the Health Belief Model (HBM), Southeast Ethiopia, 2012

Figure 1: Schematic representation of the Components of the Health Belief Model (HBM), Southeast Ethiopia, 2012

Perceived susceptibility refers to a person's perception of his or her risk of becoming infected with HIV. Perceived severity refers to feelings about the seriousness of HI infection. Perceived benefit indicates a person's beliefs regarding the efficacy of the self-protective behavior. Perceived barriers refer to an individual's perception of the negative aspects of the self-protective behaviors. Cues to action account for internal and external events that trigger performance of the behavior in this case staying abstinence and use of condom. Finally, self-efficacy refers to a person's beliefs regarding his or her ability to successfully staying abstinence and use of condom (Fig 1).

III. METHODS

a) Study Settings and Sample

A cross sectional study design was conducted in Madawalabu University located in Bale Zone, 430 KM from Addis Ababa, to Southeast of Ethiopia. Madawalabu University is one of the newly established public

higher educational institutions established in 2007. It has two campuses consisting of 10, 317 students of which 5,275 are regular students. The study was conducted from May to June 2012.

The total sample size was determined using single population proportion formula assuming 50% expected prevalence of perceived behavioral control from HIV infection and 10% non-response rate making the final sample size 422 students. Stratified sampling technique was used using health and non-health students as strata. Six (6) non-health schools and a college (College of Medicine and health sciences) totally seven faculties were selected using simple random sampling technique. The total sample size 422 was allocated proportionally to each of the randomly selected schools/college and then to each department. Finally, simple random sampling was employed to select students from each department.

b) Instrument and Measurement

The study instrument was originally prepared in English language then translated to the local language and then translated back into English to check its consistency. The questionnaire contains variables like socio-demographic variables, sexual history and risks to HIV infection, perception (perceived severity, perceived susceptibility, perceived benefits, perceived barrier, perceived self-efficacy and cues to action), abstinence for sexual intercourse and condom use. The internal consistency of items to measure perception was checked by Cronbach's alpha and it was in the range 0.61 to 0.79. Then the data were collected by self-administered questionnaire.

IV. DATA ANALYSIS

The data were entered into SPSS version 16.0. Descriptive and binary logistic regressions analyses were carried out to characterize the study participants and identify factors associated with risk perception for HIV infection. Finally multivariable logistic regression was modeled to identify the independent associated factors and a corresponding p-value <0.05(two tailed) was considered to decide statistical significance.

Letter of Ethical approval was received from Madawalabu University, Research and Community service directorate office ethical clearance committee. Official letter of co-operation was also obtained from research and community service directorate office. Informed verbal consent was secured from study participants in their own language explaining the purpose of the study, potential risk and benefits of participating in the study and the right to refuse filling the questionnaire. The participants were also assured about the confidentiality of the information they provided and it will kept anonymously.

V. RESULT

a) Socio-demographic characteristics

Of the total of 422 study participants, 390 of them making response rate 92.4%. The mean (SD) age of the respondents was 21.3(±1.5) years with the range of 18-28 years. The most of the respondents were males by sex with 4:1 male to female ratio. Majority (63.7%) of the respondents were Oromo by ethnic group. More than half of the respondents were Orthodox Christian and 2.2% of the respondents were married currently

Table 1 : Socio-demographic characteristics of Madawalabu University students, Southeast Ethiopia, 2012 (n=390)

Variables	Number	Percent
Age		
15-19	23	5.9
20-24	352	90.3
25-29	15	3.8
Mean age (±SD) years	(21.3±1.5)	
Class year		
II	279	71.5
III	111	28.5
Ethnicity		
Oromo	230	63.7
Amhara	74	20.5
Tigre	31	8.6
Others	26	7.2
Religion		
Orthodox Christian	185	52.6
Muslim	80	22.7
Protestant Christian	87	24.7
Marital Status		
Never married	307	95.3
Married	7	2.2

Have constant sexual partner	6	1.9
Monthly allowance from family		
Yes	325	83.8
No	63	16.2

* Siltie, berta, some other ethnic groups from southern nations and nationalities

b) *Sexual History and Risks to HIV Infection*

In our study, 42.3% of students were sexually active. The mean (\pm SD) age at first sexual debut was 18.6(\pm 2.2) years. Sixty five percent (65.3%) of sexually active students have initiated sex before they joined university. Participants reported that fall in love (47.5%),

had sexual desire (33.8%), marriage (5.6%), to get money and other gifts (8.1%), peer pressure (12.5%) and alcohol (7.5%) were the provided reasons to be engaged in their first sexual intercourse. Thirty percent (30%) of sexually active students practiced casual sexual (**Table 2**).

Table 2 : Distribution of risk sexual behaviors for HIV infections among sexually active Students, Madawalabu University, Southeast Ethiopia, 2012

Variables	Frequency	Percentage
Place for first sexual activity		
Before joining campus	94	65.3
After joining campus	50	34.7
Relation with first sexual partner		
Casual	40	27.6
Permanent sexual partner	84	57.9
Benefit based relationship	14	9.7
Spouse	7	4.8
Number of life time sexual partners		
One	84	52.5
Two	18	11.2
Three	21	13.1
More than three	37	23.1
Condom use for first sexual activity		
Yes	63	43.8
No	81	56.2
Number of sexual partners in the last 12months		
One	94	69.6
Two	20	14.8
Three	12	8.9
Four and above	9	6.7
Condom use for last sexual activity		
Yes	75	56.0
No	59	44.0
History of sex with CSW*		
Yes	30	24.0
No	95	76.0
Knowledge level of respondent (mean score = 7.56)**		
Poor knowledge (below mean score)	92	57.5
Knowledgeable(above mean score)	68	42.5

*Commercial sex worker (assessed for only male students), ** Cronbach's alpha 61.6% (internal consistency measure for knowledge items)

c) *Perceptions and Behavioral Control to HIV Infection*

Of the total study participants, 177 (45.4%) of them reported low perceived risk to contract HIV infection while the rest 213 (54.6%) of the respondents reported high perceived risk to acquire HIV infection. Forty four percent 173 (44.4%) of the respondents perceived that complications related to HIV infection are serious. Majority, 267(68.5%) of the respondents have high perceived benefit towards the prevention and control methods of HIV infection. On the contrary, 123 (31.5%) of the students have reported low perceived benefits of the recommended HIV infection prevention

and control methods. Fifty percent, 197 (50.5%) of the respondents reported high perceived barriers to use HIV infection prevention and control methods mainly abstinence, be faithful for one sexual friend and condom use. One hundred fifty two (39.0)of the respondents had reported low rated self-efficacy to use recommended HIV infection prevention and control methods. Only 157 (39.2%) of the respondents were knowledgeable on the mode of transmission and preventive methods while the rest 237 (60.8%) of them had poor knowledge on the mode of transmission and preventive measures of HIV infection (Table 3).

Table 3 : Reliability of perception and knowledge items used to assess students' perception toward risks for HIV infection; Madawalabu University, Southeast Ethiopia, 2012

Variables		No(%)	Cronbach's alpha
Perceived severity – 11 variables	Low	177 (45.4)	0.67
	High	213 (54.6)	
Perceived susceptibility – 6 variables	Low	173 (44.4)	0.79
	High	217 (55.6)	
Perceived benefits – 5 variables	Low	123 (31.5)	0.65
	High	267 (68.5)	
Perceived barrier – 6 variables	Low	193 (49.5)	0.61
	High	197 (50.5)	
Perceived self efficacy -11 variables	Low	152 (39.0)	0.74
	High	238 (61.0)	
Knowledge – 6 variables	Low	237 (60.8)	0.61
	High	153 (39.2)	

d) *FACTORS ASSOCIATED WITH PERCEIVED BEHAVIORAL CONTROLS*

The statistical association of knowledge of students and risk perception to HIV infection and abstinence was checked by Binary logistic regression analysis. In this analysis, perceived severity of HIV infection was statistically associated with abstinence for sexual intercourse (OR=0.61, CI [95.0%]:0.40 – 0.92)).

Perceived benefits (OR=0.60, [CI (95.0%):0.38 – 0.94]) and perceived barriers (OR=0.53, [CI (95.0%):0.35 – 0.80]) to HIV infection prevention and control measures, showed statistically significant association with abstinence for sexual intercourse Similarly perceived self-efficacy showed statistically significant association with abstinence from sexual intercourse (OR=0.38, [CI (95.0%):0.24 – 0.59]) (Table 4).

Table 4 : Factors associated with abstinence for sexual intercourse among Madawalabu University students, Southeast Ethiopia, 2012

Variables		History of sexual intercourse		COR (95% CI)	AOR (95% CI)
		Yes	No		
Knowledge	Poor	92	138	1.28(0.84 – 1.94)	
	Good	68	80		
Perceived susceptibility	Low	69	105	1.22(0.84-1.81)	
	High	91	113		
Perceived severity	Low	60	108	0.61(0.40-0.92)*	0.85(0.54-1.33)
	High	100	110		
Perceived benefits	Low	40	78	0.60(0.38-0.94)*	0.97(0.58-1.61)
	High	120	140		
Perceived	Low	66	124	0.53(0.35-0.80)*	0.53(0.34-

barriers	High	94	94	1.0	0.81)*
	Low	42	105	0.38(0.25-0.60)*	0.38(0.24-0.59)*
Perceived self- efficacy	High	118	113	1.0	1.0

* Significant at $p < 0.01$, COR= Crude Odds Ratio, AOR= Adjusted Odds Ratio

Perceiving condom can prevent HIV transmission showed statistically significant association with condom use (OR=8.05,[CI (95.0%):0.08 – 21.04]). From the health belief model (HBM) constructs perceived benefits of HIV infection prevention and control measures by sexually active students showed

statistical significant association with condom use (OR=0.46,[CI (95.0%):0.27 – 0.83]). Similarly, there was statistically significant association between perceived self-efficacy and condom use among sexually active students (OR=0.38, [CI (95.0%):0.17 – 0.82]) (Table 5).

Table 5 : Factors associated with condom use among sexually active students, Madawalabu University, Southeast Ethiopia, 2012

Variables		Ever use condom		COR (95% CI)	AOR(95%CI)
		Yes	No		
Condom can prevent HIV transmission ?	Yes	90	30	8.05(3.08-21.04)*	6.3(2.26-13.7)*
	No	7	19	1.0	1
Knowledge	Yes	59	28	1.22(0.61-2.44)	-
	No	38	22	1.0	-
Perceived susceptibility	Low	38	24	0.7(0.35-1.38)	-
	High	59	26	1.0	-
Perceived severity	Low	32	20	0.74(0.36-1.50)	-
	High	65	30	1.0	-
Perceived benefits	Low	20	15	0.46(0.27-0.83)*	0.47(0.25-0.9)**
	High	77	35	1.0	1
Perceived barriers	Low	44	17	1.61(0.79-3.27)	-
	High	53	33	1.0	-
Perceived self- efficacy	Low	17	18	0.38(0.17-0.82)*	0.72(0.35-1.47)
	High	80	32	1.0	1

*Statistically significant at $p < 0.01$, **Significant at $p < 0.05$ and adjusted for variables that are significant under crude odd ratio

Multivariable logistic regression analysis was carried out to identify the independently associated factors with condom use among sexually active students during their sexual intercourse. Perceived benefits (OR=0.47, [CI (95.0%):0.25-0.89]) and thinking that condom can prevent HIV transmission (OR=6.3, [CI (95.0%):2.26-13.68]) remained the independent factors for condom use (Table 5).

VI. DISCUSSION

In this study, Health Belief Model was used to assess perception toward HIV infection and utilization of HIV infection prevention and control methods (abstinence and condom use). Therefore, in this study 177 (45.4%) of the respondents have reported low perceived risk of contracting HIV infection. Similarly, a study conducted in African American commuter urban university, USA showed that 57.9% of students aged <19 years and 48.1% of students aged 20-19 years

have reported no perceived chances of getting HIV infection[12]. A study done in Tanzania showed that only 25% of students felt that they had a very low risk to HIV, while 53.1% felt that they were not at risk at all [18,19]. A similar study done in Cape Town, South Africa showed that only 24% of youths involved in concurrent sexual relationships consider themselves to be at risk of HIV [16]. On the other hand, a study conducted on Black African American University students, 79 % of students perceived to be at low risk for HIV infection [22].

Forty four percent (44.4%) of the respondents perceived complications related to HIV infection are severe. But 160 (41%) of the respondents were sexually active and 39.4% of them did not use condom for their past sexual intercourse. Study done on Black American University students showed that 64% of those who had at least two or more sex partners had not used a condom at last sex encounter [22]. A study done in

Nigerian students did not use HIV infection preventive measures during their sexual intercourse [10, 17].

In this study, perceived severity of contracting HIV infection, perceived benefits of behavioral control, perceived barriers to HIV infection prevention, control measures and self-efficacy to use HIV infection prevention measures were statistically significant with abstinence. Perceived barriers to HIV infection prevention and control method utilization and perceived self-efficacy were the independent predictors of sexual abstinence. Those individuals who have low perceived barriers were less likely to practice sexual intercourse (OR=0.53, [CI (95.0%):0.34 – 0.81]) when compared with those who have high perceived barriers. Similarly those of students who had low perceived self-efficacy were also less likely to practice sexual intercourse (OR=0.38, [CI (95.0%):0.24 – 0.59]).

This study also revealed that perceived benefits of HIV/AIDS prevention and control method utilization showed significant association with past condom use among sexually active students. Students who had low perceived benefits were less likely to utilize condom during sexual intercourse when compared with those who had high perceived benefits of using condom (OR=0.46, [CI (95.0%):0.27 – 0.83]).

Perceived self-efficacy and condom utilization among sexually active students were found to have statistically significant association. Students who had low perceived self-efficacy were less likely to use condom in their last sexual practice (OR=0.38, [CI (95.0%):0.17–0.82]). Other study also showed statistically significant association between perceived self-efficacy of HIV/AIDS prevention and control method and past condom usage [3, 8, 14].

