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Rat Suprachiasmatic Nucleus

Completion of Hepatitis B Vaccine

Highlights

Repetition of Parent-Adolescent

Diabetes and Metabolic Syndrome

Discovering Thoughts, Inventing Future

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Factors Associated with the Completion of Hepatitis B Vaccine among University Students in Nigeria

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Abstract- Hepatitis B virus (HBV) has been identified by WHO as one of the deadliest viral diseases in Africa due to its high prevalence and complications. Thus urgent research and policy attention is needed to stem its spread. The study assessed factors associated with the completion of hepatitis B vaccine among Babcock University students, Nigeria. Multistage sampling procedure was employed to select 360 students to participate in the study after completing all requirements for ethical clearance. Data gathered from the respondents were subjected to descriptive analysis using the Statistical Package for Social Sciences (SPSS Ver. 17). Most of the respondents are adults above 20 years and were aware of the HBV (89%). Despite serious efforts made by the University at educating the students about Hepatitis B, more than 40 % of the students still do not know the causative organism and are totally oblivious of the importance of vaccination. Also, 73% of the students had not completed the recommended 3 dosage vaccines. The findings also showed that despite the high level of awareness up to 44 percent of the students were not sure of their status or have not been previously immunized, indicating that their knowledge did not motivate their actions or practice.

Keywords: hepatitis B, immunization, student, babcock.

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Abstract- Hepatitis B virus (HBV) has been identified by WHO as one of the deadliest viral diseases in Africa due to its high prevalence and complications. Thus urgent research and policy attention is needed to stem its spread. The study assessed factors associated with the completion of hepatitis B vaccine among Babcock University students, Nigeria. Multistage sampling procedure was employed to select 360 students to participate in the study after completing all requirements for ethical clearance. Data gathered from the respondents were subjected to descriptive analysis using the Statistical Package for Social Sciences (SPSS Ver. 17). Most of the respondents are adults above 20 years and were aware of the HBV (89%). Despite serious efforts made by the University at educating the students about Hepatitis B, more than 40 % of the students still do not know the causative organism and are totally oblivious of the importance of vaccination. Also, 73% of the students had not completed the recommended 3 dosage vaccines. The findings also showed that despite the high level of awareness up to 44 percent of the students were not sure of their status or have not been previously immunized, indicating that their knowledge did not motivate their actions or practice. Factors which positively influenced completion of immunization dosage were the students' perceived need to be protected from HBV, the availability of vaccine, their knowledge of the fact that the monetary cost of vaccine had already been included in the school bills, their opinion that immunization is part of the criteria for complete registration, their knowledge of the effectiveness of the vaccine to protect against HBV, and their perceived susceptibility to HBV infection. Conversely, factors constraining respondents' completion of immunization dosage were their forgetting the date for next appointment, their perception that the vaccine was not necessary, their lack of knowledge about the total number of doses to be taken, their lack of knowledge or inability to remember having been given a vaccine for HBV and perceived fear of possible side effect of the vaccine. There is the need for the institution to re-work the awareness education for Hepatitis B to strongly highlight the need for complete dosage vaccination in order to prevent infection.

Keywords: hepatitis B, immunization, student, babcock.

I. Introduction

Hepatitis is ranked the world's eight biggest killers and one of the leading public health problems (Auduand Ujah, 2014).

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It is one of the world's most common and severe infectious diseases. Statistics according to the World Health Organisation (2014) showed that 3-6% of the world's population suffer from Hepatitis B virus, 2 billion people have been infected, and 400 million individuals are chronic carriers of the Hepatitis B virus (HBV) with 350million new cases and above one million people die each year as a result of Hepatitis B virus. Hepatitis B virus is one of the viruses that cause viral hepatitis; it is the most dreaded hepatitisvirus amongst the entire currently identified hepatitis viruses (WHO, 2014). Samuel, Aderibigbe, Salami and Babatunde (2009) stated that Hepatitis B virus has a high prevalence in sub-Saharan Africa and East Asia. Approximately 5-10% of the adult population in these regions are chronically infected with the HBV (Samuel et al., 2009). This has great social and economic implications.

Hepatitis B virus has been identified by WHO (2014) as one of the deadliest viral diseases in Africa due to its high prevalence and complications such as liver cancer, cirrhosis and hepatocellular carcinoma. This has made research into preventing it a major public health issue of global concern. Over three quarters of all the cases of HBV globally occurs in Asia, Middle East and Africa. The prevalence of HBV varies between countries even in the same continent (Samuel *et al.,* 2009).

Nigeria is one of the countries in Africa with high prevalence of hepatitis B virus (Owolabi and Ojo, 2008). According to the Nigeria Institute of Medical Research (NIMR, 2014), above twenty million Nigerians are currently living with Hepatitis B virus. This statistics, which is about 10-40% prevalence level, is said to have reached a hyper endemic level. This means that in every 10 Nigerians, 1-4 of them will test positive to HBV. Yet there is tendency for this figure to rise in the future if preventive measures are not put in place (Audu and Ujah, 2014).

According to Kesieme et al (2011), Hepatitis B virus requires less exposure to cause an infection compared to other blood borne pathogens. They compared the risk of getting an infection when exposed to various blood-borne pathogens from an infected needle prick injury. The result indicated that the risk for being infected with Hepatitis B virus was 30%, 3% for

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contracting Hepatitis C virus and 0.3% for HIV infection per 1,000 respective exposures.

Vaccination and avoiding contact with infected blood has been proven to be the most efficient ways of preventing HBV infection / transmission (WHO, 2014). The World Health Organisation has recommended that the Hepatitis B vaccine should be included in the immunization schedule /services rendered (WHO, 2014). Furthermore, it was recommended by the Advisory Committee on Immunization Practices (ACIP) that children should be given their first dose of Hepatitis B vaccine at birth, followed by a second dose at six months and the third dose at eighteen months (Centre for Disease Control & prevention [CDC], 2014). The vaccine has been made available in the global market since 1982(Hepatitis B Foundation, 2013).This has brought about significant drop in the infection cases in many countries. According to WHO (2014), the completion of the vaccine series will induce protective antibody in more that 95% of children, infants and young adults in which the protection can last for a minimum of 20 years and possibly lifelong. It was also stated that, in countries were 8-15% of their children previously used to become chronically infected with HBV, the introduction of the vaccine has drastically reduced the rate of chronic infection to less than 1% among immunized children (WHO, 2014). Paediatric Association of Nigeria in 2012 identified that as at 2004, Nigeria included the Hepatitis B vaccine into the country's immunization schedule, which is twenty-two years after it was made available in the global market (1982). This implies that individuals born before the year 2004 or those that could not afford to pay for the immunization are at risk of HBV infection posing a great threat to themselves and others, (Sadoh and Eregie,2009).

The doses recommended for adolescents and adults comes in a total of three doses as listed

- First dose - At any given time
- Second dose - At least one to two month after the first dose
- Third dose - Six months after the first dose

Various researches carried out on the acceptance and completion of Hepatitis B vaccination showed that there is generally poor acceptance and completion of Hepatitis B vaccination in the developing countries (Peterside et al., 2012; Azodo et al., 2011). In Nigeria, some studies have showed low rate of vaccination against HBV, even below 30%, and generally poor completion culture (Samuel et al., 2009; Kesieme et al., 2011). Even among health worker, who are expected to have higher awareness, the situation is not so different. In Edo state, a research conducted among 320 consenting resident doctors revealed that 50.6% received the hepatitis B vaccine, 49.4% did not receive the vaccine, 71.6% completed the vaccine doses and 28.4% did not complete the dose (Peterside et al., 2012).

As a result of under reporting and poor record keeping in Nigeria, there is a limited/ inadequate access to the appropriate incidence and prevalence of HBV in Nigeria, especially among young adults in the tertiary institutions. It was also reported by Charles and Jay (2012) that with regards to foreign support, there is no defined focus for the hepatitis in Nigeria. Shefurther added that it has not been a major priority for the foreign donor agencies; therefore, Nigeria is faced with the challenge for designing a local model to fight Hepatitis B which will require raising awareness and assessing factors determining receiving and completing the HBV vaccination as highlighted in this paper.

Vaccine against Hepatitis B virus infection has been made available in Nigeria and this should make administration possible to students of various institutions including Babcock University, (which has been selected for this study), during the registration process. According to observations and preliminary survey, majority of the Babcock University students do not return to the Babcock University Teaching Hospital (BUTH) to complete the vaccination (second and third doses) despite the fact that payment has been made for complete doses, and vaccines are also available. This is a challenging situation to health of the general public. These practices make the students susceptible to HBV infection, transmitters of this virus without knowing, and potential patients of liver cancer and so on.

It is against this backdrop that this research was aimed at identifying the various factors associated with the completion of Hepatitis B vaccine among undergraduate students of Babcock University.

II. Methodology

The study was carried out among undergraduate students of Babcock University Ilishan-Remo, Ogun state. Babcock University began anoperation as one of the first private Universities in Nigeria in 1999 and since then students' population has grown steadily. Sampled students were from 300 to 500 levels. A total of 3,485 students were in this group within the period of the survey (with respective population of 1,516[43.5%], 1,640[47.1%] and 329[9.4%] students respectively in each level). The multistage sampling technique was used in the determining sample size. The first stage was the stratification of the University into the 6 existing schools, followed by further stratification into the levels highlighted earlier and finally students that participated were randomly selected from each level (proportionate to population).

Following Yamane (1967), the sample size was calculated from a population of 3, 458 students:

$$n = N/1+ \{N (e^2)\} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where: n=minimum sample size, N=total number of population (which was 3485 for this study) and e=level of precision (e=0.05) which is constant.

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Thus, the estimated minimum sample size was 358 respondents which was approximated to 360. The questionnaire used was subjected to both validity and reliability tests. A Cronbach's alpha of above 0.74 was obtained for all constructs.

Data gathered included demographic, knowledge on Hepatitis B vaccination and Students perception towards Hepatitis B vaccination and completion data. Information collected were analyzed and presented using descriptive and inferential statistics. The Statistical package for social sciences (15th editions) was used for all data analysis. Ethical clearance was obtained from the Babcock University Health Research Ethics Committee before commencement of the survey. Furthermore, informed consent forms were also attached to the questionnaires where the respondents indicated their willingness to participate in the study by signing after reading the terms and conditions that applied to the study.

III. Results Presentation

a) Demographic data of respondents

The demographic characteristics of the respondents are presented in Table 1. The results revealed that majority (72 %) falls within the age bracket of 20-24 years. Almost half (51%) of the respondents are males while 49% of the respondents are females. The result also revealed that 98% of the students are single. Majority (79%) of the respondents are Christians.

b) Knowledge of respondents

Results in figure 1 shows that majority of students (89%) have heard of Hepatitis B while 11 percent (40 students) indicated that they have not heard of Hepatitis B before.

Further result in Table 2 revealed that the major source of information about Hepatitis were mostly from University health officials (45.6%) usually during orientations and seminars. More than half of the respondents (57.7 percent) were aware that Hepatitis B is a viral disease while up to 42.3 percent did not know that Hepatitis B is a viral disease.

