Factors Associated With Children's HIV Positive Status Disclosure in Wolaita Zone, Southern Ethiopia: Cross-sectional Study

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Abstract

Background: Children HIV-positive status disclosure is an essential component of chronic care and long-term disease management. However, children HIV-positive status disclosure is complex and varies across different communities. Although data from different settings are necessary to overcome this problem, evidences are limited particularly in case of children HIV-positive status disclosure. Therefore, this study aims to assess the prevalence of HIV-positive status disclosure and associated factors among children on antiretroviral therapy.

Methods: A facility based, mixed-method study was conducted among 203 care givers with children in Wolaita Zone, Southern Ethiopia. Structured questionnaire and in-depth interview guide was implemented to collect quantitative and qualitative data, respectively. Simple random sampling for quantitative and purposive sampling for qualitative parts was applied. Content or Thematic analysis for qualitative and Binary logistic regression was used for quantitative.

Results: In this study, the overall prevalence of children HIV-positive status disclosure was found to be 46.8%. Age of the child ≥ 12 years (AOR= 7.5, 95% CI: 2.9-15.6), duration on ART ≥ 72 months (AOR= 3.8, 95% CI: 1.7-6.7), death of parent (AOR= 2.0, 95% CI: 1.1- 3.8), and types of health facilities (AOR= 2.1, 95% CI: 1.3-4.7) were associated with disclosure. Being immature child was the commonest reason of non-disclosure. Frequent questions by the child why they are taking drugs, for better self-care, and treatment adherence were the commonest reason of care givers to make decision for disclosing their children's HIV positive status.

Conclusion: Children's HIV-positive status disclosure was low in this study. Health facility, children, and care givers related factors were mentioned as reason for disclosing or not-disclosing children's HIV-positive status. Hence, health care workers should give especial attention for children HIV-positive status disclosure which helps to increase the effectiveness of treatment out-come and prevent further HIV transmission.

Background

Globally, Pediatric human immunodeficiency virus (HIV) infection continues to be one of the major public health concerns and challenges [1]. At the end of 2019, approximately 38.0 million people were living with HIV world-wide; of which 1.8 million were children (age 0–14 years) [2]. The Sub-Saharan African (SSA) countries were suffered from the global burden of HIV- infection. By the end of 2018, an estimated 56,514 children under age of 15 were living with HIV in Ethiopia. Of which, around 2,994 were newly infected with HIV [1].

American academy of pediatrics and World Health Organization (WHO) strongly recommends the gradual process of giving age appropriate information to HIV-infected school age children by considering child's cognitive and emotional development [3, 4]. The African network for the care of children affected by HIV/AIDS (ANECCA) recommends that pediatric HIV disclosure start as early as at age of 5–7 years old

[5]. Disclosure of HIV positive status remains a major hindrance in the fight against the spread of HIV in sub-Saharan Africa [3, 6].

Disclosure helps the child to gain knowledge of his/her HIV positive status [7, 8]. Studies showed that if children living with HIV know his/her HIV status, he/she is more likely to adhere antiretroviral therapy (ART) [9]. Several studies have been documented on the clinical and psycho-social benefits of HIV positive status disclosure to infected children which helps to improve the quality of life of people infected and affected by the HIV disease [7, 9–11]. The World Health Organization (WHO) reported that the knowledge about their disease will enable individuals to make safe & healthy life choices about relationship's, sex and reproduction [12].

In Ethiopia, researchers were conducted studies on children's HIV positive status disclosure based on previous guidelines[13–15]. Factors like history of ART interruption and restart, caregiver's HIV status, care givers duration on ART, place of ART follow up, and disclosure status of care givers which contributes to the occurrence of low level of disclosure were infrequently included in the former studies. Additionally, majority of the prior studies were conducted at referral health facilities which have multidiscipline staffs to manage the disease[13, 14]. This study encompasses health centers which covers the majority of health facilities and providing pediatric ART service in Ethiopia. As to the knowledge of principal investigator, in the study area, there was no studies done on the HIV positive status disclosure and associated factors among children living with HIV. Current and up-to-date evidence regarding to children's HIV-positive status disclosure is essential for policy makers and clinicians to take appropriate measurements. Therefore, aiming to fill these gaps and to increase body of knowledge about HIV-positive status disclosure, this study was conducted to determine the prevalence and identify contributing factors of disclosure among children attending ART clinic in Wolaita Zone public health facilities.

Methods

Study settings, design, and period

A facility based, mixed-method study was conducted from July to September 2021 among HIV-infected children on ART. The study was carried out in Wolaita Zone public health facilities which provide pediatric ART service. Wolaita Sodo is the capital city of Wolaita Zone, located in Southern Ethiopia which is 338 km away from Addis Ababa. The study was done in six selected public health facilities (i.e. Bodity Health Center, Areka Health Center, Sodo Health Center, Humbo Primary Hospital, Bitena Primary Hospital, and Wolaita Sodo University Comprehensive Specialized Hospital).

Study participants, sample size, and sampling technique

All care givers of HIV-positive children (aged 6-18 years) on ART follow up in Wolaita Zone public health facilities were the target population. All care givers of HIV-positive children (aged 6-18 years) taking ART at the selected pediatric ART clinics were included. However, children who came alone or no care givers or

parent to undertaken the consent, care givers diagnosed to have mental health problem or with other serious illness were excluded.