This study is based on the health belief model (HBM) along with perceived behavioral control; these variables which would provide a better specific gaps and strength and quality information for intervention. But this study is limited to establish temporal relationship because of its cross-sectional nature.

VII. CONCLUSIONS

In this study, two out of every five students were sexually active. The knowledge of students towards the mode of transmission of HIV infection found to be unsatisfactory. Significant proportion of students reported low risk perception to HIV infection. Although majority of the respondents have high perceived benefit of HIV infection prevention and control measures, more than half of the students reported high perceived barriers to use behavioral controls. Students have reported low rated self-efficacy to use recommended HIV infection behavioral controls measures. Participants with low perceived barriers and low perceived self-efficacy were less likely to practice sexual intercourse. Similarly, students with low perceived benefits and low

perceived self-efficacy of condoms were less likely to use condom. In this study, perceived severity of HIV infection showed statistically significant association with sexual abstinence and condom use. Similarly, perceived benefits of behavioral controls, perceived barriers to HIV infection prevention and perceived self-efficacy were statistically associated with sexual abstinence and condom use. But perceived benefit of condom remained the independent factors for condom use. Therefore, university based HIV risk reduction intervention should be geared towards the identified factors.

VIII. COMPETING INTERESTS

None of the authors and other organizations has competing interest.

IX. AUTHORS' CONTRIBUTIONS

TS and AT conceptualized and designed the study. ND and BT assisted in designing the study. ND conceptualized and refined the concept, analyzed and interpreted the data, drafted the manuscript. TS assisted preparation and critically reviewed the manuscript. AT & BT critically reviewed the manuscript. All authors have re in data analysis and interpretation, manuscript ad and approved the final manuscript.

X. ACKNOWLEDGEMENTS

We would like to thank Madawalabu University for its financial support and Madawalabu University, College of Medicine and Health Sciences (CMHS) for its technical support .We are also grateful to data collectors and study participants who shared their priceless time.

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GLOBAL JOURNAL OF MEDICAL RESEARCH
DISEASES

Volume 13 Issue 5 Version 1.0 Year 2013

Type: Double Blind Peer Reviewed International Research Journal

Publisher: Global Journals Inc. (USA)

Online ISSN: 2249-4618 & Print ISSN : 0975-5888

The Role Clean Self Intermittent Catheterization Following Direct Vision Internal Urethrotomy in Reduction of Recurrence of Urethral Stricture

By Zahir Abdlegadir Mohamed Elhaj Mr. Adil Ibrahim & Professor Sharfi

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Abstract- Background: Urethral stricture is major health urological problem; urethral dilatation and internal optical urethrotomy were the only treatment. Clean Self catheterization follow direct visual internal urethrotomy has greatly decreased the recurrence of stricture.

Objectives: To investigate the effect of clean self intermittent catheterization on recurrence rate following direct vision internal urethrotomy and to assess rate of complication of direct visual internal urethrotomy (DVIU) alone versus DVIU with self catheterization.

Patients and Methods: This double blind case control study was conducted in Soba university hospital (SUH). A total of sixty two patients were selected randomly in to treatment group B (31 patients) and control group A (31 patients) all patients were treated with DVIU followed with indwelling catheter for three days.

GJMR-F Classification : NLMC Code: WJ 600



THE ROLE CLEAN SELF INTERMITTENT CATHETERIZATION FOLLOWING DIRECT VISION INTERNAL URETHROTOMY IN REDUCTION OF RECURRENCE OF URETHRAL STRICTURE

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The Role Clean Self Intermittent Catheterization Following Direct Vision Internal Urethrotomy in Reduction of Recurrence of Urethral Stricture

Zahir Abdlegadir Mohamed Elhaj ^α Mr. Adil Ibrahim ^σ & Professor Sharfi ^ρ

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Results : Twenty Four (77.41%) out of 31 patients in control group A developed urethral stricture recurrence while six (19.35%) patients in treatment group B had stricture recurrence ($p < 0.000$). In control group A 14 patients (58.3%) out of 24 had their recurrence in the first six months of follow-up while five (83.3%) out of six in group B had their recurrence in the next six months of follow-up. In control group A four patients developed urinary tract infections all were positive for E.coli. In treatment group B urinary tract infections were found in three patients, culture was positive for E.coli in two patients and Klebsiella for the third one, and one patient developed epididymo-orchitis.

Conclusion : Clean self intermittent catheterization is a simple, safe, cost effective and easy to perform procedure for prevention of urethral stricture with good acceptability, compliance, better outcome and with few complications.

I. INTRODUCTION

Urethral stricture is common urological disorder and its oldest disease known to mankind. Previously infections and gonorrhoea were the common causes of urethral stricture [1, 2]. In developed world, gonococcal strictures are rare and most strictures today are either iatrogenic or idiopathic [3, 4] because

more and more urological procedures are being done transurethrally. Dilatation was often the first intervention chosen to deal with small urethral stricture but long term results with dilatation have high failure rate. Direct vision internal urethrotomy, has greatly improved the treatment of urethral strictures. This procedure is now the preferred method of treatment of urethral strictures less than 1.5 cm in length and which are located in the bulbar or penile urethra.

Despite good immediate results there is considerable risk of recurrence between 10-50% [5, 6].

Self catheterisation has been popularised to reduce the risk of recurrent of urethral stricture disease after urethrotomy [7].

The concept of clean intermittent self catheterisation was introduced by Lapides in early 1970s who proposed that strict aseptic technique is not necessary for clean intermittent self catheterisation.

In this study we have investigated the effect CSIC on the frequency of recurrence of urethral stricture in a randomised controlled manner including complications.

II. PATIENTS AND METHODS

Sixty two adult male patients with urethral stricture disease booked for direct vision internal urethrotomy (DVIU) divided into two groups: group A (31) patients underwent DVIU where group B (31) were put on CISC following DVIU.

All the patients had the same investigation include urine general, culture and sensitivity test, renal function and ascending urethrogram.

Both groups of patients had DVIU in urology unit at soba university hospital .The DVIU performed by consultant urologist.

All patients had a size 16F urethral catheter following DVIU for 3-5days.

All were given a single dose of antibiotic with the start of the procedure.

The two groups randomized in to DVIU alone and DVIU plus CISC.

The group for CSIC performed the procedure CISC safely by well trained theatre attendant. The follow-up at 3, 6 and 12th month. In each follow up the patient history of urinary stream, urine analysis and ascending

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urethrogram (for only symptomatic one) assessed. The rate of recurrence assessed mainly by ascending urethrogram.

III. DATA COLLECTION

Data was collected by structure questionnaire for each patient, from date of operation until discharge from hospital, and out patient follow-up at three months, sixth months and 12th months. Patients or their relatives either had written or verbal consent before being enrolled in this study a flow chart will be used for data collection.

a) Study Duration

The study was conducted in the period from 2nd of July 2012 to 3rd of December 2013.

Data was analyzed by computer using statistical package for social science (spss) program. The result was in texts, tables, and figures. Post hoc multiple comparison were done with difference was considered significant when probability ($p < 0.05$).

IV. RESULTS

A total of sixty two patients were included in the study 31 patients in treatment group B and 31 patients in control group A. No drop-outs occurred apart of one patient in control group who was dead because of renal failure but after he had a recurrence of urethral stricture. The mean age in control group A (52.3 ± 13.23) range (28-79). In group B the mean age 47.03 ± 12.96 range (28-68). Incomplete bladder empty in 49 patients (79%) and poor stream in 48 patients (77.4%) were the most common presentation followed by terminal dribbling in 34 patients (54.8%), hesitancy in 27 patients (43.5%) and urine retention in eight (12.9%) of all patients with urethral stricture. The most common etiology of urethral stricture were infections and gonorrhoea in 24 patients (38.7%) followed by idiopathic in 14 patients (22.6%), instrumentation in eight patients (12.9%), post prostatectomy in 10 patients (16.1%), trauma a cause of urethral stricture in five patients (8.1%) while surgery for hypospadias being the least common cause in one patient (1.6%) (figure 1). Concerning site of strictures bulbar stricture was the commonest one found in 35 patients (56.5%) followed by membranous in 13 patients (21%), prostatic in 11 patients (17.7%) and penile stricture least one in three patients (4.8%) (figure 3). Most of patients had complications related to urethral stricture 43 patients (69.4%), of these 17 patients (39.5%) had urinary tract infections, 15 patients (34.9%) had bladder diverticulum, chronic cystitis and urine retention in four patients (9.3%) for each, renal impairment in two patients (4.7%) and vesical stone in only one patient (2.3%) (figure 2).

The result showed 24 patients (77.41%) had recurrence of stricture in control group A and six

patients (19.35%) had a recurrence in treatment group B (figure 4). At the end of the first three month of follow-up five patients (20.8%) in the control group A had urethral stricture recurrence in compare to one patient (16.7%) had recurrence in the treatment group B. At the end of six month of follow-up nine patients (37.5%) in the control group A developed recurrence while no patient (0.0%) in the treatment group B had stricture recurrence. By the end of follow-up at one year ten patients (41.7%) in the control group A had recurrence of urethral stricture while five patients (83.3%) had recurrence in treatment group B (figure 3).

In the control group A 14 patients (58.3%) their recurrence in the first six months of follow-up, while five patients (83.3%) in the group B had their recurrence in the second six months ($n=5$) 83.3% (figure 4).

In the control group A, four patients had urinary tract infections were positive for E.coli. In the treatment group B three patients had urinary tract infections two were positive E.coli and one positive for klebsiella and one patient developed epididymo-orchitis.

Figure 1 : Distribution of the study population according to the etiology of urethral stricture

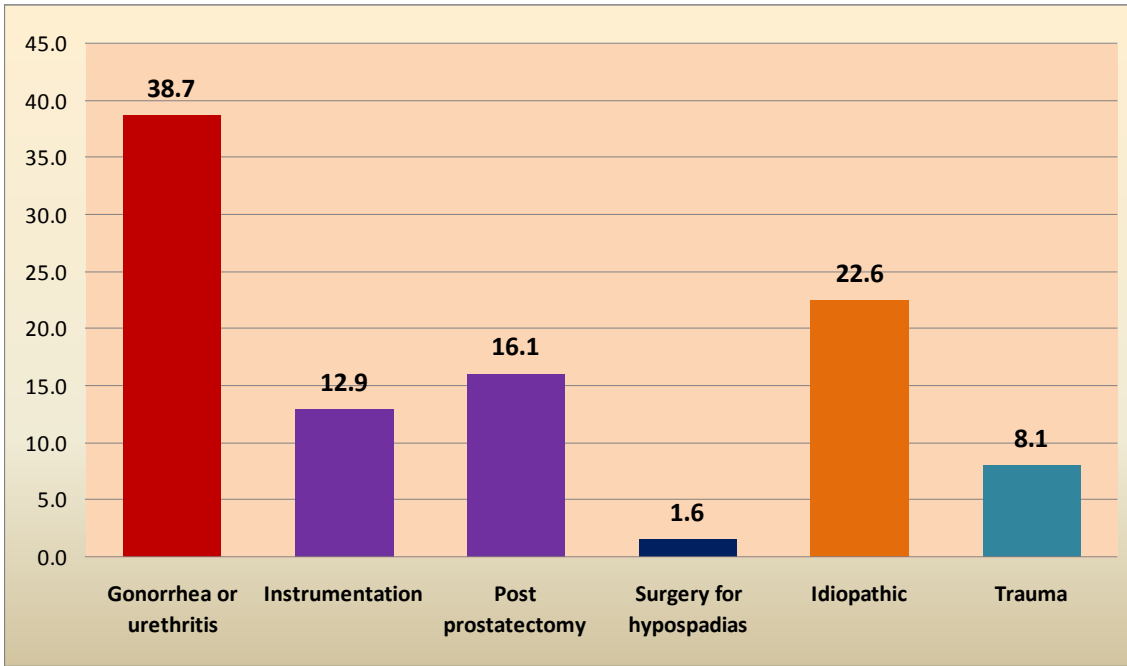


Figure 2 : Distribution of the study population according to The Complications of urethral stricture