Table 1 : Demographic characteristics of respondents

Characteristics	Categories	Frequency (n=360)	Percentage
Age (years)	15-19yrs	69	19.2
	20-24yrs	260	72.2
	25-29yrs	26	7.2
	30 and above	5	1.4
Sex	Males	185	51.3
	Females	175	48.7
Marital status			
	Single	349	98.0
	Married	10	2.8
Religion	Christian	283	78.7
	Islam	70	19.4
	Traditional	7	.9.1
Level	300 level	156	43.3
	400 level	170	47.2
	500 level	34	9.5

Source: Field survey, 2015

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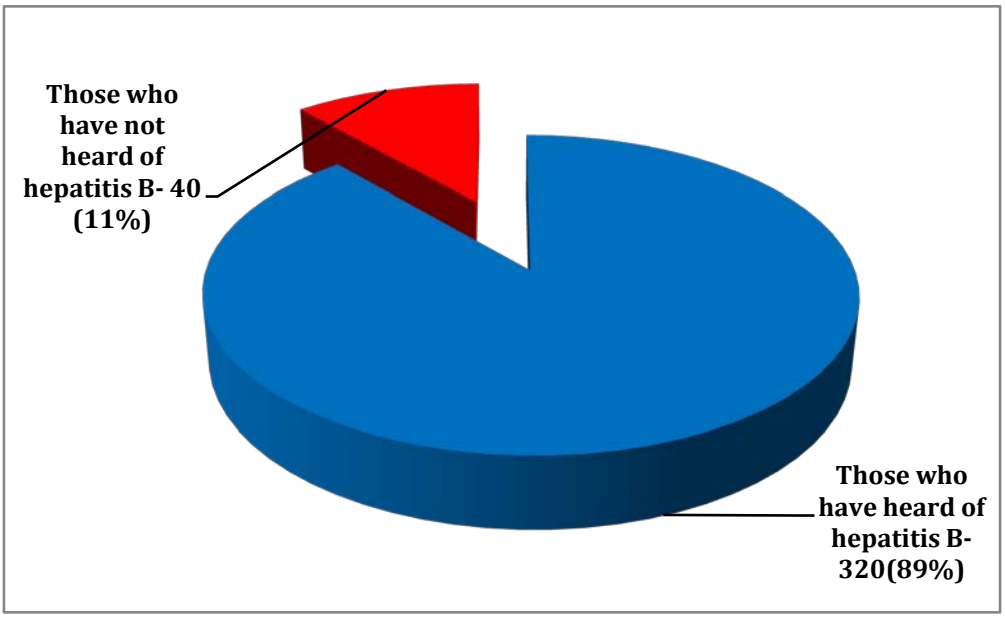

Fig. 1 : Those who have or have not heard about Hepatitis B

Also, 59.7 percent of the respondents knew the signs and symptoms of Hepatitis B while about 40 percent did not know the sign and symptoms. Three quarters (75 percent) of the respondents were aware of the vaccine for preventing HBV. However, up to 25 percent of the respondents were not aware. Some (49percent) of the respondents had been immunized in the school teaching hospital (BUTH) while some (7percent) claimed to have been immunized from other teaching hospitals and medical centres outside the University. It was observed that 56 percent of the respondents indicated to have been immunized against HBV while 26 percent have not been immunized and 18percent were not sure of having been immunized.

Further results showed that about 42 percent and 39 percent of the respondents indicated that Hepatitis B is being transmitted through sex and blood contact respectively. Though 32 percent did not have any knowledge of ways of transmission of Hepatitis B, about 34 percent and 29 percent respondents thought that kissing and sweat are ways of transmission of this infection. Result also showed that most (57 percent) of the respondents did not know the appropriate dosage for complete vaccination; however, about a quarter (27 percent) indicated the correct dosage of 3 doses. Other respondents (7 percent, 5 percent and 4 percent) indicated 6 doses, 2 doses and 1 dose respectively.

Table 2 : Responses on different aspect of knowledge about Hepatitis B

Variables	Categories	Freq	%
Source of information about Hepatitis	Parents	46	14.4
	Friends	38	11.9
	Health officials	146	45.6
	Media	71	22.2
	Others	19	5.9
Respondents' indication of what Hepatitis B is.	Viral disease	208	57.7
	Bacteria disease	25	7.0
	Fungi disease	5	1.3
	Protozoa disease	0	0
	Do not know	122	34.0
Hepatitis B has signs and symptoms	Yes	215	59.7
	No	24	6.7
	Do not know	121	33.6
There is a vaccine to prevent HBV	Yes	270	75.0
	No	1	.3
	Do not know	89	24.7
Location and status of immunization against	BUTH	176	49.0

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HBV	Other health facilities have not been immunized	24	7.0
		95	26.0
	Not sure of having been immunized.	65	18.0
Mode of transmitting HBV	Sexually transmitted	151	42
	Blood contact	141	39
	Through Kissing	123	34
	Through sweat	104	29
	Not sure	115	32
Knowledge about the complete vaccination	3 doses	97	27
	6 doses	25	7
	2 doses	18	5
	1 dose	15	4
	Not sure	205	57

Source: Field survey, 2015

c) The total number of doses taken by respondents

Results in Figure 2 showed that the majority (43percent) of the respondents did not know (cannot recall) the total number of doses they have taken. Some (30percent) have not taken the complete dose and only 27percent have taken the complete 3 dosage.

Further descriptive result in Table 3 reveals the knowledge of respondents concerning the appropriate doses of vaccination *vis-a-vis* the number of doses they have taken so far. Majority (51 percent) of the respondents did not know the actual number of doses received. A unique pattern was observed in which the total number of doses received by the respondents seemed to be anchored on the knowledge about the assumed correct number of doses for the vaccine. Results showed that the majority of respondents who indicated 1 dose (40 percent), 2 doses (73.3percent) and 3 doses (61 percent) as appropriate number are found more in the categories of 1, 2 and 3 doses of total doses taken respectively.

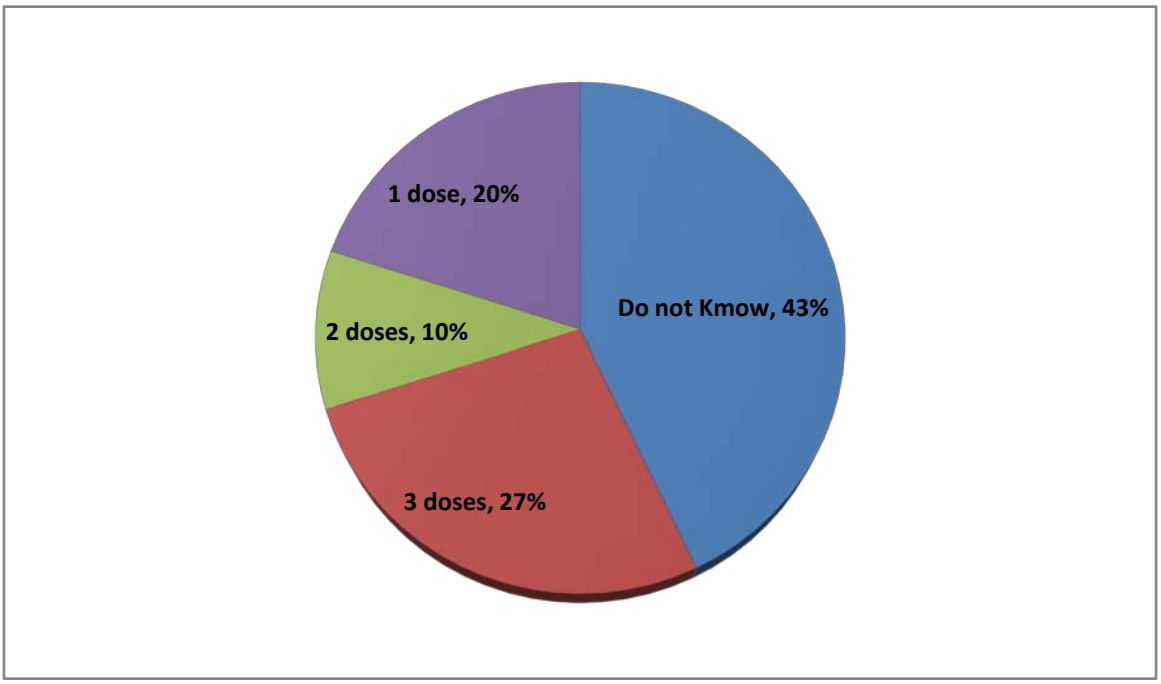

Figure 2 : The total number of doses taken by respondents

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Table 3 : A cross tabulation of knowledge about appropriate doses and total doses taken by respondents

		Knowledge about the right complete doses for vaccination					Total
		1 dose	2 doses	3 doses*	6 doses	Do not know	
Total number of doses taken by respondents	1 dose	5	2	20	7	42	76
		40.0%	6.7%	24.6%	31.6%	30.2%	27.5%
	2 doses	0	12	10	9	5	36
		.0%	73.3%	11.6%	36.8%	4.3%	13.5%
	3 doses	0	0	50	3	1	54
		.0%	.0%	60.9%	15.8%	.9%	20.1%
	6 doses	0	1	0	4	2	7
		.0%	6.7%	.0%	15.8%	.9%	2.2%
	Do not know	7	3	2	0	89	101
		60.0%	13.3%	2.9%	.0%	63.8%	36.7%
	Total	12	18	82	23	139	274
		100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

3 doses* = 3 doses are the recommended doses for complete HB vaccination.

d) Respondents' perception towards Hepatitis B vaccination

Results in Table 4 showed that 98.3 percent of the respondents agreed that healthy people need to be vaccinated against HBV. Most of the respondents (97 %) agreed that it is appropriate for them to be vaccinated with Hepatitis B vaccine at their age. Most of the respondents (98%) perceived that it is imperative to complete Hepatitis B vaccine dosage and majority (97.7 percent) disagreed that Hepatitis B vaccination is meant for only medical personnel or workers.

e) Factors influencing vaccination of students

The result of respondents' factors influencing completion of vaccination dosage for Hepatitis B is as presented in Table 5. Reasons given by the majority of those who have completed the immunization dosage, for completing the doses recommended included their perceived need to be protected from hepatitis B virus (54 percent); availability of vaccine (52 percent); knowledge of the fact that the monetary cost of vaccine had already been included in the school fees (63 percent); immunization is part of the criteria for complete registration (52percent); knowledge of the effectiveness of the vaccine to protect against HBV (61 percent) and perceived susceptibility to HBV infection (53 percent).

Prominent reasons, by those who have not completed the vaccination dosage, for not completing the doses recommended included forgetting the date for next appointment as indicated by 67 percent of the respondents; perception that the vaccine is not necessary (55%); lack of knowledge about the total number of doses to be taken (59%); lack of knowledge or cannot remember having been given a vaccine for HBV (60%) and fear of possible side effects of the vaccine (57%).

Table 4 : Respondent's perception towards the completion of Hepatitis B vaccination

Perception	Agree		Disagree	
	N	%	N	%
Healthy people need to be vaccinated against HBV	354	98.3	6	1.7
I need to be vaccinated with Hepatitis B vaccine at your age	350	97.3	10	2.7
Hepatitis B vaccination is meant for only medical doctors and nurses	26	7.3	334	92.7
Individual should receive the Hepatitis B vaccine	350	97.3	10	2.7
Individuals should complete the doses of the vaccine	352	97.7	8	2.3

Source: Field survey, 2015

Table : Factors influencing respondents' completion of Hepatitis B vaccination dosage

†Factors	Frequency	%
Factors positively influencing completion (n = 97):		
perceived need to be protected from hepatitis B virus	52	54
availability of vaccine	51	52
knowledge of the fact that the monetary cost of vaccine had already been	61	63

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included in the school fees		
immunization is part of the criteria for complete registration	51	52
knowledge of the effectiveness of the vaccine to protect against HBV	59	61
perceived susceptibility to HBV infection	52	53
Factors negatively influencing completion (n= 263):		
forgetting the date for next appointment	176	67
perception that the vaccine is not necessary	145	55
lack of knowledge about the total number of doses to be taken	155	59
lack of knowledge or cannot remember having been given a vaccine for HBV	158	60
fear of possible side effect of the vaccine	150	57

†multiple response available

Source: Field survey, 2015

IV. Discussion of Results

Most of the respondents are adults and mature enough to take informed decisions regarding health issues. Most of the students are aware of the HBV, especially those who commenced the school from first year, because it is part of the awareness program given to the students during their first year orientation. This result is similar to the conclusion of Dahlström *et al* (2013). Despite the vigorous effort of the University at educating the students about Hepatitis B, some of the students still do not know the causative organism and are totally oblivious of the importance of vaccination. Usually, people do not take health education seriously until they are down with one form of sickness or the other. Irrespective of the students' individual knowledge or attitude towards Hepatitis B, as part of registration requirement, the entire students who have not hitherto been immunized, were encouraged to take the first dose of the vaccination in their first year. Those who have not been immunized are those who either refused the immunization or enrolled in the school at upper levels. Since the hepatitis B vaccine was given along with other medical examination tests and vaccinations, some of the students whose responses showed poor knowledge of HBV and HB vaccination, probably did not pay attention to the details of their medical procedures and probably did not follow up with the subsequent doses of vaccination for Hepatitis B. The findings also showed that despite the high level of awareness, up to 44 percent of the students were not sure of their status or have not been previously immunized, indicating that their knowledge did not motivate action or practice. Further findings showed that most of the students did not know the appropriate dosage for complete Hepatitis B vaccination and in fact had not taken the complete 3 dosage according to WHO recommendation (WHO, 2012), irrespective of the fact that most of them perceived that immunization is good and complete dosage must be taken. This result clearly showed the need for intervention programme to align the knowledge, perception and practice of the students in the study area with standards regarding Hepatitis B immunization.