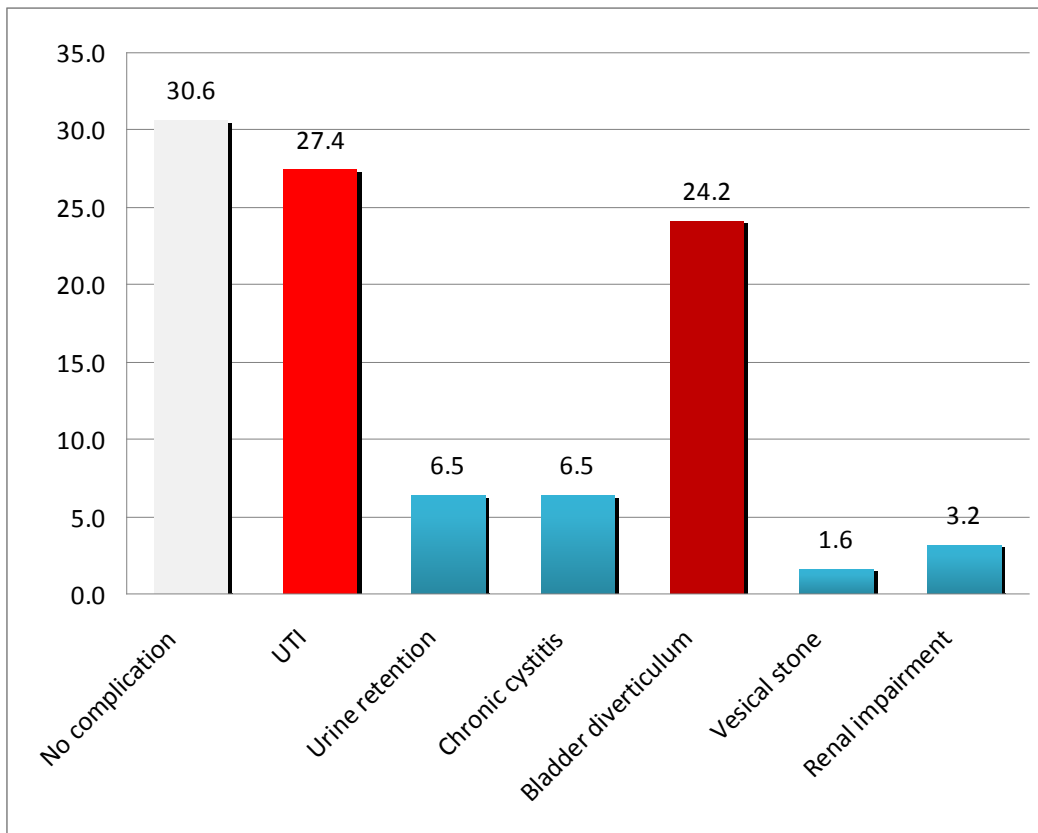


Figure 3 : Recurrence of urethral stricture in the control and treatment groups

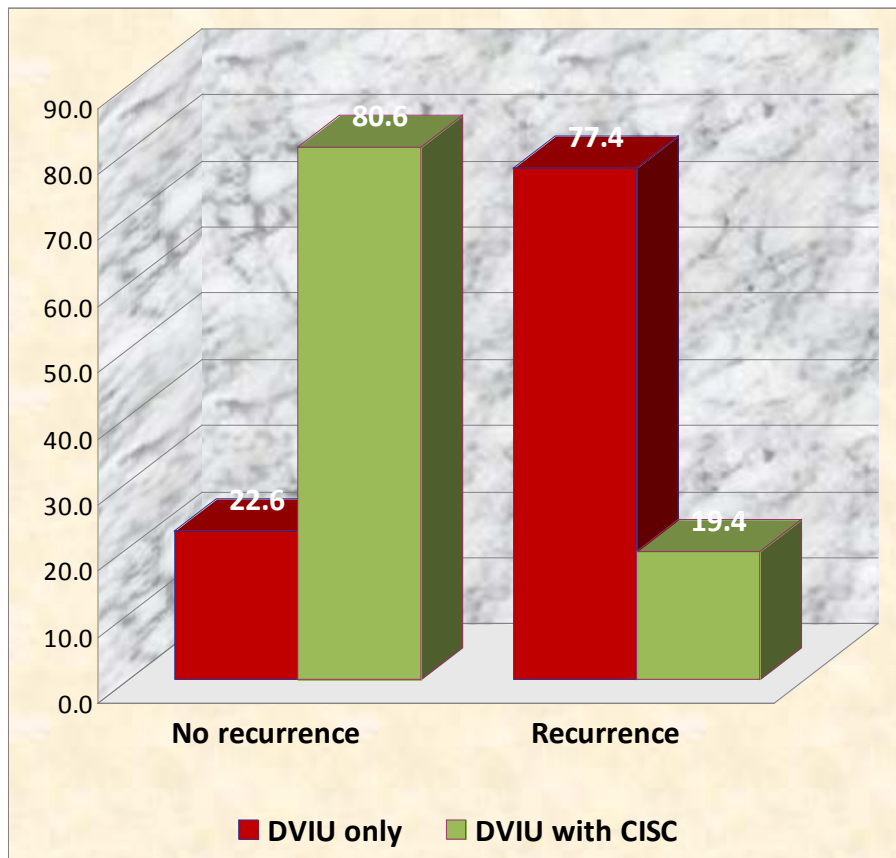
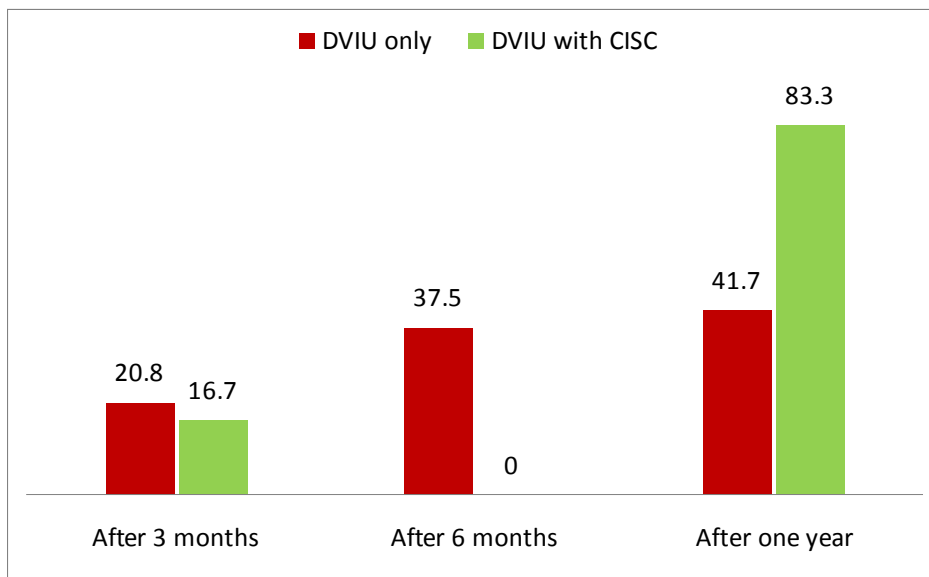


Figure 4 : Time of stricture recurrence during period of follow up



V. DISCUSSION

Analysis of age in our study showed that 28 (45.5%) of these patients were aged between 31 to 50 years old, this mean that most of patients are middle age groups may be due to the etiology in which

gonorrhoea and urethritis were the most common cause of stricture. In our study gonorrhoea and urethritis were the most common cause of urethral stricture (38.7%) and this may be attributed to unprotected sexual practice or in complete ineffective treatment of

gonorrhoea. However study in Benin, Nigeria [8], showed that 55.9% of stricture disease traumatic in origin and this similar to that reported from developed countries [3,4]. This reflects that development is not without its drawbacks while underdevelopment has its own health implications. Bulbar urethral stricture is the most common site of stricture 35% followed by membranous one 13% and this may be related to the aetiology of stricture. In this study most of recurrence occurred in the control group A 24 (77.41%) patients in compare to six (19.35%) in the treatment group B P value < 0.005 this mean that clean self intermittent catheterisation significantly reduce the rate of stricture recurrence, similar result obtained from different studies[7,9,10,11,12,13] . In the first three months of follow-up five patients in the control group A had stricture recurrence in compare to one patient in the treatment group B, this mean that no significant difference in short term of use of self catheterisation. Our observation is similar to study by Bodker A, etal [12]. By the end of six months of follow-up the total number of recurrence in the control group A is 14 in compare to one in the treatment group B. At end of follow-up at one year the overall recurrence in the control group A is 24 out of 31 and six out of 31 in the treatment group B P value < 0.005. This high rate of recurrence compare to international literature may be due to careful assessment of patients using history ascending urethrogram as an imaging to confirm urethral stricture recurrence. Most of recurrence in the control group A occurred in the first six months of Follow-up 14 (58.3%) patients in compare to only 16.7% in the treatment group B while most of recurrence in the treatment group B occurred in the next six months of follow-up 83.3%.this mean that the disease free interval is better in patients treated with clean self intermittent catheterisation[11].

In our study four patients in the control group A developed urinary tract infection all of them were positive for E.coli while in the treatment group B patients three patients had urinary tract infections and only one patient developed epididymo-orchitis, of those with urinary tract infections two were positive for Ecoli and one patient was positive for Klebsiella. No urethral bleeding or pain reported in patients on self catheterization so no difference in the two groups [14,15], this reflect that clean intermittent self catheterisation is simple, safe and easy to perform procedure with good patients compliance associated with minimal morbidity, this may be attributed to good education of all patient involved in clean intermittent self catheterization, good patients compliance, quality of catheter and proper application of good catheterisation technique.

VI. CONCLUSION

Urethral stricture is the major health urological problem among male middle age group of patients

associated with much morbidity, direct visual internal urethrotomy in spite of short effective out come, minimally invasive, with avoidance of false passage associated with high rate of recurrence. Perform of Clean intermittent self catheterization for three months following direct visual internal urethrotomy is an effective method to reduce rate of urethral stricture recurrence.

Clean self intermittent catheterization easy, acceptable procedure by the patients, less invasive and associated with minimal morbidity.

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GLOBAL JOURNAL OF MEDICAL RESEARCH
DISEASES

Volume 13 Issue 5 Version 1.0 Year 2013

Type: Double Blind Peer Reviewed International Research Journal

Publisher: Global Journals Inc. (USA)

Online ISSN: 2249-4618 & Print ISSN : 0975-5888

Immune Associations in Hashimoto's Thyroiditis and Related Disorders

By Dan Perețianu, Cătălina Poiană, Mara Carșote, Daniela Cristina Staicu, Irina Aniniși, Bogdan Opreșan, Alexandrina Clodeanu

Abstract- Material and method

a) *Diagnostic: A. Diagnostic of thyroid immune disease: ATPO and ATG investigation was considered as necessary and were correlated with ultrasound. B. Diagnostic of immune disease. The diagnostic was based on classical guides for every disease. 2. Patients: A. "Classical" Hashimoto thyroiditis (hyper-ATPO-emia, HT) = 1276, B. thyroiditis with isolated hyper-ATG-emia, with normal ATPO (T-ATG) = 85, C. thyroiditis "sero-negative" (normal ATPO and ATG, pathology diagnosis) = 9, D. idiopathic myxedema (hypothyroidism, no A,B,C) = 76; E. control = 1216 (no antibodies, when hypothyroidism, iatrogenic).*

b) *Statistical analysis: χ^2 test for comparing patients data with control data and z-test for comparing proportions.*

GJMR-F Classification : NLMC Code: WK 200, WK 265



IMMUNE ASSOCIATIONS IN HASHIMOTO'S THYROIDITIS AND RELATED DISORDERS

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Immune Associations in Hashimoto's Thyroiditis and Related Disorders

Dan Perețianu ^α, Cătălina Poiană ^σ, Mara Carșote ^ρ, Daniela Cristina Staicu ^ω, Irina Aniniși [¥], Bogdan Opreșan [§], Alexandrina Clodeanu ^x

Abstract- Material and method

- a) **Diagnostic:** A. Diagnostic of thyroid immune disease: ATPO and ATG investigation was considered as necessary and were correlated with ultrasound. B. Diagnostic of immune disease. The diagnostic was based on classical guides for every disease. 2. Patients: A. "Classical" Hashimoto thyroiditis (hyper-ATPO-emia, HT) = 1276, B. thyroiditis with isolated hyper-ATG-emia, with normal ATPO (T-ATG) = 85, C. thyroiditis "sero-negative" (normal ATPO and ATG, pathology diagnosis) = 9, D. idiopathic myxedema (hypothyroidism, no A,B,C) = 76; E. control = 1216 (no antibodies, when hypothyroidism, iatrogenic).
- b) **Statistical analysis:** χ^2 test for comparing patients data with control data and z-test for comparing proportions.

Results

- a) **Immune association – in total:** in HT = 237 (18.57%, $p < < 0.001$); in T-ATG = 23 (27.06%, $p < < 0.001$); in "sero-negative" = 1 (11.11%, NS); in idiopathic myxedema = 11 (14.47%, $p = 0.9$, NS); in control: 107 (8.80%).
- b) **Main Immune Associations were with:** A. Vitiligo: in HT = 37, $p=0.0006$; in T-ATG = 2 ($p = 0.09$); in Control = 11. B. Allergic dermatitis: in HT = 35, $p=0.0001$; in T-ATG = 2 ($p = 0.09$). C. Drug allergy: in HT: 27 ($p=0.007$); in ATG-T: 2. D. Immune ovariitis with precocious menopause: in HT = 16, $p=0.009$. E. IDDM: in HT: 15 ($p= 0.06$); F. Allergic rhinitis: in HT = 13 ($p = 0.006$); G. Biermer anemia: in HT = 12 ($p=0.0096$). H. Major collagenoses and vasculitis: in HT: 12 vs 8 in control (NS); I. Rheumatoid arthritis: in HT = 8 vs 20 in control (NS). J. Immune enteric diseases: in HT: 10 ($p = 0.025$); K. Bronchial asthma: in HT: 9 vs 10 in control (NS). L. Alopecia areata: in HT = 8 ($p = 0.06$); M. Repetitive zona zoster: in HT = 8 ($p=0.023$); N. Thrombophilia: in HT = 7 vs 3 in control (NS); O. Otosclerosis: in HT = 4 (NS), in T-ATG = 3 ($p < < 0.001$) vs 2 in controls. P. Multiple sclerosis: in HT: 4 vs 1 in controls (NS). Q. Corticoadrenal insufficiency: in HT: 4 ($p = 0.05$).
- c) **Multiple associations** (HT/T-ATG and other minimum 2 immune disorders) were recorded: in HT, no = 69 (29.11%); in T-ATG, no = 3 (13.04%). Examples of multiple association in our patients: Cerebral vasculitis with Sneddon sd, pulmonary fibrosis, cryoglobulinemia,

- virus C hepatitis (also under IFN) & sicca sd; Sarcoidosis with drug allergy, scleroderma, adenomegaly & arthritis;
- d) Asthma with postpartum trombophilia & antiphospholipidic sd; Selective alopecia areata (no eyebrows), ferriprive anemia, miopia; Sharp disease, zona zoster, dispepsia, alopecia areata & trombocytosis.

Conclusions

1. Hashimoto's thyroiditis and Thyroiditis with only ATG associate other immune diseases or immune conditions with significant increased frequency. 2. The most significant and prevalent association are with, vitiligo, allergic dermatitis, drug allergy, early menopause with immune ovaritis, allergic rhinitis, Biermer anaemia, immune enteric diseases, repetitive zona zoster, corticoadrenal insufficiency, otosclerosis. 3. Borderline could be considered alopecia areata, IDDM and thrombophilia. 4. Multiple immune associations are very common.

I. INTRODUCTION

a) About organ specific vs systemic immune disorders

Immune/Autoimmune thyroiditis is considered as a limited disease, extended only to one organ – the thyroid. There are many organ limited immune disease, named as "organ specific". On the other hand, there are immune diseases expanded to the whole body; they are called "systemic" immune diseases.

The day to day practice showed that it is possible that one organ specific disease could be associated with another organ specific disease, or a systemic immune disease could be associated with another one, or with a more organ specific disease. In this paper we will present our experience based on over 40 years of observations (DP) on our patients with immune diseases, related to immune thyroiditis.

Based on "immune network" of Jerne (1985) (Nobel Price, 1984), we suggest that there is no organ specific, nor a systemic immune disease, but, instead, the entire clinical context is an "immune network" disruption.

b) About what we understand by thyroid related disorders

Immune thyroiditis is characterized by inflammation of the thyroid, associated with specific immune mechanisms. Defining thyroiditis, the nosological Hashimoto thyroiditis has undergone a historical process.

Originally, Hakaru Hashimoto (1881-1934) described in 1912 (Hashimoto, 1912) a form of thyroid

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disease with thyromegaly with follicular inflammation and hypothyroidism different from atrophic hypothyroidism, that time called "myxedema" or "Ord's thyroiditis" [named from William Miller Ord (1834-1902) which described the atrophy of the thyroid with thyroid inflammation in 1877].

Subsequently, the pathogenesis of thyroid lesion was recognized as immunological and thus was named "lymphocytic", "chronic", and/or "autoimmune". Under clinical spectrum has been observed that patients with thyroiditis can be normothyroid (euthyroidism), not necessarily hypothyroid as originally Hashimoto described.

Investigating the pathogenesis of this disease, it has been observed that it is caused by an antibody called "antimicrosomal" because affected some thyrocyte cellular organelles, i.e. microsomes. After "antimicrosomal" antibody was discovered the antigen: thyroperoxydase. So, the name of antibody was changed from antimicrosomal to "antithyroperoxydase" (ATPO), as is now in use. Then, in some atrophic Ord's thyroiditis patients have been discovered the same antibodies.

In that moment, become obvious that the volume of thyroid is not essential in defining the disease; it is essential the immune process, which could lead, in evolution, to thyromegaly or to thyromicria even to atrophy.

So, Hashimoto's thyroiditis become that thyroiditis in which the pathogenesis was related to antithyroperoxydase antibody. It is a lymphocytic chronic inflammation of the thyroid, characterized by a specific immune mechanism, named *antibody dependent cellular cytotoxicity* (ADCC) (Rebuffat, 2008). In defining the disease, the thyroid size (bigger, as in Hashimoto's description or atrophic, as in Ord's description) has no importance, as the disease was defined by a pathogenetic mechanism.

Moreover, thyroiditis classification should not be depending on thyroid functionality. There are patients with the same pathogeny but with different thyroid function, either hyperthyroidism, or hypothyroidism. Moreover, most patients are euthyroid.

Some researchers and authors (see, for example, Clerc, 2009) make inadequate distinction between "Hashimoto thyroiditis" (thyroiditis with "goiter", i.e. thyromegaly) and "chronic lymphocytic thyroiditis" (thyroiditis without "goiter"). This distinction is not based on a proper understanding of the pathogenesis of thyroiditis due to phenomena associated with ATPO, but is based on clinical grounds. These data do not have any impact on pathogenesis, which is the one which should define a nosological status.

The existence of an immunological mechanism strictly individualized makes without meaning the broader term "immune disease / autoimmune thyroid

disease". In this broader context, some believe that Graves-Basedow disease, Hashimoto's thyroiditis, postpartum thyroiditis or silent forms of immune thyroid disease is a single disease or a continuous spectrum of disease (see Trifanescu, 2008).

Instead of viewing one immune disease, different multiple antibodies, and different multiple immune reactions should lead to consider the assertion "one mechanism - a disease" (Peretianu, 2012). In addition, other diseases have other immune mechanisms, and they are identified and clearly specified (Ganesh, 2007).

In chronic lymphocytic thyroiditis (considered as Hashimoto's thyroiditis), the presence of other antibodies (along ATPO), such as antithyroglobuline (ATG), lead to new nosological and taxonomical problems. As long as ATG are directed to another antigen and as long as the immune reaction is different (not ADCC, but, mostly, CDCC—*complement dependent cellular cytotoxicity*) (Ronco, 2009), adopting the concept "a mechanism - a disease", become that the ATG thyroiditis is another disease. If we accept that concept, we should named Hashimoto thyroiditis that immune disease based on ATPO mechanisms and related to ADCC mechanism.

Another problem occurs when thyroiditis has no antibodies; the condition could be named "seronegative" (Spina, 1990). The diagnosis was strictly pathologically and in serum were not observed any type of known antithyroid antibody. By adopting the concept of "an autoimmune thyroid mechanism – one thyroid immune disease", we could observed that "seronegative" thyroiditis could not be Hashimoto's thyroiditis. In the future, it will become another form of thyroiditis, when the antibodies and the antigens involved will be discovered.

Another condition related to immune thyroid diseases is that in which there is hypothyroidism, and ultrasound appearance, usually with thyromicria, but with no ATPO or no ATG. Since the patient do not performed a thyroid punction for a pathological exam, the condition should be named as "idiopathic myxedema".

Therefore, we analyze in this paper 4 immune thyroid conditions: thyroiditis due to ATPO (so called classical Hashimoto's thyroiditis), thyroiditis without ATPO but with high level of AGT (we named this condition ATG-thyroiditis), idiopathic myxedema (non induced hypothyroidism without ATPO and without ATG), and "seronegative thyroiditis" (thyroiditis on pathology but without any antithyroid antibody).

II. MATERIAL AND METHOD

a) Diagnostic

- i. *Thyroid disease*: ATPO and ATG investigation was considered as necessary and sufficient for

thyroiditis diagnostic. The *cut-off* was considered at 34/35 u/ml. We used usual laboratory commercial kits for both antibodies. We used electrochemiluminescence method. At this cut-off level, only 9 patients were considered as "sero-negative" thyroiditis, and only 76 patients with idiopathic myxedema could have the potential of being thyroiditis (but no puncture was performed on them).

- ii. *Immune disease*: The diagnostic was based on classical guides for every disease. The diagnosis of our patients was done by our colleagues from other clinical fields: internal medicine, dermatology, hema-tology, infectious disease, a.o. For major collagenosis, vasculitis and lupus-related disorders was used a combination from Jennette and Guillevin classifications (Jennette, 1997, Guillevin, 2008).

b) *Patients*

All 1446 patients were nonselected, and they were registered in time, in the consecutive order of visiting our clinics. Patients were considered only if they have minimum an ATPO analysis, a TSH level and an ultrasound exam. Based on this schedule, the data were registered from 1999 till September 2013 (table 1).

- 1. Patients with thyro- iditis with high antithyrop-eroxydase antibodies (hyper-ATPO-emia): total 1276; women: 1207, men: 69 (5.41%). Median age: 50.5 years.

Their function was: euthyroidism: 44.71%, hypothyroidism: 41.35%, hyperthyroidism: 13.94%.

The thyroid volume was: normal: 62.87 %, small (thyromicria or atrophy): 5.8%, high (thyromegaly): 31.33%. Patients with thyroid nodules: 6.50%.

- 2. Patients with hyper/high antithyroglobuline antibodies and with normal level of ATPO (hyper-ATG-emia thyroiditis): total: 85; women: 80, men: 5 (6.25%). Median age: 51 years. The ratio of men was similar to that in "classical" thyroiditis ($p = 0.93$, $z = - 0.08$).

There function was: euthyroidism: 62.55%, significative more that in hyper-ATPO-emia ($p = 0.001$, $z = - 3.24$), hypothyroidism: 24.71%, significative less that in "classical" thyroiditis ($p = 0.002$, $z = 3.08$), hyperthyroidism: 12.94%, no differences between HT vs TATG ($p = 0.76$, $z = 0.29$).

The thyroid volume was: normal: 67.85%, similar in both TH & TATG ($p = 0.35$, $z = -0.9$); high (thyromegaly): 29.45%, similar in both TH & TATG ($p = 0.71$, $z = 0.36$); small (thyromicria): 2.70% (2 times lower than in hyper-ATPO-emia; $p << 0.001$, $z = 5.57$).

Patients with thyroid nodule: 18.95%, 3.5 times more as in hyper-ATPO-emia ($p << 0.001$, $z = 8.56$).

- 3. Idiopathic myxedema: total: 76; women: 67, men: 9 (13.43%), more that in the other thyroid disorders (p vs HT = 0.35, NS). Median age: 60 years.

The function was 100% hypothyroidism. Thyromicria: 13.04% was twice as in Hashimoto's thyroiditis.

- 4. Seronegative thyroiditis: total: 9; all women. Median age: 53 years.

The thyroid function was: euthyroidism: 87.5%, hypothyroidism: 12.5%. All had thyroid nodules, for which they were punctured. One patient associated also ultrasound hypoechoic pattern as in classical thyroiditis.

- 5. Control group was formed by patients who were investigated for a thyroid disorder. In this group most patients were with thyroid nodules (60.46%), either macro (>1 cm) or micro. Normal thyroid was registered in 22.29% patients. In this group, around 17.26% patients presented pseudonodular hypoechogenic non-nodular homogenous/ inho-mogenous thyroid, as was observed in thyroiditis in 91.77%. In all these patients, the antibodies were normal: no high ATPO, no high ATG.

Total: 1216; women: 1088, men: 128 (11.76%). Median age: 54 years.

Their thyroid function was: euthyroidism: 92.26%, hypoth-yroidism: 2.72%, hyperthyroidism: 5.02%.

Table 1 : Clinical data of 2506 patients investigated for thyroid function and morphology

	Classic Hashimoto thyroiditis (hyper-ATPO-emia)	Thyroiditis with only hyper-ATG-emia (normal ATPO)	Idiopathic myxedema (hypothyroidism normal ATPO, normal ATG)	Sero negative thyroiditis (pathological diagnosis)	Control group
Number	1276	85	76	9	1216
Age					
Average	50.21	50.07	57.93	45.00	53.65

Standard deviation	14.79	15.73	15.87	15.22	15.18
ATPO					
Average	737.76	11.77	11.77	7.29	9.25
Standard deviation	1728.75	9.14	8.75	5.21	7.38
ATG					
Average	479.85	495.13	12.93	17.66	17.66
Standard deviation	1070.28	1075.13	10.41	53.80	53.80
Sex					
Women	1207	80	67	9	1088
Men	69	5	9	0	128
Thyroid function					
Euthyroidism	570	53	0	8	1121
Hypothyroidism	528	21	76	1	34 ***
Hyperthyroidism	178*	11**	0	0	61 ****
Ultrasound					
Hypoechoic (pseudonodular & homogenous)	1171	62	58	1	210
Nodular (> 1 cm)	83	19	8	8	735
Normal (hyperechoic)	22	4	10	0	271

* 160 patients certainly associated with Graves-Basedow disease (high TRAB; ELISA); ** 5 patients certainly associated with Graves-Basedow (with hyper-TRAB); *** 2 amiodarone-induced; **** one amiodarone-induced
 TRAB: TSH receptor antibody

c) *Statistical analysis*

Statistical analysis for our discrete data was performed with χ^2 test (usually for 2 rows and 2 columns). For percentage differences was used z-test.

III. RESULTS AND DISCUSSIONS

a) *In patients with hyper ATPO thyroiditis (Hashimoto's thyroiditis)*

Another non-thyroid immune disease (or association which could have an immune/autoimmune substrate or mechanism) was registered in 237 patients (18.57%): 224 women and 13 men (5.49%).

The prevalence of men with thyroiditis was not different compared with the prevalence of men with an

immune association and thyroiditis (5.49 vs 5.41%) [p = 0.07, z = - 0.03]. That suggests that the immune association was not characterized especially for women. When appeared, thyroiditis is the same accompanied by an immune association irrespective of sex.

In the control group, an immune disease was registered in only 107 patients (8.80%). These ratios (18.57% vs 8.80%) lead to a very high statistical significance [p << 0.0001, χ^2 >> 24]. With other words: Hashimoto's thyroiditis associate more probable another nonthyroid immune disease than controls.

All the clinical situations were tabulated (table 2).