Factors which positively influenced completion of immunization dosage were the students' perceived need to be protected from hepatitis B virus, the availability of vaccine, their knowledge of the fact that the monetary cost of vaccine had already been included in the school bills, their opinion that immunization is part of the criteria for complete registration, their knowledge of the effectiveness of the vaccine to protect against HBV, and their perceived susceptibility to HBV infection. By implication, increasing or improving on these factors will contribute to enhancing uptake of complete immunization dosage. This result is consistent with the submissions of Pathak et al. (2013). Conversely, factors constraining or negatively influencing respondents' completion of immunization dosage were their forgetting the date for next appointment, their perception that the vaccine was not necessary, their lack of knowledge about the total number of doses to be taken, their lack of knowledge or inability to remember having been given a vaccine for HBV and perceived fear of possible side effects of the vaccine. Amelioration of these factors is expected to increase uptake and completion of immunization for Hepatitis B. The study conducted by Obi et al. (2013) also posited similar results.

V. Conclusion and Recommendation

The study assessed the response in terms of knowledge and perception of students in Babcock University towards the Hepatitis B immunization. The factors which influenced completion of immunization dosage were also assessed. Majority of the respondents had heard about Hepatitis B and knew that there is a vaccine which can prevent HBV, yet more than half of them have not completed the dosage. Based on the findings, the following recommendations are made:

1. The result showed discrepancy between knowledge and practice among the students. There is the need for the institution to re-work the awareness education with respect to Hepatitis B and the need for complete dosage vaccination in order to prevent infection.
2. Vaccine given to students and other clients at Babcock University Teaching Hospital (BUTH) should be explained, documented and a card given

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to each client stating the date of the next appointment/dose. Follow-up action can be done in form of text messages that can be sent out to remind all clients/students of the dates of subsequent doses.

3. Evidence of complete vaccination can be part of higher level registration requirement to motivate completion.

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Immunohistochemical Localization of Hyperpolarization Activated Current Channel Subunits in Rat Suprachiasmatic Nucleus

By Nermien E. Waly & Richard Hallworth
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Abstract- *Background:* The hyperpolarization-activated current (I_h) is a mixed Na^+/K^+ inward current that is believed to regulate a wide variety of physiological function in both the central nervous system (CNS) and in the heart. The mammalian I_h is encoded by four members of the Hyperpolarization-activated and Cyclic Nucleotide-gated non-selective cation channel (HCN) gene family (HCN1-4). There was a conflict in previous reports regarding the fraction of suprachiasmatic nucleus (SCN) neurons that exhibited I_h. We first tested the hypothesis that channel subunits are found within the SCN.

Methods: Using immunohistochemistry, the distribution of HCN1, HCN2, and HCN4 in the SCN was studied. Both HCN1 and HCN2 subunits were present in the SCN but with different patterns of localization. HCN4 was not detected in the SCN. I_h was also recorded from SCN neurons using whole cell voltage clamp.

Results: The results show that I_h is functionally well expressed in SCN neurons. 84% of SCN neurons exhibited I_h. I_h recorded had the activation constant (τ) of 236±2 ms and amplitude of 25±1 pA at -40 mV step. At -60 mV step, τ was significantly reduced to 167±3 ms ($p<0.5$) while the current amplitude was significantly increased to 34±2 pA ($p<0.01$).

GJMR-K Classification: *NLMC Code: WL102*

IMMUNOHISTOCHEMICALLOCALIZATIONOFHYPERPOLARIZATIONACTIVATEDCURRENTCHANNELSUBUNITSINRATSUPRACHIASMATICNUCLEUS

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Nermien E. Waly [α] & Richard Hallworth [σ]

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Conclusion: Our results show that I_h plays a major role in the SCN excitability and therefore may regulate its circadian function.

I. Introduction

The suprachiasmatic nucleus (SCN) is the mammalian circadian pacemaker. As a part of the central nervous system (CNS), SCN neurons exhibit different ionic currents e.g. sodium currents, potassium currents, and calcium currents (1, 2). Of particular interest is the hyperpolarization-activated current (I_h), as it has been proposed to play a role in the regulation of spontaneous firing as well as the excitability of SCN neurons (3). This current has been identified in different tissues including the brain and the sino-atrial node (SAN) of the heart (4). Depending on the tissue, the current has different names, e.g. I_f and I_q (5, 6). In pacemaker cells, I_h plays an important role in cell depolarization and the generation of rhythmic activity (7).

I_h is a slowly activating inward mixed Na^+/K^+ current. It activates at hyperpolarizing steps more negative than the resting membrane potential (-50 to -70 mV) and slowly depolarize the cell membrane towards its equilibrium potential (8). This current may be further identified by its sensitivity to Cs^{++}, which is known to substantially block I_h at concentrations of 1-3 mM (8, 9). It has been suggested that the physiological role of this current is to limit the duration of hyperpolarizing events and maintain the membrane potential close to the firing threshold (3). In addition, McCormick and Pape (10) have shown that I_h promotes spontaneous firing in thalamic neurons.

The mammalian Hyperpolarization-activated and Cyclic Nucleotide-gated non-selective cation channels (HCN) that generates I_h is encoded by four members of a gene family (HCN1-4) (11-14), three of which are expressed in rodent hippocampus (HCN1, 2 and 4) (15). Each HCN is composed of six trans-membrane domains with a pore region between S5 and S6 and a cyclic nucleotide-binding domain in the cytoplasmic C-terminal (9, 11, 16). Studies have shown that four HCN subunits can co-assemble to form a homomeric channel with different functional characteristics. For example, homomeric HCN1 activates rapidly (tens of milliseconds) on hyperpolarization and is modulated by c-AMP, while HCN2 is slower to activate (hundreds of milliseconds) with less sensitivity to c-AMP (11, 13). HCN4 homomeric channels show strong modulation by c-AMP and even slower activation (seconds) (12, 14).

There has been inconsistency in the reports regarding the percentage of cells expressing I_h in the SCN (3, 17). Also, while immunohistochemical studies have reported the localization of HCN channel subunits in the brain in general (7, 18, 19), but to date no one has specifically described the pattern of distribution of these subunits in the SCN. Therefore, this study aimed to determine the distribution of HCN channel subunits in the SCN to have a better understanding of the role of I_h in the circadian time keeping function in the SCN.

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II. Methods

a) Animals

Long Evans rats (Charles River, Wilmington MD) 4-6 weeks old were entrained for at least 2 weeks to a 12/12 light/dark (LD) cycle prior to experiments. Animals were housed in groups of 4 rats per cage and provided with rodent chow and water ad libitum.

For immunohistochemistry, rats were anesthetized at circadian time (CT) 10-14 with Nembutal Sodium Solution (100 mg/kg) (Abbott Laboratories, Chicago, IL, USA) then fixed by cardiac perfusion using heparinized saline followed by 4% paraformaldehyde fixative in phosphate buffer saline (PBS). Brains were removed and kept in fixative for at least one hour at room temperature. Brains were then rinsed several times with PBS and kept at 4° C until used. At the time of the experiment brains were further cut into blocks containing the hypothalamus. Coronal slices (thickness 70 μm) containing the SCN were prepared from each brain using a vibrating tissue slicer (Vibratome, TCI, St Louis, MO).

All animal care, handling and sacrifice were in accordance with a protocol approved by the Creighton University Institutional Animal Care and Use Committee.

b) Immunohistochemistry (IHC)

IHC was performed on free-floating sections (70 μM) using standard avidin–biotin complex methods as previously described (20). Coronal brain sections were incubated at 4°C in PBS in 24-well plates (Corning Inc., Corning, NY). Sections were washed several times with 0.3% Triton X-100 (Sigma, St. Louis, MO) in PBS (PBS-T) before treatment with 0.3% H_2O_2/PBS 30 minutes. Non-specific binding sites were blocked using 2% normal goat serum in PBS for 1 hour. After rinsing thoroughly with PBS-T for at least 15 minutes, sections were incubated for about 36 hours at 4°C with rabbit anti HCN1, 2 or 4 polyclonal antibodies (Chemicon International, Temecula, CA) at concentrations of 1:500 or 1:1000 in PBS then washed three times with PBS-T for at least 10 minutes. Sections were then incubated with biotinylated goat anti-rabbit IgG (1:200; Vector Laboratories, Burlingame, CA) for 1 hour. After washing for 10 minutes with PBS-T, sections were incubated for 2 hours in avidin-biotin-peroxidase complex (ABC) solution (1: 100; Vector Laboratories). The reaction product was visualized by incubating the sections with 0.04% 3, 3' diaminobenzidine (DAB) containing 0.01 H_2O_2 for 4 to 5 minutes with PBS. Specimens were visualized using an Axioskop II microscope (Carl Zeiss Jena, Jena, Germany) equipped with 40x and 100x objectives. Negative controls were performed by omission of the primary antibody. Images were obtained using a Spot RT digital camera (Diagnostic Instruments, Sterling Heights, MI). Images were prepared using Adobe Photoshop (Adobe Systems, San Jose, CA).

c) Electrophysiological recording

i. *Hypothalamic slice preparation*

Rats were decapitated quickly after a brief exposure to CO_2 and brains were rapidly removed and cut into blocks containing the hypothalamus. Blocks were chilled on ice-cold artificial cerebrospinal fluid (a-CSF). One 400-500 μM thick slice containing the SCN was prepared from each brain using a vibrating tissue slicer (TCI, St Louis, MO). Slices were prepared as described in Hallworth et al. (21) but in a coronal orientation.

Slices were then incubated in warm a-CSF at room temperature under atmosphere of 95% O_2, 5% CO_2. The a-CSF contained (in mM) NaCl 122, KCl 3.8, $MgSO_4$ 1.2, $KHPO_4$ 1.2, $NaHCO_3$ 25, $CaCl_2$ 25, Dextrose 10, and bubbled with 95% O_2, 5% CO_2. After incubation for at least one hour, the slice was transferred, and secured to the floor of the recording chamber (RC-25 Warner Inst. Hamden, CT). The slices were continuously perfused with a-CSF and maintained at 32°C using a single channel heater controller (TC–324B, Warner Inst., Hamden, CT). The slice was viewed using a Nikon upright microscope (Japan) and the entire preparation was mounted on an air table.

ii. *Whole cell voltage clamp*

The blind whole cell patch clamp method (22) was used to obtain voltage clamp recordings from SCN neurons. Patch pipettes were pulled from borosilicate glass (Dagan Corp., Minneapolis, MN) using Flaming/ Brown electrode puller (Sutter P-97, Novato, CA). Pipettes were polished using a Narshige microforge (MF–830) (Japan) and filled with an intracellular solution containing in mM: EGTA 5, HEPES 10, $MgCl_2$ 1, K-gluconate 130, NaCl 1, $CaCl_2$ 1, K_2ATP 2, osmolarity adjusted to 280 mOsm and pH 7.2. The polished and filled electrode was advanced slowly into the SCN, using an MP–285 (Sutter Inst., Novato, CA) micromanipulator. To keep the tip clean positive pressure was maintained on the electrode interior. Pipette resistance was measured from the current response to a 5 mV step voltage command. Once in contact with the cell, the positive pressure was relieved and gentle suction was applied intermittently. Sealing was indicated by the increase of pipette resistance to at least 1 GΩ. A holding potential of – 70 mV was applied to the pipette, then the interior of the cell was accessed by a brief pulse of suction to the pipette and the cell was maintained under voltage clamp for recording. Data acquisition was performed using a Warner Instruments (Warner Inc., Hamden, CT) patch clamp amplifier and recorded using a PC equipped with a Keithley data acquisition board (Keithley Inst. Inc., Cleveland, OH) and software written in TestPoint (CEC, Corp., Bedford, NH). Series resistance was compensated for as much as possible (about 60-80%). Series resistance was monitored from the height of the initial transient response to the same 5

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mV step voltage command that was used to measure seal resistance.

iii. *Data analysis*

Currents waveforms, in voltage clamp mode, were fitted to exponential function using Microcal Origin software 6.0 (Northampton, MA). Averaged data are presented as means ± SE.