Table 2 : Immune associations in Hashimoto's thyroiditis comparing with a control group (no. cases *)

Immune association in Hashimoto's thyroiditis	Immune disease in control group
Vitiligo without other associations (19)	Vitiligo without any other associations (10)
Vitiligo plus exophthalmia and Graves-Basedow disease (1)	Vitiligo plus rheumatoid arthritis (1)
Vitiligo plus Graves-Basedow (2)	
Vitiligo with immune hepatitis and hepatic cirrhosis (1)	
Vitiligo plus skin allergy (1)	
Vitiligo and acoustic neuroblastoma (1)	
Vitiligo plus alopecia areata (1)	
Vitiligo plus allergic rhinitis (1)	

Vitiligo plus Biermer anemia (1)	
Vitiligo and facial zoster (1)	
Vitiligo and thrombocytopenia (1)	
Vitiligo and retinian thrombosis (1)	
Vitiligo with hypophysitis and isolated STH deficit (1)	
Vitiligo plus NIDDM (1)	
Vitiligo with allergy to penicillin (1)	
Vitiligo plus xantelasma (1)	
Dermatitis, allergic without other associations (21): examples: dust, sun, herbal soap,	Allergic dermatitis with no other associations (7): examples: propolis, balsam, egg, cacao, apple, dust, injection sc, hay, strawberries,
Eczema plus allergy to penicillin (1)	Allergic syndrome to cold with hepatitis B (1)
Dermatitis plus allergic rhinitis (2): dogs, cats, pollen	
Dermatitis plus vitiligo (1)	
Dermatitis plus Paget disease of breast and an additional kidney (1)	
Dermatitis with high DNA double stranded antibodies (1)	
Dermatitis plus NIDDM and prostatectomy for bladder retention (1)	
Chronic rush (5)	
Dermatitis and Quincke syndrome (1)	
Dermatitis and alopecia areata (1)	
Drug/Medication allergy (27): examples: methimazol (3), sulfamides, penicillin (7), ampicillin, nalidixic acid, xylene, epointol, iron, co-trimoxazole, cefuroxim, genatmicin, oxiccillin, acetylsalicylic acid, ibuprofen, ketoprofen, betablockers	Drug/Medication allergy (10): examples: doxycycline, penicillin, ampicillin, NSAID
Precocious menopause with no other association (11)	Precocious menopause without any association (4)
Precocious menopause with alopecia areata (1)	
Precocious menopause with plus IDDM (1)	
Precocious menopause with plus hepatitis (1)	
Precocious menopause with plus allergy (nonspecified) and lichen planus (1)	
Precocious menopause with otosclerosis (1)	
Biermer anemia and no other associations (6)	Biermer anemia without other associations (1)
Biermer anemia with multiple drug, dust allergies (asthma), multiple abortions (1)	Biermer anemia with allergy to doxycycline (1)
Biermer anemia with pericarditis and bilateral kidney litiasis (1)	
Biermer anemia plus hepatitis C (1)	
Biermer anemia plus polymyozitis (1)	
Biermer anemia plus multiple sclerosis (1)	
Biermer anemia plus vitiligo (1)	
IDDM without other associations (7)	IDDM without other associations (2)
IDDM plus nonsecreting pituitary macroadenoma (1)	IDDM associates with bronchic asthma (1)
IDDM with multiple drug allergies (1)	IDDM associates with cytolysis to statines (2)
IDDM with precocious menopause (1)	



IDDM plus scleroderma (1)	
IDDM with dermatitis (1)	
IDDM with multiple sclerosis (1)	
IDDM, Cushing disease due to pituitary adenoma ACTH-secreting with bilateral suprarenalectomy and iatrogenous hypocorticism, bilateral kidney lithiasis, cholecystic lithiasis, hyposomatotropism (hypo-IGF-emia) (1)	
Allergic rhinitis without other associations (2)	Allergic rhinitis with no other associations (1)
Allergic rhinitis plus dermatitis (2)	
Allergic rhinitis plus vitiligo (1)	
Allergic rhinitis plus bronchic asthma (2)	
Allergic rhinitis and B hepatitis (1)	
Allergic rhinitis with both dermatitis and bronchic asthma (1)	
Systemic lupus erythematosus with no other association (3)	Systemic lupus erythematosus without other association (2)
Systemic lupus erythematosus with Raynaud syndrome and multiple abortion (1)	Systemic lupus erythematosus with rheumatoid arthritis (1)
Systemic vasculitis without other specification (1)	Buerger arteriopathy with rheumatoid arthritis (1)
Subacute nodular vasculitis plus cryoglobulinemia, and non C non B hepatitis (1)	Systemic vasculitis associated with anticardiolipinic antibodies, demyelinating areas in white substance in subcortical frontal area with paralysis and tetraplegia (1)
Cerebral vasculitis with Sneddon syndrome, pulmonary fibrosis, cryoglobulinemia, C hepatitis (IFN), sicca syndrome (1)	Systemic vasculitis unspecified (1)
Sharp disease with alopecia areata, dyspepsia, repetitive zona zoster, allergy to betablockers (1)	Sjögren syndrome (1)
Sharp disease with cryoglobulinemia (1)	Henoch-Schoenlein purpura (1)
Sjögren syndrome with rheumatoid arthritis (1)	
Lupic hepatitis (1)	
Henoch-Schoenlein thrombocytopenic purpura with corticosuprarenal (CSR) insufficiency (1)	
Bronchic asthma without other associations (1)	Bronchic asthma with hepatitis B (1)
Bronchic asthma at pollen (1)	Bronchic asthma without other immune associations (8), one with NIDDM
Bronchic asthma with allergic rhinitis (3)	Bronchic asthma and IDDM (1)
Bronchic asthma at dust with zona zoster (1)	
Bronchic asthma (dust, pollen), with Biermer anemia ao (see above) (1)	
Bronchic asthma, thrombophilia postpartum, and antiphospholipidic syndrome (1)	
Bronchic asthma, dermatitis (1)	
Rheumatoid arthritis without other associations (3)	Rheumatoid arthritis and systemic lupus erythematosus (1)
Rheumatoid arthritis plus hepatitis C (2)	Rheumatoid arthritis with Buerger obliterans arteriopathy and NIDDM (1)
Rheumatoid arthritis and Sjögren syndrome(1)	Rheumatoid arthritis with hepatitis C (2)
Rheumatoid arthritis and lupic hepatitis (1)	Rheumatoid arthritis with hepatitis B (1)

Rheumatoid arthritis, hepatitis and rhinitis (1)	Rheumatoid arthritis without other associations (15)
	Rheumatoid arthritis and otosclerosis (1)
Zona zoster, all repetitive (8)	Zona zoster (3), repetitive (1)
Thrombophilia antepartum with gene 675 4G/5G (1)	Thrombophilia with hyperthyroidism and amiodarone administration (1)
Thrombophilia intrapartum with antiphospholipidic antibodies (1)	Thrombophilia related to pregnancy (1)
Thrombophilia postpartum (1)	
Thrombophilia with spontaneous abortions (1)	
Thrombophilia and retinian thrombosis (1)	
Thrombophilia with proven protein S deficit (1)	
Thrombocytosis with dyspepsia, Sharp syndrome, repetitive zona zoster and recidivant alopecia areata (1)	
Alopecia areata without associations (1)	Alopecia areata without associations (2); one patient had hypotestosteronemia (?) **
Alopecia areata postabortum with precocious menopause (1)	
Alopecia areata, dyspepsia (enteritis), Sharp syndrome, thrombocytosis and recidivant zona zoster (1)	
Alopecia areata with vitiligo (1)	
Alopecia areata, selective to eyelashes plus ferriprive anemia and myopia (1)	
Alopecia areata with erythema nodosum (1)	
Alopecia areata and dermatitis (1)	
Alopecia totalis (1)	
Dyspepsia, Sharp syndrome, alopecia areata, and repetitive zona zoster (1)	Crohn disease with non B, non C hepatitis (1)
Enteritis and allergy to spinach, including dermatitis (1)	Ultero-hemorrhagic rectocolitis (1)
Enteritis no specification (2)	
Glutenic enteropathy (2)	
Diarrhea without explanation for 2 months with allergic syndrome and double stranded DNA antibodies (1)	
Dyspepsia syndrome without other association (2)	
Ultero-hemorrhagic rectocolitis with drug allergies (1)	
Autoimmune hepatitis with no other association (2)	Autoimmune hepatitis with Crohn disease (1)
Autoimmune hepatitis with lupus erythematosus and rheumatoid arthritis (1)	Autoimmune hepatitis with PCT
Autoimmune hepatitis with rheumatoid arthritis (1)	
Autoimmune hepatitis (and chirosis) with vitiligo (1)	
Lymphomas (3)	Hodgkin lymphoma with radiotherapy and secondary hypothyroidism (1)
Multiple myeloma (1)	
Monoclonal benign gammopathy (with antinuclear antibodies positive) (1)	
Otosclerosis without other associations (2)	Otosclerosis and rheumatoid arthritis (1)



Otosclerosis with precocious menopause (1)	Otosclerosis without other association (1)
Otosclerosis with multiple sclerosis (1)	
Multiple sclerosis with facial paralysis (1)	Central demyelination (1)
Multiple sclerosis with IDDM (1)	
Multiple sclerosis with Biermer anemia (1)	
Multiple sclerosis with otosclerosis (1)	
Costicosuprarenal insufficiency without other associations (Schmidt syndrome) (2)	
Costicosuprarenal insufficiency with ovarian insufficiency and precocious menopause (1)	
Costicosuprarenal insufficiency plus thrombocytopenic purpura (1)	
	Autoimmune hepatitis (2), one with Crohn disease, other with porphyria cutanea tarda
Quincke edema (4), one to bee venom and propolis, one to lidocaine, one to penicilline and one to acetylsalicylic acid	
Psoriasis (2)	Psoriasis (3)
Dupuytren disease (2)	Dupuytren disease (2)
Lichen planus (2)	
Hypophysitis follow by empty sella (1)	
Hypophysitis plus vitiligo (1)	
Sarcoidosis with glomerulonephritis (1)	
Sarcoidosis associated with several drug allergies (methylprednisolone), scleroderma, adenomegaly, arthritis (1)	
Myasthenia gravis (2), one with thymectomy	
Ankylosing spondylitis with hydrocele and NIDDM (1)	Ankylosing spondylitis (4)
Sicca syndrome with NIDDM (1)	Sicca syndrome and allergy to ampicillin (1)
Carpian tunnel syndrome (1)	
Scleroderma plus IDDM (1)	
Dermatopolymyositis with Biermer anemia (1)	
Glomerulonephritis with chronic kidney insufficiency (1)	
Histiocytosis X (1)	
Infertility by antispermatic antibodies (1)	
Autoimmune hemolytic anemia (1)	
Chronic nonspecific inflammatory syndrome with high RCP, gammaglobulins and IgM (1)	
Chronic nonspecific inflammatory syndrome with high ESR (60 mm/h) and fibronogenemia (600mg/dl), with extended xantelasma (1)	

* ! By listing the association at two or more clinical situations, the number of total cases is apparently higher than the number of patients !

** see also Rovensky, 2010

Association of Hashimoto's thyroiditis with vitiligo: Vitiligo was observed in 37 patients (prevalence = 2.90%), 3 times higher than in controls (no = 11), with an increased significance ($\chi^2 = 11.48$; $p = 0.0003$), showing that vitiligo is very specific to thyroiditis. If added the patients from ATG-thyroiditis and idiopathic myxedema (see table 3 and table 4), vitiligo could be considered observed in 40 patients (prevalence in all thyroid immune disorders = 2.77%).

All our patients with thyroiditis-vitiligo associations were women. In the control group were 2 men with vitiligo (W:M ratio 5.5:1). Usually, in general population vitiligo is a women disease but only with 1.8 ratio (Schallreuter, 1994). Thus, vitiligo and thyroiditis was very specific to women.

Thyroid function of our patients with thyroiditis and vitiligo was: euthyroidism: 15 (40.54%); hypothyroidism: 14 (37.84%); hyperthyroidism: 8 (21.62%). The general ratio of thyroid function in all patients (44.71%, 41.35%, respectively 13.94%) is slightly respected also in vitiligo patients, with an insignificant small amount of hyperthyroidism ($z = -1.3$; $p = 0.18$), suggesting that thyroid function did not influence the appearance of vitiligo in thyroiditis.

Concerning the appearance, 2 women presented very widespread vitiligo. Both cases were euthyroid. On the other hand, one woman from control group had the same. Moreover, one man from the control group had vitiligo, widespread only to penis.

Association of Hashimoto's thyroiditis with dermatitis: Allergic dermatitis (presented as chronic rash, eczema, prurigo, papules), sole (only with thyroiditis) or with other more complex associations (see table 2) is very frequent in our patients (no = 35; prevalence 2.74%). In control group we registered only 8 patients. The difference was very significant ($\chi^2 = 15.96$; $p = 0.0001$). Therefore, dermatitis should be considered as a clinical condition very associative with thyroiditis. If added the 4 patients with only hyper-ATG thyroiditis and 1 in idiopathic myxedema (see below), the prevalence of this condition in thyroid immune disorders could be closer to that observed in vitiligo (total prevalence = 2.70%).

Concerning sex ratio, the association thyroiditis-dermatitis in our patients was over 6 times more in women than it was usually described for dermatitis (W:M ratio 2 :1) (Peiser, 2012), since only 2 men were registered (W:M ratio 17.5 : 1).

The thyroid function of our patients with thyroiditis-dermatitis association was: euthyroidism: 43%; hypothyroidism: 43%; hyperthyroidism: 14%. This ratio fit the general thyroiditis functional ratio, suggesting that dermatitis could appear with any thyroid function. As unusually appearance, one man presented association thyroiditis-dermatitis with high double stranded DNA antibodies.

Association of Hashimoto's thyroiditis with drug allergy: In our patients, we observed very frequently allergy to different drugs/medications (no = 27; prevalence 2.12%). In control group there were only 10 patients. This fact showed that drug allergy is very specific to Hashimoto's thyroiditis ($\chi^2 = 7.12$; $p = 0.0076$).

Allergy to penicillin is quite frequent (7 patients), being most registered antibiotic. Other antibiotics with allergy are: oxacillin, cefuroxime, and sulfamides.

Sometimes, severe forms of allergy were observed: anaphylactic shock to xyline/lidocaine and/or with Quincke edema (4 patients, table 2) (see also one patient with Quincke syndrome in only hyper-ATG thyroiditis – table 3).

An interesting drug allergy was observed in 3 patients with thyroiditis (ATPO increased) and Graves-Basedow (TRAB increased) associations in which *per orem* antithyroidian drug (methimazole, especially) triggered some forms of allergies.

Association of Hashimoto's thyroiditis with precocious menopause, probably due to immune ovariitis: We observed 16 women with precocious menopause (under 35 years). (prevalence = 1.25%). The prevalence of this condition in control group was much lower (no = 4), suggesting that precocious menopause could be considered as a clinical conditions very associative to thyroiditis ($\chi^2 = 6.69$; $p = 0.0097$). An additional patient with idiopathic myxedema was also observed (table 4).

As appearance, in one case, the menopause appeared at 15 years old !

Association of Hashimoto's thyroiditis with diabetes mellitus type 1: Insulin dependent diabetes mellitus (IDDM) was observed in 15 patients (prevalence = 1.18%). However, in the control group, the prevalence of IDDM was 6 patients. These data suggest that IDDM could be a significant association in our patients, but was NOT achieved the statistical significance ($\chi^2 = 3.47$; $p = 0.06$ – missing one patient).

As concerning the thyroid function, 5 patients were euthyroid (33.33%), 7 patients were hypothyroid (53.33% vs 41.35 in all patients), and 3 were hyperthyroid (20%). Association IDDM-thyroiditis had presented mainly as hypothyroidism (but not reaching statistical threshold, $z = -0.936$, $p = 0.35$).

Association of Hashimoto's thyroiditis with allergic rhinitis: We registered 13 patients, most of them associated also with other immune conditions (see table 2). In control group was observed only 2 patients. Therefore, allergic rhinitis appeared as very associative with Hashimoto's thyroiditis ($\chi^2 = 7.6$; $p = 0.0059$).

Three patients had both allergic rhinitis and bronchic asthma. 8 patients were euthyroid and 5 patients were hypothyroid.

Association of Hashimoto's thyroiditis with Biermer's pernicious anemia: Biermer's anemia was observed in 12 patients with thyroiditis, and with other clinical situations (see table 2). In control group we observed 2 patients with this disease. These data suggest that Biermer's pernicious anemia is a clinical condition very associative with thyroiditis ($\chi^2 = 6.71$; $p = 0.0096$).

As concerned the thyroid function, 6 patients were hypothyroid, 5 patients were euthyroid and 1 was hyperthyroid. This specific association presented also with increased hypothyroid appearance (50% vs 41.35% in all patients).

Association of Hashimoto's thyroiditis with systemic lupus erythematosus, other major collagenosis and vasculitis: 12 patients could be viewed from major

collagenosis point of view (see table 2), most of them presenting multiple and unusual association.

One particular case, woman, had cerebral vasculitis (with changes in behaviour, with initiating a childish spelling), with Sneddon syndrome, pulmonary fibrosis, cryoglobulinemia, C hepatitis and sicca syndrome. Thyroid function was normal. The onset was at 35 years with a neurological disorder due to cerebral vasculitis: a childish spelling. Corticoids were tried at onset, without effect. After 6 months, the treatment was changed: cyclosporine 250 mg/day was used, under creatinine control, because of the negative effect of the drug on kidney. After hepatitis C discovering, interferon and ribavirin were administrated. ATPO antibodies decreased less than 34 mu/ml after IFN. The patient is still on cyclosporine; stopping cyclosporine lead to cerebral vasculitis with childish spelling behaviour.

Another woman presented Sharp syndrome, with multiple associations, including repetitive zona zoster, allergies to drugs, and repetitive alopecia areata. Thyroid function was normal. Thyroiditis was diagnosed at 21 years old. Dyspepsia appeared at 30 years. Sharp diseases appeared after menopause, at 48 years old. After that, 3 episodes of repetitive zona were registred. At 50 years was discovered thrombocytosis and the first alopecia episode appeared. The patient was (and is) in euthyroidism. When polyarthritis, dyspepsia, drug allergy and alopecia area symptoms appeared, was used only symptomatic treatment with NSAID, antiallergics, and dermatological topics.

Even there were a lot of patients with major vasculitis, these conditions did not lead to statistical significance, because in the control group we registered also a lot of patient (no = 8) with the same and other interesting immune disorders and associations (see table 2) ($\chi^2 = 0.62$; $p = 0.43$).

Association of Hashimoto's thyroiditis with immune enteric disease: Immune enteritis, in different clinical forms, either as celiac syndrome or as simple dyspepsia without obvious cause, was observed in 10 patients (see table 2). In the control group were observed 2 patients with enteric diseases. Therefore, enteric diseases was a close association with thyroiditis ($\chi^2 = 4.98$; $p = 0.025$).

All our patients with thyroiditis-enteritis association were women. All, except one, were euthyroid. Therefore, we had not the possibility to search if the enteral disease diminished absorption of thyroxin, as others point out (Centanni, 2012). One patient was hyperthyroid, but not associated with Graves-Basedow disease (no TRAB).

In the control group was one man with Crohn disease, and one woman had ulcerative rectocolitis.

No patient with *helicobacter pylori* was registered as in other cases (Cammarota, 1997).

Association of Hashimoto's thyroiditis with bronchic asthma: We registered 9 patients with this clinical condition, mostly associated also with rhinitis and other conditions (see table 2). Our patients presented several crisis linked on exposure to their specific allergens (pollen, dust, dog or cat fur).

However, the disease was with the same prevalence in the control group (no = 10). Therefore, in our patient, asthma is NOT a condition associated preferentially with thyroiditis ($\chi^2 = 0.73$; $p = 0.12$). Even added the patients with only hyper-ATG and asthma (no = 2) (table 3), the statistics did not change.

Association of Hashimoto's thyroiditis with rheumatoid arthritis: We registered 8 patients with this association (prevalence: 0.63%). However, the prevalence among control group was higher (no = 20). That fact suggests that rheumatoid arthritis is not a specific association for thyroiditis. On the contrary: if a patient has rheumatoid arthritis, she/he could be protected against Hashimoto's thyroiditis ($\chi^2 = 5.81$; $p = 0.01$). Even we add the 4 patients from hyperTAG-emia thyroiditis, "seronegative thyroiditis" and idiopathic myxedema to increase the prevalence of rheumatoid arthritis in thyroiditis (all related diseases), the number did not reverse the data. Therefore, our data are contrary to other authors who said that thyroiditis-arthritis was very prevalent (Boelaert, 2010).

Association of Hashimoto's thyroiditis with alopecia areata: Alopecia areata, either localized (strictly areata) or *universalis*, was registered in 8 patients, including a woman with *alopecia totalis*. One 16 old year man had only eyelashes alopecia.

In our patients, alopecia and thyroiditis association have a particularity: while the prevalence of alopecia in the general population is similar to equality in both sexes (1.15 females: 1 male) (Seyrafi, 2005), alopecia from thyroiditis is clearly in favour of women (ratio 7:1).

However, because alopecia in control group was registered in 2 patients, the significance of the association was borderline ($\chi^2 = 3.33$; $p = 0.06$), missing 1 case.

Association of Hashimoto's thyroiditis with repetitive zona zoster: We registered 8 patients with this clinical condition. In the control group was 3 patients with zona zoster, but only one had repetitive zona. If we consider the repetitive aspect of zona, therefore, this clinical condition is highly associative with thyroiditis ($\chi^2 = 5.13$; $p = 0.023$).

Repetitive zona zoster could be considered as an immunodeficient condition, and was described in association with a multitude of autoimmune disorders (O'Connor, 2013). Usually, the disease appear in older people. In our patients, the conditions was registred even at 26 year old (average age of zona onset in our patients was 55.62 y; SD 16.7).

Association of Hashimoto's thyroiditis with thrombophilia and the deficit of protein S: We registered 7 patients with these clinical conditions (see table 2). All were women. Most of the clinical conditions were related to pregnancy, either antepartum, intrapartum or postpartum. In the control group were registered also 3 women with this clinical condition, 2 in relation with pregnancy, the other in relation with amiodarone administration. The statistical significance was not achieved ($\chi^2 = 1.42$; $p = 0.23$).

None of our patients were on the two conditions known to favour thromboembolism (Wu, 2006): estropro-gestative oral medication and surgical procedure (especially orthopedic).

Even the statistical difference was not achieved, our patient are different from the general population related to thromboembolism accidents. They were all women, and it was known that sex ratio on thromboembolism was equal among sexes (Moore, 2004).

Association of Hashimoto's thyroiditis with autoimmune hepatitis: Autoimmune hepatitis was observed in 5 patients with thyroiditis, 3 of them associated with lupus, rheumatoid arthritis and vitiligo (see table 2). In the control group we registered also 2 patients with autoimmune hepatitis, a man who associated also with Crohn disease and a woman with another association, porphyria cutanea tarda.

Therefore, there is NO significant increase of autoimmune hepatitis in thyroiditis ($\chi^2 = 1.15$; $p = 0.28$). In the context of the fact that immune therapies were used for viral C hepatitis, we observed 6 patients with interferon therapy (Pegasus^R, Pegintron^R, plus ribavirin). In 2 cases ATPO decreased, in 2 cases ATPO increased and in 2 cases ATPO behave undulatorious.

Association of Hashimoto's thyroiditis with multiple sclerosis: This clinical condition was registered in 4 patients, all women and all with other associations (see table 2). One patient in the control group had central demyelination. Therefore the association of thyroiditis with multiple sclerosis could be considered as a convincing association, even it was NOT reached the statistical significance ($\chi^2 = 1.66$; $p = 0.197$).

Association of Hashimoto's thyroiditis with otosclerosis: In our thyroiditis patients, we found 4 patients, all women. In control group there were 2 patients (see table 2). No statistical significance could be registred ($\chi^2 = 0.58$; $p = 0.44$). If added the 3 patients with otosclerosis and hyper-ATG-emia thyroiditis (see below), the significance of this association did not change ($\chi^2 = 2.26$; $p = 0.13$).

As in other clinical associations (see above), otosclerosis in our patients has a particularity: much more in women, since usually, the sex prevalence of otosclerosis is 1 man vs 1.15 women (Perez, 2009).

Association of Hashimoto's thyroiditis with corticoadrenal insufficiency: This clinical situation was

registered in 4 patients, as Schmidt syndrome along with other immune associations (see table 2). Corticoadrenal insufficiency-thyroiditis association is quite strong, since in the control group was no such a patient ($\chi^2 = 3.82$; $p = 0.05$).

Three patients were hypothyroid (75%) and one was euthyroid. An additional hypothyroid case was registred in idiopathic myxedema.

Association of Hashimoto's thyroiditis with hematological proliferation diseases, including lymphomas: Hodgkinian and nonhodgkinian lymphomas occur in 3 patients with thyroiditis. The association appears to be weak since in the control group we registered 1 patient ($\chi^2 = 0.91$; $p = 0.34$). All lymphomas were extrathyroid.

If we add more 2 patients with thyroiditis associated with multiple myeloma (1 case) and benign monoclonal gammopathy (1 case), then the mathematical test is changed ($\chi^2 = 2.48$; $p = 0.11$), but not reaching the statistical significance.

Association of Hashimoto's thyroiditis with other clinical situations with an immune/autoimmune condition: Other clinical immune associations with thyroiditis with only 2 patients or 1 patient were registered (see table 2). They are interesting only from description point of view. Obviously, no one have any statistical relevance.

Moreover, in the control group were registered more patients with other immune disorders. For example, in the control group we registred 4 patients with ankylosing spondylitis vs. 1 in thyroiditis, fact which are contrary to other studies (e.g. Peluso, 2011).

Considering hypophysitis diagnosed as such (no = 2), we observed in our patients 9 additional patients with hypo-IGF-1, three with exophthalmia, hyperthyroidism and Graves-Basedow association, and one with diplopia and hypothyroidism. A pituitary lesion was not diagnosed in these patients.

On the other hand, we registered 5 patients with high levels IGF-1, without acromegaly, 4 of them associated exophthalmia, hyperthyroidism and Graves-Basedow association.

No speculation or conclusion can be done.

Only one vs. multiple associations: We observed 168 patients with only one immune disorder associated with Hashimoto's thyroiditis. The prevalence was 70.89%. The other patients (no = 69, prevalence = 29.11%) presented multiple associations. Some of them were registred with 5 or 6 associations.

Some interesting association to Hashimoto's thyroiditis could be described (see also table 2):

- Cerebral vasculitis with Sneddon syndrome, pulmonary fibrosis, cryoglobulinemia, C hepatitis (treated with IFN), and sicca syndrome in a woman (see above).
- Sharp disease with repetitive alopecia areata, dyspepsia, repetitive zona zoster, allergy to betablockers and thrombocytosis in a woman (see above).

b) Immune associations in only hyper ATG thyroiditis

Another non-thyroid immune disease (or association which could have an immune/autoimmune substrate) was registered in 23 patients from 85 (27.06%). All the patients were women.

The prevalence of this association compared with that in "classical" thyroiditis (with high ATPO

(18.50%) was higher. Statistical significance was at border ($p = 0.054$, $z = -1.93$). However, the clinical significance could be considered as hidden, since in this form of thyroiditis there were more patients with euthyroidism than hypothyroidism (see above, table 1).

All the clinical situations were tabulated (table 3)

Table 3 : Immune associations in hyper-ATG thyroiditis (no. cases *)

HyperATG thyroiditis (without hyper/high ATPO)	
Vitiligo without other association (1)	Rheumatoid arthritis with vitiligo (1)
Vitiligo plus rheumatoid arthritis (1)	Systemic lupus erythematosus, pulmonary fibrosis and celiac disease (1)
Dermatitis to cat fur (1)	Phospholipidic syndrome (1)
Dermatitis, eczema to nickel (1)	Celiac disease with systemic lupus erythematosus and pulmonary fibrosis (1)
Allergic dermatitis without other associations (2)	Neutropenia post blood (1)
Drug/Medication allergy (2), to acetylsalicylic acid and nonspecified	Quincke edema to acetylsalicylic acid (1)
IDDM without other associations (1)	Multiple sclerosis (1)
Bronchial asthma without other associations (2)	Thrombocytemia (1)
Allergic rhinitis (1)	Psoriasis (1)
Otosclerosis (3)	

* ! By listing all the associations, from both/many points of view, the number of cases is (apparent) bigger than that of patients !

The most frequent associations were with: dermatitis (4 patients), otosclerosis (3 patients), vitiligo (2 patients), and bronchial asthma (2 patients).

From these data, we could point out that this thyroid immune condition (i.e. hyper-thyroglobuline thyroiditis) was highly associated with otosclerosis ($\chi^2 = 23.50$; $p << 0.0001$) and dermatitis ($\chi^2 = 18.65$; $p << 0.0001$) [compared with the control group]. Vitiligo, bronchial asthma and drug allergy were associated with

a nonstatistical threshold ($\chi^2 = 2.79$; $p = 0.09$) [missing only one patient for attending the threshold of 0.05].

c) Immune associations in idiopathic myxedema

In idiopathic myxedema (no = 76), we registered 11 patients with a nonthyroid immune association; prevalence = 14.47%. The prevalence is lower than that observed in "classical" thyroiditis, and is between the prevalence of the control group.