III. Results

a) HCN1 immunoreactivity in the brain

To demonstrate the specificity of the antibodies, the distribution of the immunoreactivity in the rat brain with HCN1, HCN2 or HCN4 was compared to previous studies (7, 18, 19). Labeling, which consisted of dark brown reaction product, was found in the cerebral cortex as well as the hippocampus and exhibited a distribution in these areas consistent with the results of those studies. In the cerebral cortex, there was strong immunoreactivity for HCN1 in layers 1, 2, 3 and 4. The labeling was more intense towards the apical dendrites (Fig.1A). Also the cell bodies did not show any labeling. In the hippocampus, intense labeling was observed in both CA1 and CA2 mainly in the apical dendrites as with cortical neurons the cell bodies were also immune negative (Fig.1B). In the SCN, intense HCN1 labeling was observed within the SCN as well as toward the optic chiasm (Fig.1C).

b) HCN2 immunoreactivity in the brain

HCN2-immunopositive cell bodies were scattered throughout cortical layers however labeling was less intense than HCN1 (Fig.2A). In the hippocampus, labeling was observed in a similar pattern but with less intensity (Fig.2B). In the SCN, immunopositive cell bodies were also observed but more towards the third ventricle and the center of the SCN rather than the optic chiasm. The labeling appeared to be more localized to cell bodies and was scattered throughout the SCN (Fig.2C).

c) HCN4 immunoreactivity in the brain

HCN4-immunopositive cell bodies were observed in layers 2, 3 and 4 (Fig. 3A) while in the hippocampus we observed less intense label, mainly in CA1 region (Fig.3B) in the SCN. However, no labeling with HCN4 was observed (Fig.3C).

d) I_h in the SCN

Using the whole cell voltage clamp method, I_h was recorded from 113 cells within the SCN.84% of the SCN neurons exhibited I_h (Fig. 4A). I_h was identified as being activated at hyperpolarizing potentials more the -70 mV as a slow inward current (Fig. 4B). The activation time constant in these cells exhibited voltage dependence, as it was on average 236±2 ms at -40 mV step and 167±3 ms at -60 mV step. This difference in voltage dependence was statistically significant ($p< 0.5$). The current amplitude was on average 25±2 pA at – 40 mV, while at – 60 mV the current recorded was 34±2 pA this voltage dependence was also statistically significant ($p< 0.01$) (Fig.5).

IV. Discussion

Our results show that HCN1 and HCN2 channel subunits are present in the rat SCN and have different localization patterns. HCN1 is present in the ventral aspects of the SCN toward the optic chiasm with a diffuse pattern probably due to labeling of the neuronal processes. In contrast, HCN2 has a more scattered pattern of distribution labeling throughout the SCN, but was mainly observed in somas. These data agree with Notomi and Shigemoto (7) who found labeling of both subunits in the SCN but they did not describe any labeling pattern.

I_h was present in the majority of SCN neurons (84%). Its activation time constant (τ) was 236±2 ms and the current amplitude was 25±2 pA for a -40 mV step. For a -60 mV the current amplitude was significantly increased to 34±2 pA while τ was significantly reduced to 167±3 ms. These voltage dependence data agrees with what has been reported about I_h (for reviews (8, 9)). This is also in agreement with other electrophysiological data (3, 9, 17, 23). For example, De Jeu and Pennartz (3) reported that the activation time constant for I_h ranged from 107 to 467 ms. Jiang et al. (17) also reported that I_h in the majority of SCN neurons had an amplitude of 5-35 pA.

The activation kinetics of I_h recorded here are closer to those for HCN2 homomeric channels that have a slow activation properties (11). In vivo, the activation kinetics of I_h channels does not always correspond to those of homomeric ion channels. For instance, Stevens and co-workers (23) characterized I_h in the taste buds that exhibited τ in the range of 103 to 478 ms that did not conform with either of the subunits identified (HCN1 and 4) using immunohistochemistry. Another possibility is that both HCN1 and HCN2 homomeric channels co-exist in neurons and that the recording reflects the sum of the properties of the two channels. Finally, it is also possible that both HCN1 and HCN2 may co-assemble to form a heteromer within the SCN with the resultant I_h channel having different properties different from those of the homomer (24, 25). This, however, does not exclude the possibility that the results presented here could be attributed to the blind approach used in this study and the technical constraints on recording from the areas of the SCN that express HCN1.

On the other hand, no labeling for HCN4 channel subunit was observed within the SCN in this study. This result supports electrophysiological results that showed that I_h channel in the SCN are of the relatively fast-activating type, compared to the very slow HCN4 homomeric channels (12, 14). Although Notomi and Shigemoto (7) found HCN4 labeling in the SCN, this

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could be attributed to the fact they used different strain of rats (Sprague Dawley, versus Long Evans used in this study).

In conclusion, I_h is functionally well expressed in the SCN. Both HCN1 and HCN2 channel subunits are present within the rat SCN however; they have a different pattern of distribution. Together with the fact that melatonin can inhibit I_h at dusk (our unpublished results) this suggests that I_h may play an important role in modulation of SCN circadian function. These results support the model we proposed describing the role of melatonin in the regulation of SCN circadian function(26). In this model melatonin, via MT1 receptors activation, inhibits I_h. This effect may reduce SCN neurons excitability and decrease its spontaneous firing, which alters SCN rhythm at dusk and dawn. This model is further supported by the fact that MT1 receptors exhibited circadian variation in their expression while there is no evidence of such a variation in I_h (3, 26, 27).

List of abbreviation:

- I_h The hyperpolarization-activated current
- CNS Central nervous system
- SCN Suprachiasmatic nucleus
- HCN Hyperpolarization-activated and Cyclic Nucleotide-gated non-selective cation channel
- CT Circadian time
- SAN Sino-atrial node
- PBS Phosphate buffer saline
- ABC Avidin-biotin-peroxidase complex
- DAB 3, 3' diaminobenzidine
- a-CSF Artificial cerebrospinal fluid

Availability of data and material: All data are presented in the main paper.

Competing interest: The authors declare no competing interest.

Figure Legends

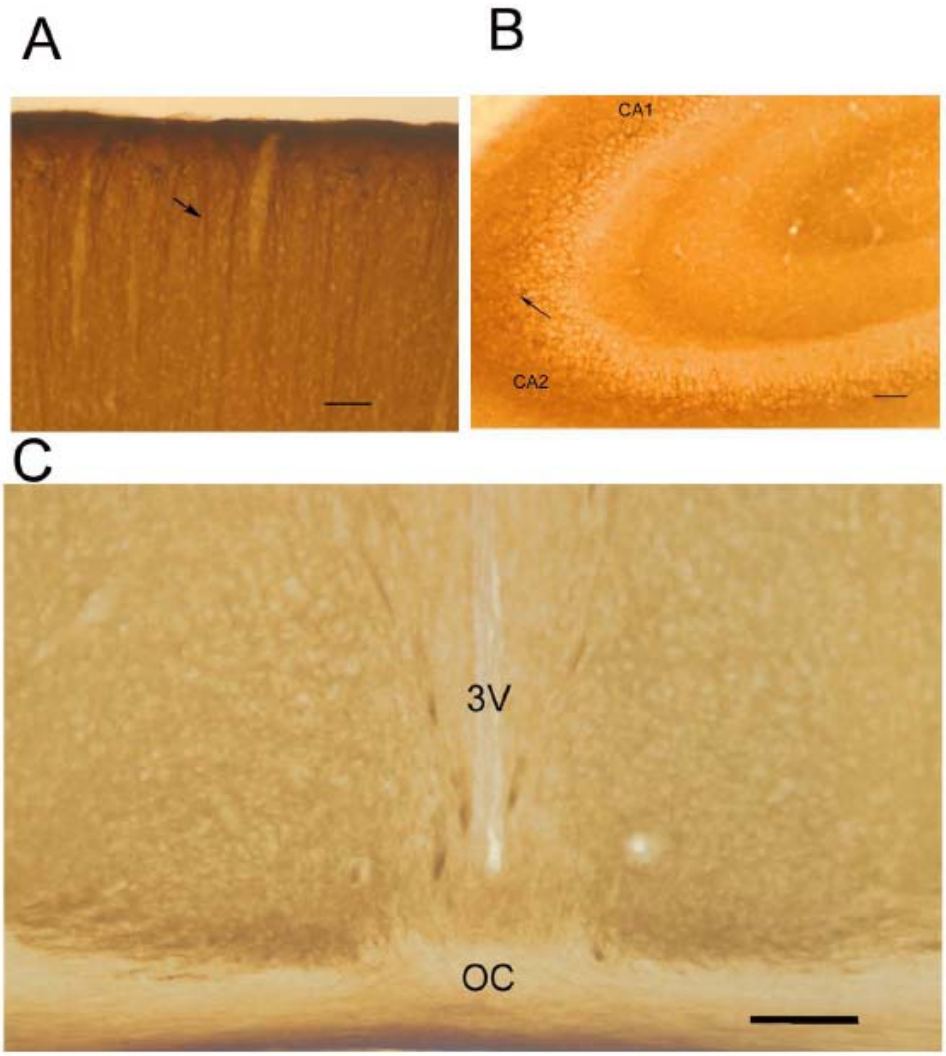

Figure 1 : HCN1 immunostaining in the brain. A) Shown is HCN1 staining in the cortex, B) in the hippocampus and C) in the SCN. 3V is the third ventricle, OC is the optic chiasm. Scale bar is 20 μm.

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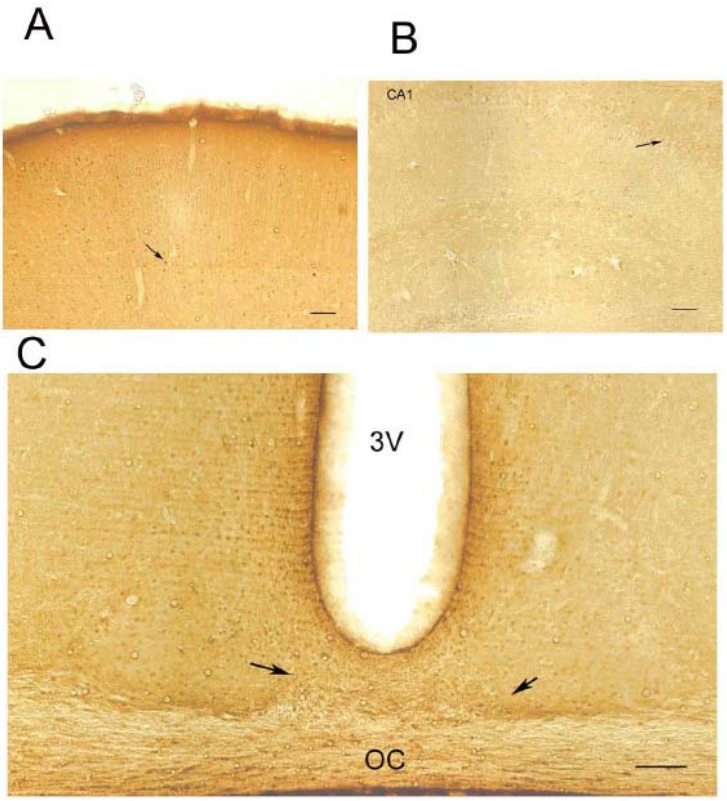

Figure 2 : HCN2 immunostaining in the brain. A) Shown is HCN2 labeling the cortex, B) hippocampus and C) SCN. The arrows points at somas. 3V is the third ventricle, OC is the optic chiasm. Scale bar is 20 μm.

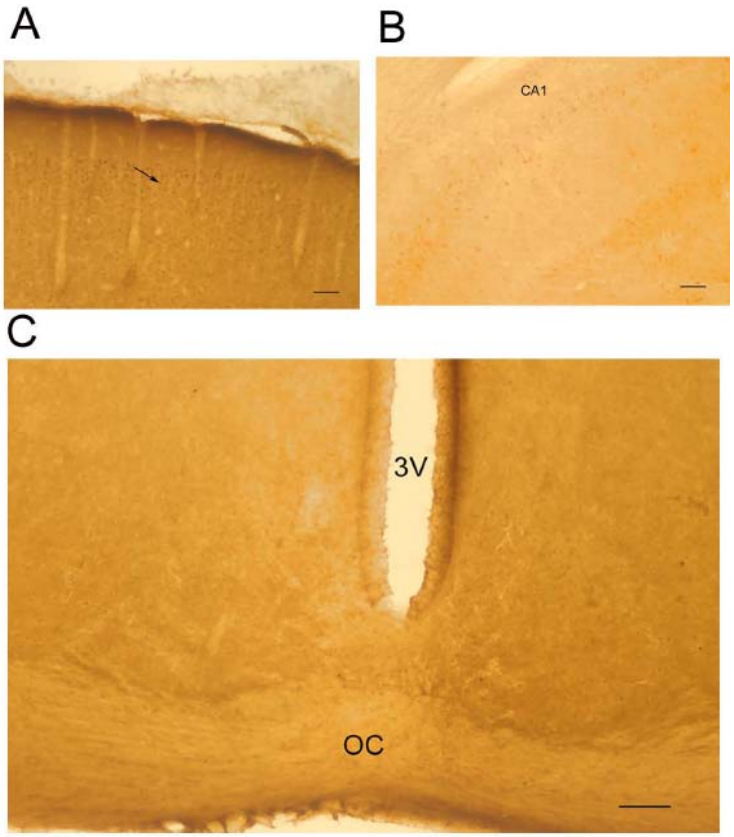

Figure 3 : HCN4 immunostaining in the brain. A) Shown is labeling with HCN4 antibody in the cortex, B) hippocampus and C) SCN. 3V is the third ventricle, OC is the optic chiasm. Scale bar is 20 μm.