All the clinical situations were tabulated (table 4)

Table 4 : Immune associations in idiopathic myxedema (no. cases *)

Immune associated disease with idiopathic myxedema
Rheumatoid arthritis (2)
Systemic lupus erythematosus and C hepatitis (1)
Dermatitis (1)
Bronchic asthma (2)
Idiopathic neutropenia (1)
Precocious menopause (probable due to immune ovaritis) (1)
Biermer anemia (1)
Schmidt syndrome (hypothyroidism with Addison disease), decreased IGF-1, ferriprive anemia by lack of Fe absorption (1)
Vitiligo (1)

No specific conclusions could be done from these ases.

d) *Seronegative thyroiditis*

From 9 patients, only 1 woman presented an immune association: rheumatoid arthritis.

IV. GENERAL DISCUSSION AND CONCLUSIONS

In the literature, there are many papers in which authors found thyroiditis in another specific immune disease: e.g.: Sjögren (Zeher, 2009), pemphigus (Pitoia, 2005), celiac disease (da Silva Koetze, 2006), rush (Zauli, 2001), dermatitis (Irani, 2012), alopecia areata (Seyrafi, 2005), vitiligo (Daneshpazhooh, 2006), ankylosing spondylitis (Peluso, 2011).

Moreover, the literature is full of isolated cases, in which multiple associations, including thyroiditis are presented, and described as "unusual". Usually, the starting disease is not thyroid. Some of our current patients are also very interesting, and could be viewed like "spectacular", even they are simply a case of "immune network disruption".

We published also isolated patients with immune associations, including a thyroid one, e.g.: Peretianu, 2006, concerning Graves-Basedow-systemic lupus erythematosus-psoriasis-vitiligo-alopecia areata, all in 2 patients; Peretianu, 1989, thyroiditis-rheumatoid arthritis-hypogonadism; Peretianu, 1990, Graves-Basedow diseases with ulcerative recto-colitis.

Much rare, researchers analyzes the patients starting from the thyroid point of view (like eg, Boelaert, 2010, Centanni, 2012), searching the immune conditions associated with a known thyroid disorders. For exemple, Boelaert found a prevalence of 14.3% immune disorders in thyroiditis and 9.67% in Graves-Basedow disease (Boelaert, 2010). Centanni found a prevalence of 16.2% immune disorders in thyroiditis (Centanni, 2012).

Our data showed slightly higher values on the prevalence thyroiditis-immune association: 18.57% for classical (hyperATPO) thyroiditis, or 27.06% for hyperthyroglobuline thyroiditis (without hyper-ATPO). If we considered all our patients, with "classical" thyroiditis, ATG-thyroiditis, seronegative thyroiditis and idiopathic myxedema, the prevalence of an immune (autoimmune) disease could be registered in 18.81% patients.

The differences on specific association prevalence between authors could have as origin bias reasons. For example, Centanni, could be bias on gastric and intestinal associations, since his group was involved in searching how thyroxin is absorbed (Centanni, 2012). That could led make more easily to a diagnosis of gastric atrophy (with Biermer's anemia) or/and celiac disease (in his study 34.8%, respectively, 11.1%). Boelaert (2010) could be bias on rheumatoid arthritis, maybe a disease widespread in England.

We tried to bring a new approach; we analysed the immune associations comparing with a group of patients without an immune thyroid disease. From this point of view, we showed that it is not important the

number of cases registered but the comparison with the same diagnosis in the "control" population.

From this point of view, in our patients, the most associative (and significant) immune disorders with thyroiditis were (in this order): vitiligo, dermatitis, drug allergies, precocious menopause (immune ovaritis), allergic rhinitis, Biermer's anemia, repetitive zona zoster, and corticosuprarenal insufficiency. Borderline could be considered multiple sclerosis, alopecia areata, IDDM, and thrombophilia.

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GLOBAL JOURNAL OF MEDICAL RESEARCH
DISEASES

Volume 13 Issue 5 Version 1.0 Year 2013

Type: Double Blind Peer Reviewed International Research Journal

Publisher: Global Journals Inc. (USA)

Online ISSN: 2249-4618 & Print ISSN : 0975-5888

Ovitrap Surveillance of Aedes Mosquitoes (Diptera: Culicidae) in Selected Areas of Dehradun District, Uttarakhand, India

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Abstract- Background & Objective: Dengue, a major public health problem in India is caused mainly by *Aedes aegypti* and *Ae. albopictus*. In Uttarakhand State (India), there has been a heavy increase in dengue cases in the year 2010 and thereafter in 2011-12, there was a decline. Keeping in view a change in climatic scenario i.e., heavy rainfall during June to September, we are expecting more and more cases of dengue this year too. Since there is lack of information on the bionomics of the recognized vectors of dengue from this region, it has been planned to determine the efficacy of ovitraps in monitoring the distribution and abundance of *Aedes* species in different urban and suburban areas of district Dehradun, Uttarakhand.

Methods: Ovitrap were placed at three sites viz., Sahastradhara, Garhi Cantt. and Karanpur of Dehradun city during August 2012 - July 2013 and examined weekly.

Keywords: *ovitrap indices, aedes mosquitoes, dehradun, uttarakhand, india.*

GJMR-F Classification : NLMC Code: QX 510, QX 525



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Ovitrap Surveillance of *Aedes* Mosquitoes (Diptera: Culicidae) in Selected Areas of Dehradun District, Uttarakhand, India

N. Pemola Devi^α, R.K. Jauhari^ο & Ritwik Mondal^ο

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Methods: Ovitrap were placed at three sites *viz.*, Sahastradhara, Garhi Cantt. and Karanpur of Dehradun city during August 2012 - July 2013 and examined weekly. Collected paddles were submerged into a bowl of water containing larval food. The hatched larvae were subsequently counted and reared in the cages to emerge into adults and identified upto species level using respective Keys and Catalogues. The estimation of ovitrap indices was done following the protocol developed by FEHD. and Lim et al.(2010)

Result: As many as 6 species of *Aedes viz.*, *Aedes aegypti*, *Ae. albopictus*, *Ae. edwardsi*, *Ae. pseudotaeniatus*, *Ae. unilineatus* and *Ae. vittatus* were collected during the study period. *Ae. aegypti* shared highest (37.28%) followed by *Ae. albopictus* (33.27%), *Ae. pseudotaeniatus*, (15.68%) and *Ae. vittatus* (8.33%). The mixed breeding comprised larvae of *Culex*, *Anopheles* and some unidentified species and shared least percentage (3.10%). In indoor, overall mosquito accounts low percentage (18.82%) in all three localities as compared to outdoor percentage (19.47%). Maximum ovitrap index was encountered from Garhi Cantt. (48.75) followed by Karanpur (45.00) and Sahastradhara (43.75) during August 2012. Outdoor indices of area ovitraps index were in the range of 17.30±1.83 to 21.88±2.10, while in indoors the range was 12.30±1.67 to 15.42±1.56. Monthly ovitrap index of the study period ranged from 0.00 to 45.83.

Conclusion: Ovitrap provide a very sensitive and economical method for detecting container breeders when the population density is low. A high density of dengue vectors in the residential area warrants the vector surveillance activities in time along with awareness programme.

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Keywords: ovitrap indices, aedes mosquitoes, dehradun, uttarakhand, india.

I. INTRODUCTION

Dengue, a major public health problem in India is an arbo-viral disease caused by the dengue virus (DENV) (Family: *Flaviviridae*) comprising four serotypes (DEN-1, DEN-2, DEN-3 and DEN-4) and female *Aedes* mosquito, mainly *Ae. aegypti* and *Ae. albopictus* play a role in the transmission of disease. Dengue Virus Infection (DVI) cause a spectrum of disease ranging from mild infection (dengue fever, DF) to a severe deadly disease - dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS) [1]. About 40% of the global population is living in the areas where transmission of dengue occurs. In an estimate, 50 million dengue infections, including 5,00,000 cases of DHF require hospitalization every year [2]. Earlier, estimated 3.46-3.61 billion people live in areas at risk of dengue from 124 countries which correspond to 53.0-55.0% of the world population [3]. Due to global warming *Ae. aegypti* and *Ae. albopictus* moved northward and had more rapid metamorphosis, the WHO expects millions more to be affected in the coming years [4].

In district Dehradun, the Dengue infections are well established from the year 2006 onwards. The abundance of vectors species have been reported by earlier works [5,6] who observed the breeding of *Ae. aegypti* in both natural habitats and domestic containers. Larval population of *Ae. aegypti* has been recorded in drains, pits, streams, canals, containers and tree holes while the breeding of *Ae. albopictus* was recorded in tanks, ponds, streams, containers and tree holes from district Dehradun [7]. The occurrence of *Ae. vittatus* in Garhwal region in Uttarakhand state was recorded in the past [8,9] and in recent years too [10]. Moreover, from Nainital district in Kumaon region of Uttarakhand, entomological investigations during an outbreak of Dengue fever in Lal Kuan town revealed larval and adult stages of *Ae. aegypti* and *Ae. albopictus* in transmission season [11].

Ae. aegypti is an urban mosquito that breed almost entirely in man-made containers (cistern, flower pots, tanks, tyres and cans) found in and around

households, construction sites, factories etc. On the contrary, *Ae. albopictus* breeds in both man-made containers as well as in natural containers such as bamboo, tree holes and leaf axils. Ovitrap surveillance is the most common sampling method to monitor *Ae. aegypti* and *Ae. albopictus* populations through their egg laying activities [12]. It has been claimed to be a more effective and sensitive technique as compared to the conventional larval surveys, especially when the *Aedes* infestation rates were very low [13].

Keeping in view that for the last 3-4 years, on one hand there is an increase in Dengue cases in Dehradun (India) while on the other hand, lack of information in bionomics of *Aedes* sp. involved in Dengue transmission, it was decided to determine the

efficacy of ovitraps in monitoring the distribution and abundance of *Aedes* species in different urban and suburban areas of district Dehradun in Uttarakhand state, located in the northern India.

II. METHODS

a) Study Area

The present study was carried out mainly in urban area of Doon Valley (latitude 30° 19'N, 78° 04'E, longitude 77°35'E to 78°20'E) in district Dehradun (Uttarakhand). Ovitrap surveillance was conducted at three sites of Dehradun city: Sahastradhara, Garhi Cantt. and Karanpur from August 2012 - July 2013. The ecological description of the study sites is being provided as under –

Table 1 : Ecological description of the study site

Study site	Ecological description
Sahastradhara	Abundant natural vegetation like trees and shrubs, clean environment and mainly two-storied newly made buildings.
Garhi Cantt.	Lush green vegetation, environment is clean and in general newly made buildings exist.
Karanpur	Less vegetation, environment partly clean and highly populated and both old and new buildings are common.

b) Ovitrap surveillance

Each ovitrap was placed indoor and outdoor in randomly selected houses scattered over the study area. The paddles were collected individually from the ovitraps on weekly basis. Thereafter, fresh paddles were put in the ovitraps jar and the water level was adjusted so that they would remain moist. Collected paddles were submerged into a bowl of water containing larval food. The hatched larvae were subsequently counted and reared in the cages to emerge into adults. The adults were identified upto species level using respective Keys and Catalogues [14,15,16]. The estimation of ovitrap indices was done following the protocols developed [17,18]. The following indices were work out:-

- i. *Ovitrap Index (OI)*: The percentage of *Aedes* positive trap.
- ii. *Area Ovitrap Index (AOI)*: Calculating the extensiveness of the distribution of the *Aedes* mosquitoes in a particular area.
- iii. *Monthly Ovitrap Index (MOI)*: Monthwise *Aedes* positive trap (average of all AOIs).

III. RESULTS

During the study period, 20 ovitraps (10 indoors and 10 outdoors) were installed for each week in each locality and observed the ovitrap index on monthly basis (Table 2). Maximum index was encountered from Garhi

Cantt (48.75) followed by Karanpur (45.00) and Sahastradhara (43.75) during the month of August. During January and February, the breeding index was found nil. In all selected localities, the ovitrap indices were high during June to September. The mean indices were 18.65, 15.63 and 15.94 at Sahastradhara, Garhi Cantt and Karanpur respectively.

Fig.1 shows the Area Ovitrap Index (AOI) of the selected sites during the study period. The outdoor indices were in the range of 17.30±1.83 - 21.88±2.10. Highest index was found at Sahastradhara (21.88±2.10) followed by Karanpur (19.58±1.94) and Garhi Cantt (17.30±1.83). All the indoor ovitraps showed low index in all three localities in comparison to outdoor ovitraps (12.30±1.67- 15.42±1.56).

Fig. 2 shows the MOI of the study period ranging from 0.00 to 45.83. Highest MOI was found during August (45.83) followed by July (36.66) and September (30.41). During the winter months like January and February, the index was recorded nil.

Fig. 3 shows the composition of *Aedes* mosquitoes in ovitraps at selected sites. A total of 6 *Aedes* species viz., *Aedes aegypti*, *Ae. albopictus*, *Ae. edwardsi*, *Ae. pseudotaeniatus*, *Ae. unilineatus* and *Ae. vitattus*. were collected. Of these, *Ae. aegypti* shared highest (37.28%) followed by *Ae. albopictus* (33.27%), *Ae. pseudotaeniatus*, (15.68%) and *Ae. vitattus* (8.33%). The mixed breeding comprising larvae of *Culex*, *Anopheles* and some unidentified species shared 3.10%

only. In indoor, overall mosquito accounts low percentage (48.2%) in all three localities as compared to outdoor percentage (51.8%).