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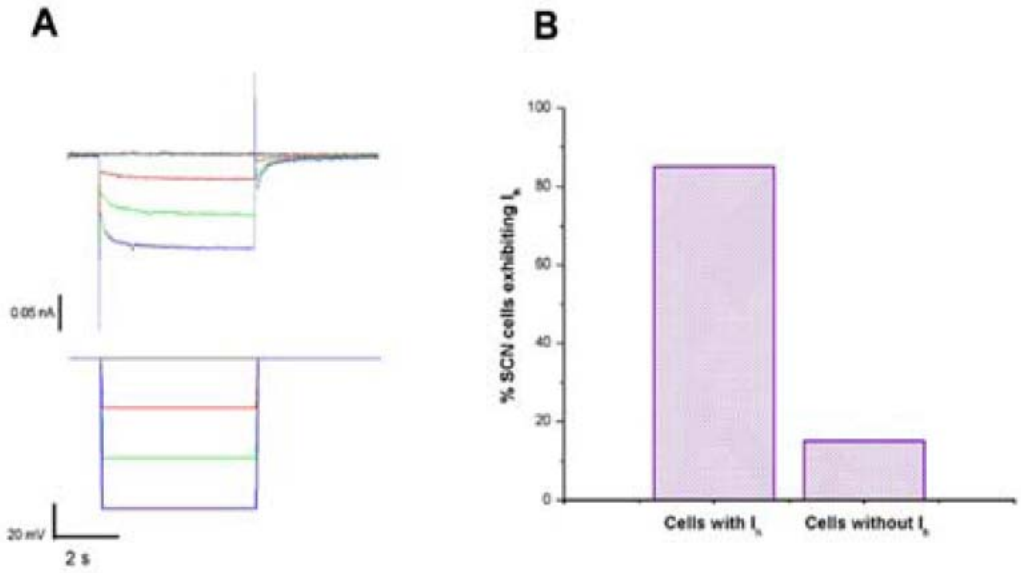

Figure 4 : Hyperpolarization activated current (I_h) in the SCN A) Activation of I_h in the SCN neurons. In voltage command mode, hyperpolarizing steps of 20 mV magnitudes from a holding potential of –70 mV were applied to activate the inward current. The top panel shows a voltage clamp record in a neuron in the suprachiasmatic nucleus. The lower panel shows the protocol used for eliciting I_h. B) Frequency of I_h recorded in the SCN at dusk.

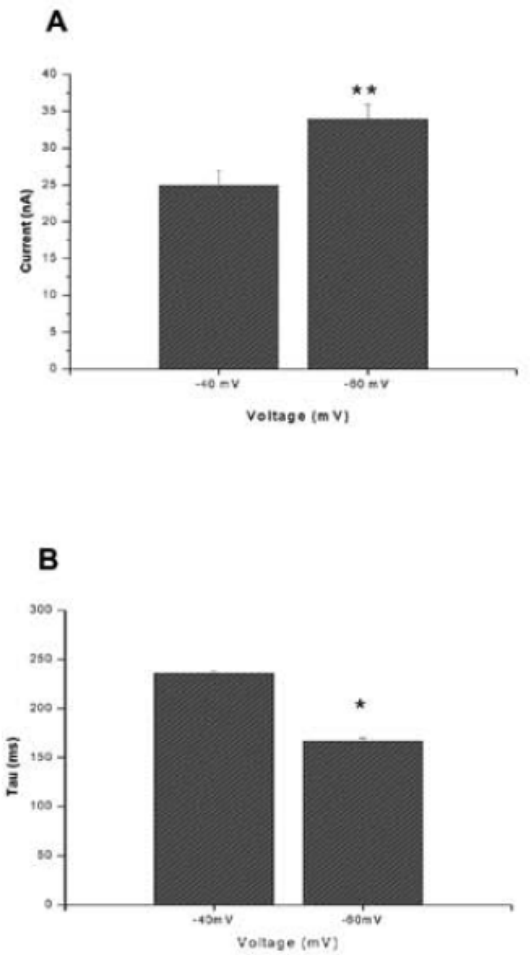

Figure 5 : Voltage dependence of I_h magnitude A) and B) activation time constant in SCN neurons. * indicates a p value less than 0.05.

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A Cross Sectional Study to Assess Liver Fibrosis in Patients with Diabetes and Metabolic Syndrome in India

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Abstract- *Background:* Liver fibrosis is now being considered as a reversible process which is characterized by excessive accumulation of extra cellular matrix. The use of non-invasive methods to assess liver fibrosis in patients with HCV, Non- Alcoholic Fatty liver Disease (NAFLD) and alcohol abuse has been well validated. However use of these noninvasive methods in patients with diabetes mellitus and metabolic syndrome has not been assessed who might develop fibrosis during asymptomatic stage. Hence we tried to use these noninvasive methods in patients with diabetes and metabolic syndrome who are at high risk of developing NAFLD or liver fibrosis in routine clinical practice.

Aim: To evaluate liver fibrosis in patients with diabetes and metabolic syndrome.

Methodology: This was a single center, prospective, 50 patients with diabetes and metabolic syndrome attending the endocrinology department of Osmania General Hospital were assessed for fatty liver and enrolled in to the study. NAFLD fibrosis score was used to assess liver fibrosis and BARD score was used for staging of fibrosis as per metavir classification.

Keywords: *liver fibrosis, enhanced liver fibrosis, non alcoholic fatty liver disease, cirrhosis, NAFLD fibrosis score.*

GJMR-K Classification: *NLMC Code: WI 700*

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Mohammed Alim [α], Dr. Rakesh Sahay [σ], Hafsa Khalid [ρ], Manasa Reddy Chintapalli [ω], Dr. Prabhakar [¥] & Dr. Mohammed Ibrahim [§]

Abstract- Background: Liver fibrosis is now being considered as a reversible process which is characterized by excessive accumulation of extra cellular matrix. The use of non-invasive methods to assess liver fibrosis in patients with HCV, Non-Alcoholic Fatty liver Disease (NAFLD) and alcohol abuse has been well validated. However use of these noninvasive methods in patients with diabetes mellitus and metabolic syndrome has not been assessed who might develop fibrosis during asymptomatic stage. Hence we tried to use these noninvasive methods in patients with diabetes and metabolic syndrome who are at high risk of developing NAFLD or liver fibrosis in routine clinical practice.

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Results: The mean age of the patients was 50.8 ± 8.2 with 22 males and 28 female's. 90% of the population was found to have some degree of fibrosis. 56% of patients were at advance fibrosis stage as per the BARD score based on metavir classification.

Conclusion: Patients with diabetes and metabolic syndrome should be constantly evaluated for liver fibrosis apart from development of diabetes and other complications to prevent any adverse effects due to waning of liver function.

Keywords: liver fibrosis, enhanced liver fibrosis, non alcoholic fatty liver disease, cirrhosis, NAFLD fibrosis score.

I. Introduction

Liver fibrosis or hepatic fibrosis is a reversible process, results due to chronic tissue damage characterized by excessive accumulation of extracellular matrix (ECM).[1] It is the first stage of liver scarring and slowly builds up and take over most of the liver diminishing the normal activities of the liver. If not treated or reversed may lead to cirrhosis the final stage of fibrosis.[2] The most common causes for cirrhosis are viral infections such as hepatitis C, alcohol abuse, non-alcoholic fatty liver disease (NAFLD), Non-alcoholic steatohepatitis (NASH), the extreme form of NAFLD is recognised to be the major cause.[3]

Liver fibrosis progresses to cirrhosis based on the etiology of liver diseases accelerated by environmental and genetic factors.[4]Oxidative stress is well documented cause for liver fibrogenesis clinically.[5] The process is initiated by cell injury with activation of hepatic stellate cells (HSC) producing ECM.[6]It is a dynamic process with many changes in the cell physiology of liver.

Treatment for liver fibrosis is not standard globally, mainly due to proper diagnostic issues and lack of understanding of its pathophysiology which is mainly derived from in vitro studies. Reversal of liver fibrosis has been observed in patients after successful treatment of underlying disease. However treatment of liver fibrosis involve treating the causative mechanism or agent by use of anti-inflammatory drugs, antioxidants, growth factors, gene therapy, insulin sensitizers, anti-fibrotic agents and renin angiotensin inhibitors. The clinical management of patients with chronic liver disease is still not up to the mark due to lack of translation of basic research.[7]

Liver biopsy is the standard for evaluating liver fibrosis with histopathological examination, it has limitation in interpreting the fibrosis stage with intra and inter observer variability, pain during the procedure and development of major complications.[8] Ultrasonography is quick and low cost method to detect increased echogenicity, but has got much operator dependency. The focus has been shifted to non-invasive methods based on biochemical and radiological test.[9] Routinely available serum test not related directly to extracellular matrix metabolism has been evaluated for prediction of liver fibrosis. These parameters are known as indirect markers while the direct biomarkers are hyaluronic acid,tissue inhibitor of metalloproteinases 1 (TIMP-1), amino-terminal propeptide of type III procollagen (PIIINP)[10, 11] The need for simple, reliable non-invasive

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method for assessing liver fibrosis will be much useful. With combination of biochemical parameters few studies have validated results for liver fibrosis and some studies have used serum fibrotic markers.Among these methods the AST-to-platelet ratio index (APRI) test, Forns test, and FibroTest (FT) have given some satisfactory results.[12, 13, 14]

Patients with diabetes mellitus (DM) and Metabolic Syndrome (MS) hasa significantly higher prevalence of NAFLD compared to those without diabetes and metabolic syndrome.[15]Previous studies has suggested NAFLD may progress to cirrhosis and failure of liver. Thus identifying liver fibrosis in this population will be of significant value and early detection may help in starting the treatment and management earlier.

NAFLD fibrosis score has been validated for presence of fibrosis in NAFLD patients where biopsy can be avoided for confirmation of fibrosis. The prevalence rates of NAFLD in general population is 32% while in population with diabetes and metabolic syndrome is 90%.[16] Since NAFLD is more prevalent in patients with diabetes mellitus and metabolic syndrome with a high risk of cardiometabolic syndrome, we tried to evaluate liver fibrosis in this population.

II. Materials and Methods

This was a single center, prospective cross sectional observational study. Patients with diabetes mellitus and metabolic syndrome were evaluated for fatty liver by ultrasound imaging and out of 68 screened, 50 patients with fatty liver were enrolled in to the study visiting to endocrinology department of Osmania General Hospital.

Patients having type II diabetes mellitus and metabolic syndrome were included with age ≥ 18 years. Diabetes was confirmed with patient's medical history and fasting blood glucose. Modified National Cholesterol Education Program Adult Treatment Panel III criteria[17] was used to define Metabolic Syndrome. Patients with waist circumference (>90 cm in men and >80 cm in women), increased TGs and low HDL cholesterol, high blood pressure (>130/ 85mmHg; or on anti-hypertensive drugs), and high fasting blood glucose (FBG) (>110 mg/dL; or a known diabetic) were applied and metabolic syndrome was defined by the presence of three or more of these criteria.[18] Patients body mass index, was calculated and biochemistry assessment were done. A qualitative test to determine the presence of anti-nuclear antibody test was done to rule out any autoimmune disorders and viral screening for Hepatitis B surface antigen (HbsAg) and Hepatitis C virus (HCV) was done. Liver fibrosis assessment was done using online NAFLD fibrosis score calculator (http://nafldscore.com) and staging of fibrosis was done using BARD scoring system (http://gihep.com/calculators/hepatology/bard/). A Bard score of 2 to 4 is associated with F3 or F4 stages of fibrosis and a score of less than 2 was considered as strong negative predictive value of advanced fibrosis F0 or F2 as per metavir scoring system.

NAFLD fibrosis score is a validated simple noninvasive scoring system comprising of easily available clinical and laboratory variables. Using the cutoff values a prediction can be made of absence or presence of liver fibrosis in patients with NAFLD and liver biopsy can be avoided. NAFLD is the common cause for chronic liver disease in general population with increasing prevalence of obesity, diabetes mellitus and metabolic syndrome. This score has been validated in patients with diabetes mellitus and metabolic syndrome with fibrosis confirmed with liver biopsy.[19]

BARD score is a very simple non-invasive method for staging of liver fibrosis in patients with NAFLD that can be used in routine practice. It was introduced in 2008 and involves ALT/AST ratio, BMI and diabetic assessments. It has been validated in biopsy proven NAFLD patients.

The study has been approved by the institutional ethics committee and is registered at clinical trial registry of India (CTRI/2014/07/004725).The study was carried out in accordance with the "Ethical Guidelines for Biomedical Research on Human Participants, 2006" by the Indian Council of Medical Research and the Declaration of Helsinki, 2008.

a) Data Analysis

Descriptive statistics was done using Microsoft Excel 2013.

III. Results

All results has been expressed as means± standard deviation (S.D) in Table 1. 50 patients were enrolled with diabetes and metabolic syndrome, mean age of the population was 50.8 ± 8.2 with 22 men and 28 women participants. The mean age of menand women was 52.1±8.2and 49.7±8.1 respectively. Mean NAFLD fibrosis score was 0.4 ±1.2. The high cut off value (>0.676) as per NAFLD fibrosis score was 73% and 17% in males and females respectively, while 27% in males were at indeterminate cutoff value (-1.455 - 0.676) and 21% in females. 62% were at low cutoff point in females as per the NALFD fibrosis score. Figure 1 and 2 shows the distribution patients as per the cut off values of NAFLD fibrosis score and assessment of liver fibrosis respectively. Viral screening for HbsAg, HCV and Anti-nuclear antibody test was negative for all patients. 56% of patients were at advance fibrosis stage i.e., F3 or F4 while 44% were having fibrosis score of F0 –F2 as evaluated by BARD score (Figure 3).

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IV. Discussion

Many non-invasive methods has been developed for liver fibrosis assessment following the limitations for liver biopsy. These methods can be used for primary evaluation for the population at risk or undiagnosed fibrosis in outpatient departments.[20] The popularity of these noninvasive scores is increasing for evaluation of non-significant and advanced liver fibrosis.[21] Transient elastography is a rapid non-invasive method for evaluation of liver fibrosis with high cost and limited to specialist.[22] Most of the non-invasive methods has been validated in population with hepatitis C and few in NASH/NAFLD.[11,23] In a exploratory study advanced fibrosis was diagnosed with high accuracy in patients with NAFLD using non-invasive parameters.[24]

Direct serum markers for liver fibrosis are not done routinely in all labs, the results might not be reliable in patients characterized by fibrogenesis in organs other than liver and are relatively expensive. Radiological assessments include computed tomography (CT) scan, magnetic resonance imaging (MRI) scan, Acoustic Radiation Force Impulse (ARFI) imaging and transient elastography which are quite costly. Ultrasound imaging is a cheap and easily available imaging technique widely used for fatty liver detection. Considering the merits and demerits imaging techniques alone, a combination of serum direct and indirect markers and imaging techniques helps is identifying liver fibrosis is patients at risk.[25]

Our results shows that 90% (Figure 1) of patients with diabetes mellitus and metabolic syndrome have some degree of liver fibrosis which are consistent with previous epidemiological studies for NAFLD,[15, 26, 27] however further evaluation needs to be done for classifying these patients based on the pathophysiological mechanism. These non-invasive methods can be routinely used to follow up these patients as these are highly reproducible.[11]It is recommended to use the non-invasive biomarkers along with transient elastography which increases diagnostic accuracy for liver fibrosis. Compared to liver biopsy these methods has no contraindication, less risk to the patients, has high applicability and reproducibility with a demerit of accurately staging liver fibrosis, non-specific surrogates of liver. These non-invasive methods with proper physical examination will help in identifying patients for further screening, evaluation and treatment.[20] In our study, males (73%) were at high cutoff value suggesting of advance fibrosis. While in females it was only 21%, suggesting men to have more prevalence of fibrosis than women, however earlier studies which was done in type 2 diabetes patients shows more prevalence in females than males.[26] The BARD score is another non-invasive method for assessing liver fibrosis and it stages the liver fibrosis as per metavir scoring system. We used NAFLD fibrosis score and BARD score to assess liver fibrosis in this population and to identify staging of liver fibrosis as per metavir scoring system respectively suggesting 56% of this population is at either F3 or F4 stage of fibrosis.

Oxidative stress being widely considered as one of the pathophysiological mechanism, [27] patients with diabetes and metabolic syndrome are at more risk and use of these non-invasive assessments will help in early identification of patients having moderate to advanced liver fibrosis. While liver fibrosis is considered to be reversible, [28] this population should be studied further for classifying the various pathophysiological mechanism that can occur so as to follow the treatment guidelines accordingly.

There is a need to identify patients underlying with liver fibrosis as early as possibly to start the treatment early. The non-invasive methods are well validated and should be used in day to day clinical practice for population at risk. We tried to evaluate liver fibrosis in fatty liver patients with diabetes mellitus and metabolic syndrome using their routine biochemical test. The main limitation of our study is that it's a cross sectional study with less sample size, however due to various pathophysiological mechanism and reversibility of liver fibrosis this population should followed further for any complication that can happen due to decreased performance of liver.

V. Conclusion

Liver fibrosis is the first step in progression to liver cirrhosis with different etiologies lacking effective therapy. Liver plays an important role in detoxifying chemicals, metabolizing drugs and producing important proteins for body functions. Patients with diabetes and metabolic syndrome are at risk of developing diabetes complications and cardiovascular disease. A decline in liver function may attribute to increase risk of developing diabetic complication and other cardiometabolic disease in this population. Further studies are needed to evaluate the association of liver fibrosis and cardiometabolic disease in patients with diabetes and metabolic syndrome due to its increasing global burden.

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Table 1 : Baseline Parameters

Baselines Parameters	
Variable(units)	N=50 (Mean±S.D)
Age(years)	50.84±8.18
Height(cm)	156.02±7.6
Weight(Kg)	67.26±10.16
BMI(kg/m2)	27.59±3.40
SBP(mmHg)	132.98±20.59
DBP(mmHg)	81.38±11.11
FBG (mg/dL)	160.72±68.98
SGOT (U/L)	24.52±9.74
SGPT (U/L)	36.02±17.26
GGT (U/L)	44.42±30.63
Creatinine (mg/dL)	1.01±0.43
HbA1C(%)	8.27±1.89
TotalCholesterol (mg/dL)	176.32±37.62
LDL (mg/dL)	103.85±35.49
HDL (mg/dL)	42.54±8.85
Triglycerides (mg/dL)	151.9±69.1
Total Proteins (g/dL)	7.68±0.42
Haemoglobin (g/dL)	12.04±1.87
Platelet count (lacs/Cmm)	3.04±0.88
Bilirubin(mg/dL)	0.46±0.23
Albumin (g/dL)	4.09±0.35

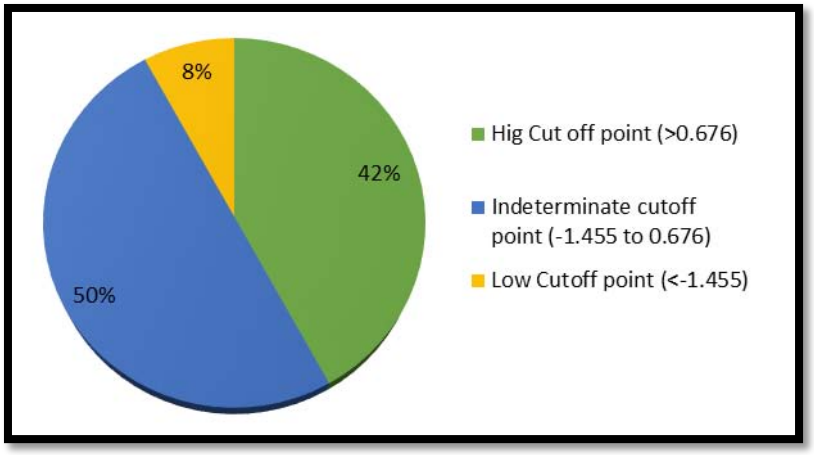

Figure 1 : Distribution of NAFLD Fibrosis Score

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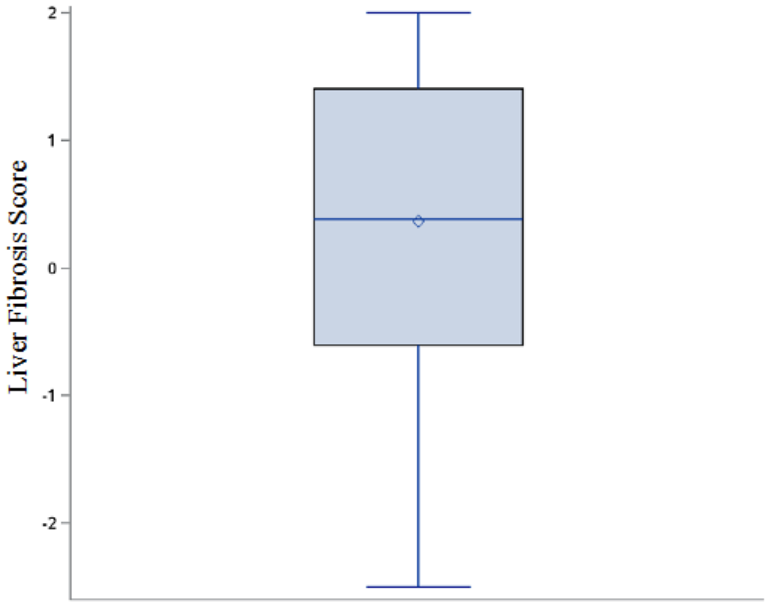

Figure 2 : Box plot for Liver Fibrosis Assessment

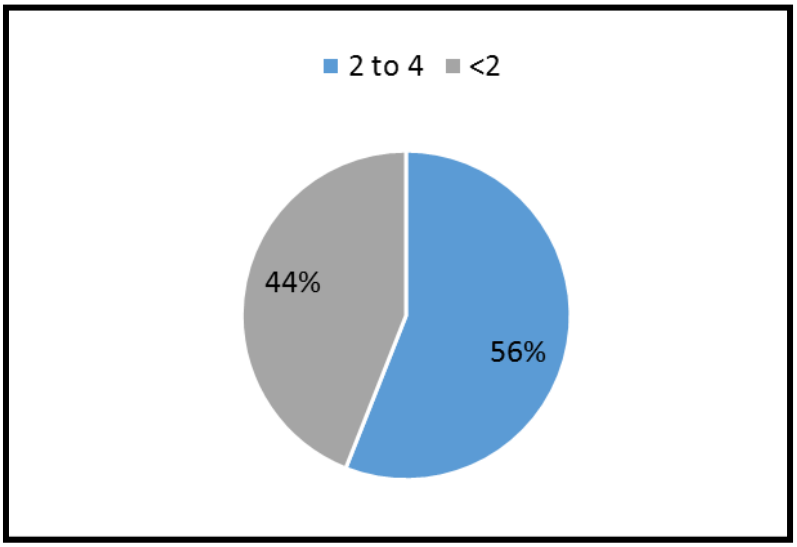

Figure 3 : BARD score

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Repetition of Parent-Adolescent Communication on Sexual and Reproductive Health Matters in High School Students in Yirgalem Town, South Ethiopia

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Abstract- *Back ground:* Adolescent is an experimental and transitional time to adulthood, they also susceptible to different sexual and reproductive health problems. Almost all of studies weren't addressed on repetition of parent–adolescent communication on sexual and reproductive health issues. This study aimed to assess repetition of parent-adolescent communication on sexual and reproductive health matters among secondary and preparatory school students in Yirgalem Town, South Ethiopia.

Methods: An institution based cross sectional study was conducted in 2015. A 684 high school adolescents were recruited by simple random sampling method in Yirgalem Town. Focus group discussion qualitative was used through separately for female and male parents. Data were entered using Epi Info version 3.5.1 was exported and analyzed by SPSS version 20.Bivariate and multivariate logistic regression was used to identify repetition of adolescent- parent communication.