IV. DISCUSSION & CONCLUSION

Owing to inherent human behaviour and some traditional habits, detection of the presence of different mosquito vectors in urban situations has been a difficult task. It has been observed that the vector species are common in most areas on account of deficient water management, presence of non degradable and long-lasting water holding containers and materials, as well as increasing urban agglomerations and inability or lack of mobilization to the population to the need to eliminate mosquito breeding sites. In a study conducted on dengue vector surveillance at Malaysia, the mosquito abundance was found related to population and human activity [19]. Occurrence of positive ovitraps in sampled houses positive ovitraps is an indication of human activity that provides a suitable environment for the propagation of these vector species in the residential area.

Earlier, it was stated that *Ae. aegypti* is strictly domiciliary, preferring less vegetation, biting indoors and primarily found indoors, while *Ae. albopictus* is found outdoors and breeds in all types of natural containers [20,21]. In these aspects, there is a bit similarity with the results of our study.

Dengue is a disease associated with the slum areas, where breeding of *Aedes* mosquitoes is most prevalent [22]. However, the ovitrap surveillance in the selected areas showed that *Aedes* mosquitoes are not only associated with the slum areas, but they are also associated with the residential area. As per the gathered observations, the settlement site had numerous natural and artificial containers providing good larval habitats. But the residential sites had a clean environment, with minimal natural containers. As all the houses had piped water supply, thus there was no necessity for the residents to store water. From our observations, the residential sites had minimal natural containers. The only possible habitat for *Aedes* mosquitoes was the concrete drainage system outside the houses. The drains had clear stagnant water with fallen leaves and other debris. *Aedes* larvae require clear, but not necessarily clean water and this was provided by the clear stagnant clear water of the drain [23,24]. In this way the drains served as good artificial larval containers for *Ae. aegypti*.

In the past, it was found out that *Ae. aegypti* rests in secluded locations inside homes such as under beds, in closets and on curtains [25]. In contrast, *Ae. albopictus* which breeds in both man-made containers such as cans, tires and water jars; as well as natural containers such as bamboo, bromeliads and coconut shells is more cosmopolitan in its feeding habitats and

rests both inside and outside homes, making control difficult.

Aedes population has been observed in the ovitraps in both indoor and outdoor placement in urban residential sites, through the positivity of ovitraps was more in outdoor than indoor [26], thus resembling with our studies. Further, similar results were obtained in a study on surveillance of *Aedes* mosquitoes in a University Camps in Kuala Lumpur [27]. This may be due to availability of natural potential breeding sites such as bamboo tree, tree holes and mudden broken containers in outdoor environment.

Conclusively, the prevalence of a high density of dengue vectors in an urban area inspires an intensification of the vector surveillance activities jointly with community participation.

V. ACKNOWLEDGEMENTS

Authors are thankful to University Grants Commission (UGC) and Council of Scientific & Industrial Research (CSIR), New Delhi, India for financial support and to Dr. J.P. Bahuguna, Distt. Vector Borne Disease Control Officer, Dehradun (Uttarakhand) for Co-operation.

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Table 2 : Month-wise statement about ovitraps installed at selected localities in district Dehradun during Aug 2012- July 2013.

Months	Sahastradhara		Garhi Cantt.		Karanpur	
	Ovitraps		Ovitraps		Ovitraps	
	Installed	Positive index	Installed	Positive index	Installed	Positive index
Aug. 12	20/ turn	43.75	20/ turn	48.75	20/ turn	45.00
Sept. 12	20/ turn	27.50	20/ turn	33.75	20/ turn	30.00
Oct. 12	20/ turn	26.25	20/ turn	16.25	20/ turn	23.75
Nov. 12	20/ turn	16.25	20/ turn	7.50	20/ turn	5.00
Dec. 12	20/ turn	1.25	20/ turn	2.50	20/ turn	1.25
Jan. 13	20/ turn	0.00	20/ turn	0.00	20/ turn	0.00
Feb. 13	20/ turn	0.00	20/ turn	0.00	20/ turn	0.00
Mar. 13	20/ turn	1.25	20/ turn	1.25	20/ turn	1.25
Apr. 13	20/ turn	16.25	20/ turn	10.00	20/ turn	8.75
May 13	20/ turn	21.25	20/ turn	16.25	20/ turn	17.50
Jun. 13	20/ turn	27.50	20/ turn	20.00	20/ turn	21.25
July 13	20/ turn	42.50	20/ turn	31.25	20/ turn	36.25

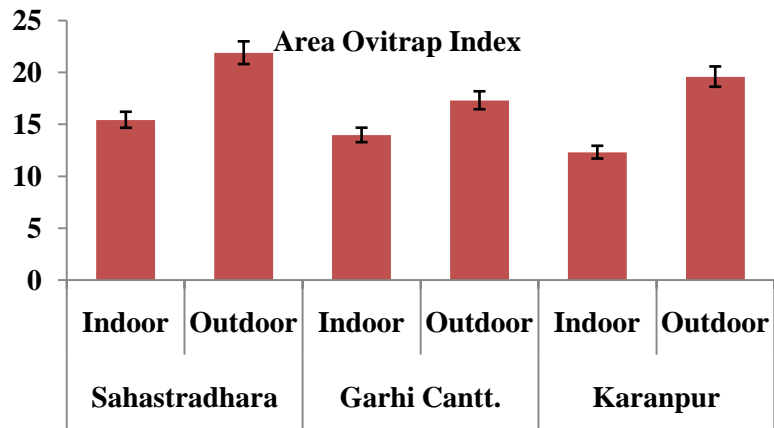


Figure 1 : Showing Area Ovitrap Index (AOI) at three localities in district Dehradun.

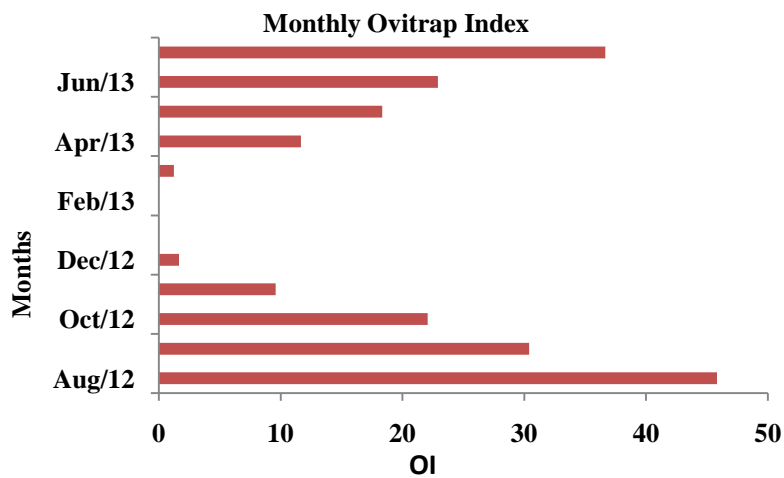


Figure 2 : Showing Monthly Ovitrap Index (MOI) at selected localities in district Dehradun during Aug 2012- July 2013.

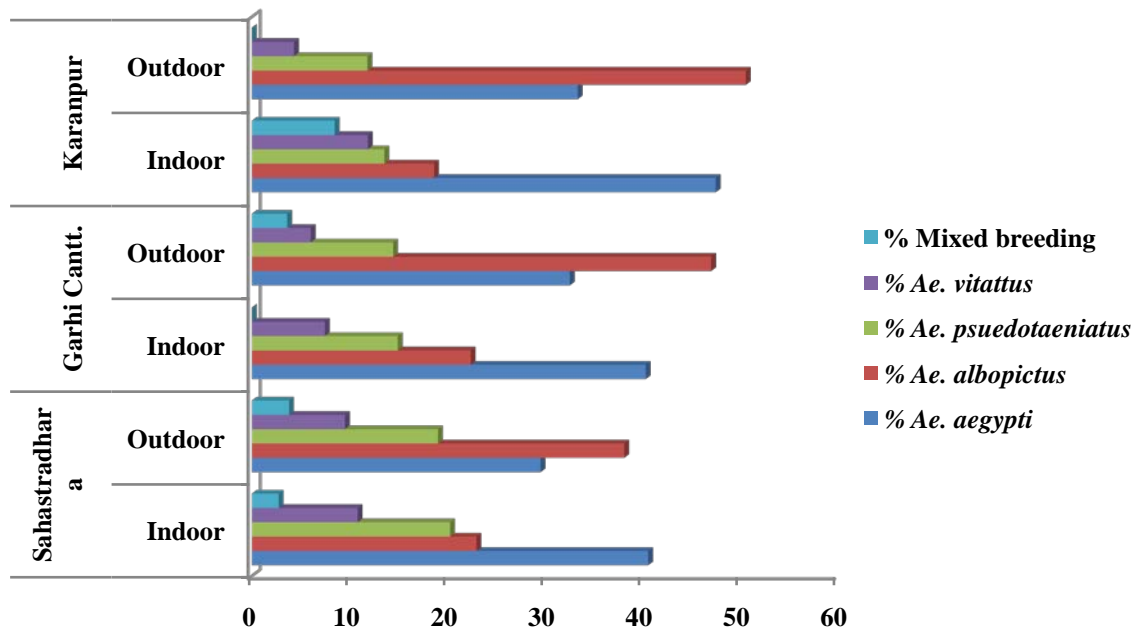


Figure 3 : Composition of *Aedes* population in ovitraps at selected localities in district Dehradun during Aug 2012- July 2013.

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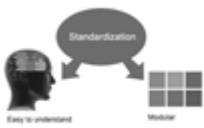
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- (b) A brief Summary, "Abstract" (less than 150 words) containing the major results and conclusions.
- (c) Up to ten keywords, that precisely identifies the paper's subject, purpose, and focus.
- (d) An Introduction, giving necessary background excluding subheadings; objectives must be clearly declared.
- (e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition; sources of information must be given and numerical methods must be specified by reference, unless non-standard.
- (f) Results should be presented concisely, by well-designed tables and/or figures; the same data may not be used in both; suitable statistical data should be given. All data must be obtained with attention to numerical detail in the planning stage. As reproduced design has been recognized to be important to experiments for a considerable time, the Editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned un-refereed;
- (g) Discussion should cover the implications and consequences, not just recapitulating the results; conclusions should be summarizing.
- (h) Brief Acknowledgements.
- (i) References in the proper form.

Authors should very cautiously consider the preparation of papers to ensure that they communicate efficiently. Papers are much more likely to be accepted, if they are cautiously designed and laid out, contain few or no errors, are summarizing, and be conventional to the approach and instructions. They will in addition, be published with much less delays than those that require much technical and editorial correction.



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It is vital, that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

Format

Language: The language of publication is UK English. Authors, for whom English is a second language, must have their manuscript efficiently edited by an English-speaking person before submission to make sure that, the English is of high excellence. It is preferable, that manuscripts should be professionally edited.

Standard Usage, Abbreviations, and Units: Spelling and hyphenation should be conventional to The Concise Oxford English Dictionary. Statistics and measurements should at all times be given in figures, e.g. 16 min, except for when the number begins a sentence. When the number does not refer to a unit of measurement it should be spelt in full unless, it is 160 or greater.

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Key Words

A major linchpin in research work for the writing research paper is the keyword search, which one will employ to find both library and Internet resources.

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Search engines for most searches, use Boolean searching, which is somewhat different from Internet searches. The Boolean search uses "operators," words (and, or, not, and near) that enable you to expand or narrow your affords. Tips for research paper while preparing research paper are very helpful guideline of research paper.

Choice of key words is first tool of tips to write research paper. Research paper writing is an art. A few tips for deciding as strategically as possible about keyword search:



- One should start brainstorming lists of possible keywords before even begin searching. Think about the most important concepts related to research work. Ask, "What words would a source have to include to be truly valuable in research paper?" Then consider synonyms for the important words.
- It may take the discovery of only one relevant paper to let steer in the right keyword direction because in most databases, the keywords under which a research paper is abstracted are listed with the paper.
- One should avoid outdated words.

Keywords are the key that opens a door to research work sources. Keyword searching is an art in which researcher's skills are bound to improve with experience and time.

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Acknowledgements: Please make these as concise as possible.

References

References follow the Harvard scheme of referencing. References in the text should cite the authors' names followed by the time of their publication, unless there are three or more authors when simply the first author's name is quoted followed by et al. unpublished work has to only be cited where necessary, and only in the text. Copies of references in press in other journals have to be supplied with submitted typescripts. It is necessary that all citations and references be carefully checked before submission, as mistakes or omissions will cause delays.

References to information on the World Wide Web can be given, but only if the information is available without charge to readers on an official site. Wikipedia and Similar websites are not allowed where anyone can change the information. Authors will be asked to make available electronic copies of the cited information for inclusion on the Global Journals Inc. (US) homepage at the judgment of the Editorial Board.

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12. Make all efforts: Make all efforts to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in introduction, that what is the need of a particular research paper. Polish your work by good skill of writing and always give an evaluator, what he wants.

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21. Arrangement of information: Each section of the main body should start with an opening sentence and there should be a changeover at the end of the section. Give only valid and powerful arguments to your topic. You may also maintain your arguments with records.

22. Never start in last minute: Always start at right time and give enough time to research work. Leaving everything to the last minute will degrade your paper and spoil your work.

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24. Never copy others' work: Never copy others' work and give it your name because if evaluator has seen it anywhere you will be in trouble.

25. Take proper rest and food: No matter how many hours you spend for your research activity, if you are not taking care of your health then all your efforts will be in vain. For a quality research, study is must, and this can be done by taking proper rest and food.

26. Go for seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.



27. Refresh your mind after intervals: Try to give rest to your mind by listening to soft music or by sleeping in intervals. This will also improve your memory.

28. Make colleagues: Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

29. Think technically: Always think technically. If anything happens, then search its reasons, its benefits, and demerits.

30. Think and then print: When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

31. Adding unnecessary information: Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

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33. Report concluded results: Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

34. After conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium through which your research is going to be in print to the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects in your research.

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- Fundamental goal
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- Significant conclusions or questions that track from the research(es)

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The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently. You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.



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- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
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Approach

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- Give details all of your remarks as much as possible, focus on mechanisms.
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Approach:

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