Keywords: *repetition, parent- adolescent communication, yirgalem, ethiopia.*

GJMR-K Classification: *NLMC Code: WA 330*

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Repetition of Parent-Adolescent Communication on Sexual and Reproductive Health Matters in High School Students in Yirgalem Town, South Ethiopia

Zemenu Yohannes [α] & Zelalem Tenaw [σ]

Abstract- ***Back ground:*** ***Adolescent*** is an experimental and transitional time to adulthood, they also susceptible to different sexual and reproductive health problems. Almost all of studies weren't addressed on repetition of parent–adolescent communication on sexual and reproductive health issues. This study aimed to assess repetition of parent-adolescent communication on sexual and reproductive health matters among secondary and preparatory school students in Yirgalem Town, South Ethiopia.

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Results: Three hundred ninety (59.1%) respondents were discussed sexual and reproductive health issues with their parents. Parents 2.3 times monthly had discussed on HIV/AIDS than others [AOR = 2.296, 95% CI: 1.500-3.514].

Conclusion: Repetition of parent-adolescent communication on sexual and reproductive health matters were low. The mass media give coverage related to parent-adolescent communication, encourage role model families communication and community level to increase parent-adolescent communication.

Keywords: repetition, parent- adolescent communication, yirgalem, ethiopia.

I. Introduction

Adolescent is experimental, transitional and enjoyment time, they also susceptible to different sexual and reproductive health problems. Neglect of this group will not progress and achieve to sustainable development goal, meanwhile parent-adolescent communication on sexual and reproductive health is pivotal to reduce reproductive health problems and develop self-confidence for future [1]. There are 1.2 billion adolescents live in the world. Half of the population in 17 developing countries were under the age of 18.Currently over 20.19 million (24.1%) of the adolescents live in Ethiopia [2, 3, 4]. Now days 11% of birth and 14% of maternal death was occurred under the age of 19, almost 95% of adolescents birth was happened in developing countries [5, 6]. Every year in the world, adolescents are experiencing 7.4 million unintended pregnancies and 3 million unsafe abortions, especially in sub African every day 270 teenage pregnancies [7, 8]. In the other hand in the world, an estimated 1,300,000 adolescent girls and 780,000 adolescent boys are living with human immune-deficiency virus (HIV) [9]. Sexual and reproductive health matter communication is taking a lion shares' to transmit sexual values, beliefs, expectations, knowledge and experience between parents and adolescents [10]. Likewise, parent-adolescent communication is a fundamental means to transmit ideas, real situations, existing things, expectations, knowledge, their life experiences and the current conditions of parent–adolescent relations'. Parents are spent most of the time with their adolescent; they have an opportunity to communicate with their adolescents on a daily basis and can play a critical role in shaping their adolescents transitioned to adulthood. Most of the parents would like to communicate their adolescent about sexual matters superficially, due to lack necessary communication skills, knowledge, or comfort [11, 12, 13]. Over all the past five decades children mortality among under five decrease by 80%, meanwhile adolescents mortality rate were improved by 41-48%[9]. Generally to decrease significantly adolescents morbidity and mortality Parent-adolescent communication about sexual and reproductive health issues were crucial and can greatly reduce adolescents' sexual risk [14]. There is very little data available in the study area. Therefore, this study was planned to determine the prevalence of repetition parent-adolescent communication on sexual and reproductive health matters among secondary and preparatory school students in Yirgalem Town, South Ethiopia.

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II. Methods

a) Study setting and populations

The study was a cross sectional quantitative design and qualitative study were triangulated. conducted from February to March 2015, 2 secondary and preparatory schools in Yirgalem, Southern Nation Nationalities regional state of Ethiopia .It covers 28 square kilometers and had an estimated population of 38,438[15].The study population was all students from grade 9 to grade 12 who unmarried adolescents in the age group 10–19 years were included in the study and sick and unable to read local language were excluded in the study. Among 7035 students in the academic years 2014/2015. From this, 54.9% were females and 45.1% were males [16]. There are 684 study participants were selected by simple random sampling technique.

b) Sample size determination

Sample size was determined by using single population proportion formula by considering assumption of parent-adolescent communicating on sexual and reproductive health issues were 69.5% [6], desired precision of 5%, 95% confidence level. Ten percent for non-response rate, 684 students were required for the study.

c) Data Collection

Pre-tested an anonymous self-administered structured questionnaires were prepared after reviewing different relevant literatures [17, 18, 19]. The questionnaires were first prepared in English and then translated to Amharic, the local language of the respondents in the study area. The data were collected using self-administered structured questionnaire. The questionnaires were administered to all students during the data collection period, and who met the inclusion criteria.

d) Data Quality Control

Data were collected by two days trained eight Diploma nurse on the objectives of the study, sampling procedure, checking the completeness of questionnaires. Questionnaires were pre-tested at Leku high school to assess clarity, flow and consistency and revised prior to start data collection.

e) Focus group discussion

A series of four focus group discussions were carried out among purposively selected parents who have adolescents age 10-19 years enrolled in high school in Yirgalem Town. The criteria to select study participants on focus group discussion was purposively sampling techniques were used. The kebele leader was told about the objective of the study and then selected those parents who have adolescents age 10-19, who can explain /express their ideas thoroughly. Moreover, the characteristics of the study participants were similar in socio-demographic like (age, sex etc). The facilitators /moderators were principal investigators and trained health extension workers who can take note and as well as moderates the female parents to increase the quality of information. The focus group discussion was conducted separately mothers and fathers increase the quality of information that could be generated ideas and the confidence of the respective parents. To understand /to get their opinion fully tape recorder was used. There were eight participants in each group. A semi structured questionnaire guideline was used to lead the discussion.

f) Data analysis

Data were entered using Epi Info version 3.5.1 and exported to analyze SPSS version 20.0. Bivariate analysis was done to see the association of each independent variable with the outcome variable. Potential confounders (important) variables were entered into binary logistic regression model to identify the effect of each independent variable with the outcome variables. A p-value of less than 0.05 was considered statistically significant, and adjusted odds ratio with 95 % CI was calculated to determine association. Finally, the result was presented in texts, tables and graphs. For Qualitative, data were transcripts and translated to English. FGD study components were present by using quotes and explanations.

g) Ethical consideration

Ethical approval and clearance was taken from institutional review board of College of Medicine and Health Sciences, Hawassa University. Regional Education Bureau also gave permission to conduct the study. After explaining the purpose of the study, verbal informed consent was obtained from respondents before data collection. The right to withdraw the study at any time was also assured. Coding was used to eliminate names and other personal identification of respondents throughout the study process to ensure participants confidentiality.

III. Results

a) Socio demographic characteristics of the respondents

A total of 660 participants were recruited for the study, which makes the response rate 96.5 %.

Among the respondents 339(51.4%) were females, 316 (47.9%) were from grade 9 followed by grade 10, 11 and 12 accounting 243(36.8%), 49(7.4% and 52(7.9%) respectively and 50.6% were aged 13-16, while the rest were aged 17 to 19 years old. Their living arrangement 532 (80.6%) o were living with their both parents and 64 (9.7%) were living with others (Table 1).

The educational status of parents were 49 (7.4%) of fathers and 99 (15%) of mothers could not read and write, while 146 (22.1% and 129 (19.5%) of fathers and mothers had attend secondary school. The

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occupations of fathers 215 (32.6%) were farmers, 253 (38.4%) were Employee and 126(19.1%) had their own private business. Meanwhile the occupations of mothers were 278 (42.1%) housewives, 176 (26.6%) Employee and 118 (17.9%) had their private business (Table 2).

Table 3 : Socio-demographic characteristic of adolescents (N=660)

Variables	Frequency	Percentage
Sex		
male	321	48.6
female	339	51.4
age		
13-16	334	50.6
17-19	326	49.4
education		
Grade 9 & 10	559	84.7
Grade 11 & 12	101	15.3
Religion		
Protestant	374	56.7
Orthodox	233	35.3
Muslim	35	5.3
Others*	18	2.7
Ethnicity		
Sidama	521	78.9
Amhara	76	11.5
Oromo	24	3.6
Guragiyie	18	2.7
others†	27	3.2
Living condition		
Father and mother	532	80.6
mother only	64	9.7
Father only	29	4.4
Relatives/friends/Alone	35	5.3

*Others like catholic, Adventists †Others like Tigre, wolyita, silti

Table 4 : Parent's educational and occupational status among (N=660)

Variables	Frequency	percentage
Mother's ed. status (n=660)		
Illiterate	99	15
Read & write	130	19.5
Primary school	116	17.6
Secondary school	129	19.5
Diploma	81	12.3
Degree and above	66	10
Not live	39	5.9
Father's ed. status (n=660)		
Illiterate	49	7.4
Read & write	105	15.9
Primary school	60	9.1
Secondary school	146	22.1
Diploma	121	18.3
Degree and above	114	17.3
Not live	65	9.8
Mother's occupation (n=660)		
House wife	278	42.1
Employee	176	26.6
Merchant	118	17.9
Farmer	51	7.7
Not alive	37	5.6
Father's occupation (n=660)		
Employee	253	38.4
Merchant	126	19.1
Farmer	215	32.6
Not alive	66	10
Family size (n=660)		
<5	249	37.7
5 and above	411	62.3
Estimated family income (n=660)		
<1000	33	5%
1000-2000	25	3.8%
>2001	73	11.1%
Don't know	529	80.2%

b) Repetition of parent-adolescent communication on sexual and reproductive health issues

One hundred eighty seven (24.3%) respondents' were discussed sometimes or monthly on HIV/AIDS, 39(5.9%) were discussed respondents' sometimes or monthly on physiological change during adolescent (Table 5). One hundred forty six respondents' grade 9-10 adolescents were discussed sometimes or monthly on HIV/AIDS, 4 respondents' grade 11-12 adolescents were discussed sometimes or monthly have not sex until marriage (Table 6). One hundred fifty literate mothers were discussed sometimes

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or monthly on HIV/AIDS (Table 7).One hundred fifty six literate fathers were discussed sometimes or monthly on HIV/AIDS (Table 8). This is evident from the parent response, "I discuss my adolescents related to reproductive health problems especially HIV/AIDS …and its consequences….like school drop, social stigmatization, meanwhile, I discussed my male adolescent sexual intercourse made underage with girl might be accused and went to prison as that time, school drop, their vision will become dark" a 50-year-old male discussant. Another parent discussant "we have daily discussion regarding their activities, everyone have daily reports where, with whom, after that every things discuss before dinner, we have also "betseb gubaye" which means daily dairy reports from adolescents and how to overcame the problems a 54-year-old male" discussant.

A 46 years female discussant "we have family meeting & discussion with my adolescent open dialogue about reproductive health issues like the advantage of abstinence, STI and consequence, menstruation, puberty or sexual intercourse negative consequence and sexual intercourse positive consequence throughout on their life. On the other hand every my adolescents have weekly reports regarding their activities". "He said that I am desired to communicate with my adolescents regarding sexual and reproductive health matters, but difficult to communication lack of skill and the topics how to discuss." A 60 years male discussant.

Table 3 : Repetition of parent-adolescent communication on sexual and reproductive health matters (N=390)

RH issues	Always	Weekly	Some times
Contraceptive	56(8.5)	58(8.8)	124(18.8)
HIV/AIDS	87(13.2)	68(10.3)	187(28.3)
Sexual intercourse	46(7)	59(8.9)	116(17.6)
Unwanted pregnancy	70(10.6)	51(7.7)	117(17.7)
Premarital sex	68(10.3)	52(7.9)	91(13.8)
condom	46(7)	82(12.4)	91(13.8)
Puberty	54(8.2)	39(5.9)	160(24.2)

In bracket is percent

Multiple responses are possible

Three hundred eighty respondents had discussed about addictions most of parent-adolescent communication on chat chewing (Figure 1).

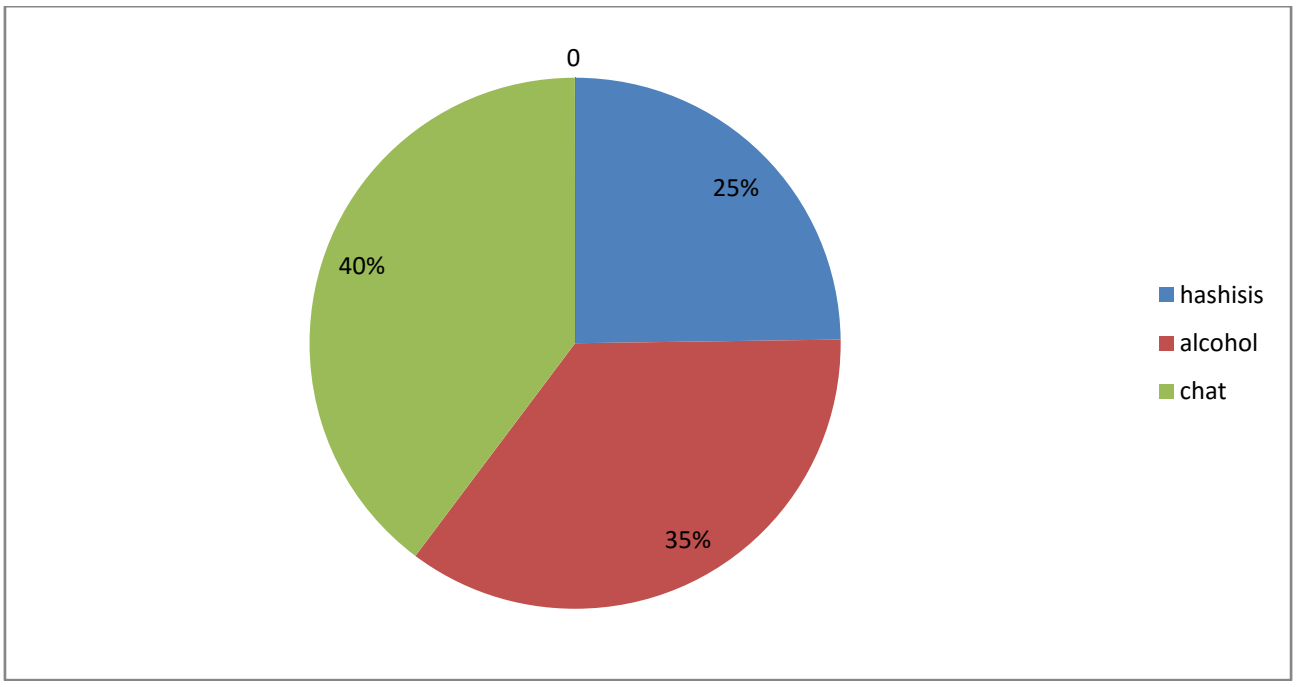

Figure 2 : Parent-adolescent communication on addictions (N=660)

Multiple responses are possible

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Table 4 : Parent–adolescent communication on sexual and reproductive health issues by grades (N=660).

RH issues	Grade	Frequency of parent- adolescent communication		
		Always	Weekly	Some times
Contraceptive	9-10	51(16.4)	52(16.7)	108(34.7)
	11-12	5(6.3)	6(7.6)	16(20.3)
HIV/AIDS	9-10	73(23.5)	61(19.6)	146(46.9)
	11-12	14(17.7)	7(8.9)	41(51.9)
Sexual intercourse	9-10	36(11.6)	54(17.4)	99(31.8)
	11-12	10(12.7)	5(6.3)	17(21.5)
Unwanted	9-10	61(19.6)	40(12.9)	98(31.5)
pregnancy	11-12	9(11.4)	11(13.9)	19(24.1)
Do not having sex	9-10	58(18.7)	48(15.4)	66(21.2)
until marriage	11-12	10(12.7)	4(5.1)	25(31.6)
Condom	9-10	41(13.2)	76(24.4)	80(25.7)
	11-12	5(6.3)	6(7.6)	11(13.9)
Puberty	9-10	43(13.8)	32(10.3)	80(25.7)
	11-12	11(13.9)	7(8.9)	11(13.9)

In bracket is percent

Multiple responses are possible

NB. Total numbers of students who communicate their parents in grade 9-10 are 311.

Total numbers of students who communicate their parents in grade11-12 are 79.

Table 5 : Mother–adolescent communication on sexual and reproductive health issues (N=660).)

RH issues	Mothers educational status	Frequency of parent- adolescent communication		
		Always	Weekly	Some times
Contraceptive	Illiterate	12(30)	11(27.5)	14(35)
	Literate	43(20.7)	43(20.7)	107(51.4)
HIV/AIDS	Illiterate	14(35)	3(7.5)	24(60)
	Literate	73(35.1)	60(28.8)	150(72.1)
Sexual intercourse	Illiterate	5(12.5)	6(15)	17(42.5)
	Literate	38(18.3)	48(23.1)	88(42.30
Unwanted pregnancy	Illiterate	15(37.7)	3(7.5)	16(40)
	Literate	54(26)	43(20.7)	93(44.7)
Do not having sex until marriage	Illiterate	16(40)	6(15)	10(25)
	Literate	49(23.6)	44(21.1)	70(33.7)
Condom	Illiterate	4(10)	20(50)	15937.5)
	Literate	42(20.2)	59(28.4)	66(31.7)
Puberty	Illiterate	8(20)	8(20)	27(67.5)
	Literate	44(21.1)	27(13)	121(58.2)

In bracket is percent

Multiple responses are possible

NB total numbers of literate mothers are 208.

Total numbers of illiterate mothers are 40.

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Table 6 : Father-adolescent communication on sexual and reproductive health issues (N=660).

SRH issues	Fathers educational status	Frequency of parent- adolescent communication		
		Always	Weekly	Some times
Contraceptive	Illiterate	5(27.8)	7(38.9)	6(33.3)
	Literate	50(40.3)	45(36.3)	99(79.8)
HIV/AIDS	Illiterate	10(55.6)	2(11.1)	12(66.7)
	Literate	70(56.5)	58(46.8)	120(96.8)
Sexual intercourse	Illiterate	2(11.1)	2(11.1)	8(44.4)
	Literate	39(31.5)	54(43.5)	94(75.8)
Unwanted pregnancy	Illiterate	7(38.9)	3(16.7)	9(50)
	Literate	61(49.2)	42(33.9)	96(77.4)
Do not having sex until marriage	Illiterate	7(38.9)	3(16.7)	4(22.2)
	Literate	59(47.6)	45(36.3)	81(65.3)
Condom	Illiterate	2(11.1)	12(66.7)	7(38.9)
	Literate	40(32.3)	63(50.8)	74(59.7)
Puberty	Illiterate	2(11.1)	5(27.8)	16(88.9)
	Literate	49(39.5)	30(24.2)	100(80.6)

In bracket is percent, multiple responses are possible NB: Total numbers of literate fathers are 124 and total numbers of illiterate fathers are 18.

c) *Factors associated with repetition of parent-adolescent communication on sexual and reproductive health issues*

Three hundred ninety (59.1%) of adolescents recognized the importance to discuss about sexual and reproductive health issues with their parents. However, most of students were discussed some times or monthly at least one topic sexual and reproductive health issues. Parents 2.3 times monthly were discussed on HIV/AIDS than others [AOR = 2.296, 95% CI: 1.500-3.514]. Parents 1.4 times were discussed on chat than others [AOR = 1.379, 95% CI: 1.175-2.574]. Parents 1.5 times were discussed on alcohol than others [AOR = 1.496, 95% CI: 1.003-2.232] (Table 9).

Table 7 : Factors associated with repetition of parent-adolescent communication on sexual and reproductive issues (N=660).

variable	Communications on SRH issues		95% CI	
	yes	no	COR	AOR
Those who sometimes discuss on contraceptive	88	36		
yes	302	234	1.894(1.240-2.893)	1.323(0.828-2.114)
Those who always discuss on HIV/AIDS	60	27		
yes	330	243	1.636(1.009-2.654)	1.581(0.925-2.703)
Those who sometime discuss on HIV/AIDS	140	47		
yes	**250**	**223**	**2.657(1.823-3.872) ***	**2.296(1.500-3.514) ****
Those who weekly discuss on unwanted pregnancy	38	13		
yes	352	257	2.134(1.114-4.088)	1.886(0.950-3.744)
Those who sometimes discuss on unwanted pregnancy	83	34		
yes	307	236	1.877(1.216-2.895)	1.121(0.661-1.900)
Those who always discuss on having not premarital until marriage	48	20		
yes	342	250	1.754(1.016-3.030)	1.628(0.883-2.999)
Those who sometimes discuss on having not premarital until marriage	64	27		
yes	326	243	1.767(1.094-2.854)	1.113(0.628-1.974)
Those who always discuss on condom	32	14		
yes	358	256	1.634(0.855-3.125)	1.194(0.577-2.473)
Those who sometimes discuss on condom	60	31		
yes	330	239	1.402(0.881-2.230)	1.053(0.617-1.797)
Those who always discuss on Puberty	40	14		
yes	350	256	2.090(1.113-3.922)	1.429(0.691-2.956)
Those who weekly discuss on Puberty	27	12		
yes	363	258	1.599(0.795-3.215)	1.478(0.701-3.116)
Those who sometimes discuss on Puberty	108	52		
yes	282	218	1.606(1.103-2.336)	1.325(0.851-2.061)
Chat	166	65		
yes	**224**	**205**	**0.428(0.303-0.603) ***	**1.379(1.175-2.574) ****
alcohol	147	59		
yes	**243**	**211**	**2.163(1.519-3.082) ***	**1.496(1.003-2.232) ****

Reference category is no

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IV. Discussion

The prevalence of parent-adolescent communication on sexual and reproductive health issues among adolescents in this study was 59.1%. This finding is slightly lower than the study was conducted in Nekmete 65.5% [18]. But higher than compared to the studies were done in other parts of Ethiopia [19, 20, 21]. This might be due to demographic and cultural difference. Parents 2.3 times monthly were discussed on HIV/AIDS than others [AOR = 2.296, 95% CI: 1.500-3.514]. Inconsistently the study was done in USA adolescent discussed their parent about sex 52.4% of parents said that very comfortable, but 25 % parents said that somewhat less comfortable [10]. This finding, Parents 1.4 times were discussed on chat than others [AOR = 1.379, 95% CI: 1.175-2.574]. Similar study was done in USA adolescents were communicated atleast one topics in the past six month [22]. In this study, Parents 1.5 times were discussed on alcohol than others [AOR = 1.496, 95% CI: 1.003-2.232]. Another study was done Caribbean family connectedness, school connectedness religious and individual values of reduced the likelihood sexual activity [1].In this study parent adolescent communication 71(10.2%) were made sexual intercourse. Other findings in USA parents were discussed with telling family culture to increase parent adolescent bond [23]. In this finding, from parent adolescent did not discuss 74(11.2%) were made sexual intercourse. Another study was done in USA adolescents who viewed religions as very important 27% were less likely to ever have had sex compared to adolescents who did not view religion as very important [OR =0 .75, 95% CI: 0.67-0.86] [24,25].Approximately one third of (N=1,076 or 32% of respondents reported frequent attendance (atleast one per week) at religious services. Those adolescents who attended services frequently were 46% less likely to ever have had sex compared to adolescents who attended religion services less frequently or not at all [OR = 0.55, 95% CI: 0.49-0.63]. Among (N=1,4233 or 62% respondents reported that they had had abstinence plus education .The first topic they had discussed to their parents about were 15% less likely to ever have had sex [OR =0 .85, 95% CI: 0.77-0.95][24].

V. Conclusion

In this study repetition of parent-adolescent communications on sexual and reproductive health, issues were very low. Parents 2.3 times monthly were discussed on HIV/AIDS than others [AOR = 2.296, 95% CI: 1.500-3.514].

VI. Recommendation

The community would be established reproductive health club. The mass media also give coverage regarding this issue. Sensitize the community to encourage open discussion among family members in general and between parents and adolescents in early age. It is important to encourage and empower parents to start to communicate with their adolescents on sexual matters while the adolescents are still in late childhood or early teenage years, before they become sexually active. The health extension workers train parents how to communication their adolescents. Role model families' and adolescent shares their experience. Stake holders encourage social norms like waiting sex intercourse until marriage and begin to give scholarship like short term training those especially delay sexual intercourse until youth. Further qualitative and analytical study design is recommended on adolescents and parents communication.

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Competing interests

All authors declare that they have no competing of interests.

Abbreviation

AIDS: acquired immune deficiency syndrome
AOR: adjusted odds ratio
HIV: human immune deficiency viruses
USA: United State of America

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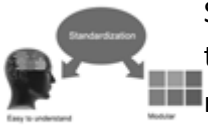

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- All figure and table must be adequately complete that it could situate on its own, divide from text

Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

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Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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