The minimum required sample size was determined using a single and double population proportion formula. To compute the sample size for the first objective, the following statistical assumptions were considered:- 95% confidence level (CI), Proportion = 43.6% taken from a study conducted in western Ethiopia [16], margin of error =5% and the value of $Z_{\alpha/2} = 1.96$, which is the corresponding Z score of 95% confidence interval (CI).

$$n = \frac{(Z_{\alpha/2})^2 p(1 - p)}{(d)^2} = \frac{(1.96)^2 * 0.436(1 - 0.436)}{(0.05)^2} = 378$$

Where, n= the required sample size, $Z_{\alpha/2}$ = Standard normal variation for type 1 error, p=prevalence (0.436) & d= Margin of sampling error tolerated (0.05).

Yet, the final estimated sample size was 203 by considering a 10% non-response rate and using population correction formula as the total study population was below 10,000 in the study area.

This study was carried out in six randomly selected public health facilities. The list of participants who had regular ART follow up visit was obtained from electronic-database and their registration book in each health facility. Sampling-frame was prepared based on the child's medical registration number (MRN) from documented files of each health facility. After determining total children in each health facility, the total sample size was allocated for each health facility proportionally. Lastly, a simple random sampling method was applied to select the allocated study participants. A purposive sampling technique was implemented to select participants for qualitative study.

Data collection tool and procedure

Both quantitative and qualitative data collection tool was developed from different related literatures. Questionnaire was prepared in English and translated to local language then back to English to ensure consistency. Quantitative data was collected using structured questionnaires through face to face interview and supplemented by chart review. Twelve trained nurses were participated in the quantitative data collection. The questionnaire was pretested out of the study area before the actual data collection. Training was given for data collectors and supervisors on the objectives of the study, methods of data collection and how to obtain informed consent. Additionally, brief introduction was given to study participants before and during the data collection process. Supervision was conducted during data collection time.

Qualitative data collection was conducted concurrently with quantitative data collection. The in-depth interview (IDI) (from the care givers and health care workers) was conducted, translated and transcribed for qualitative data collection. The audio recorded interview was carried out by the Principal Investigator.

The interviews were aimed at triangulating the issues raised by the care givers towards disclosing or not disclosing and suggestions for improving disclosure.

Operational definitions

Disclosure: was defined when caregivers said that the child knows his/her HIV/AIDS diagnosis status regardless of who told to the child [17].

Care giver: was defined as a person who was knowledgeable about the child's HIV care, responsible for the well-being of the child, and who brought the child to the clinic [18].

Adherence: was defined as HIV-positive child who attends to his/her regularly clinical follow-up cares, periodic laboratory monitoring, and avoiding practices which interferes treatment effectiveness [18].

Data management and statistical analysis

Data were entered into Epi Data Version 3.1 software packages and exported to Statistics Package for Social Science (SPSS) Version 25 for further analysis. Tables and graphs were used to present descriptive data. Moreover, frequencies, percentages, and proportions were used to describe the study population characteristics.

Goodness of fit of the model was checked using Hosmer-Lemeshow goodness of fit test and enter logistic regression method was applied to select significant variables. Variables with p-values < 0.25 in the bivariable analysis were entered into the multivariable analysis to control the effects of confounders. Before identifying the significant factors, the presence of multicollinearity was examined using Variance Inflation Factor (VIF). In the multivariable analysis, variables with p-values less than 0.05 was considered as statistically significant factors. Finally, an odds ratio with its correspondence 95% CIs was used to assess the strength and the direction of association between outcome and study variables.

Recorded data from in-depth interviews were transcribed and translated to English word by word. Thematic analysis was done to analyze the qualitative data. Transcription was analyzed using the principles of content analysis by applying appropriate codes, sorting data, and looking for differences and similarities. Lastly, Representative quotes covering the range of summarized data were used to present the data.

Results

Socio-demographic characteristics of care givers

A total of 203 study participants were eligible for this study. The majority (84.7%) of care givers were female, and 70.9% of care givers were HIV positive. Among the care givers 38.4 % had no formal education and nearly half (48.8%) of the care givers were married. Most (86.7%) of the care givers were first degree relatives (Table 1).

Socio-demographic characteristics of the children

Nearly half of the children (54.2%) were females. Almost (89%) of the children had started formal education. Additionally, about 87.7% of the children were living with their parents. Among the children about 34.5% had lost any of her/his parent, and majority (89.2%) of children had good ART adherence level. Finally, majority (82.8%) of participants had no history of ART interruption and restart (Table 2).

Prevalence of HIV positive status disclosure

A total of 95 HIV-infected children were disclosed to their HIV-positive status. The overall prevalence of disclosure among HIV-infected children was found to be 46.8%.

Factors Associated with HIV-positive status disclosure

Bivariable and multivariable logistic regression analysis were done to assess factors associated with children HIV positive status disclosure. In the multivariable logistic regression analysis: types of health facility, age of the child, death of any of his/her parent, and child's duration on ART were factors significantly associated with HIV positive status disclosure. Thus, children whose age 12 year and above were 7.54 times more likely (AOR= 7.54, 95% CI: 2.87-15.62) to be disclosed their HIV positive status as compared to their counterparts. Similarly, those children who had taken ART for ≥ 72 months were 3.84 times more likely (AOR= 3.84, 95% CI: 1.65-6.72) to be disclosed their HIV positive status as compared to those children who had taken ART for < 72 months. Moreover, children who had lost any of his/her parent were nearly 1.96 times more likely (AOR= 1.96, 95% CI: 1.05-3.84) to be disclosed their HIV positive status as compared to their counterpart children who had both of his/her parent. Finally, types of health facility were 2.13 times more likely (AOR= 2.31, 95% CI: 1.33 - 4.67) to disclose their children HIV positive status than those whose age less than thirty eight years (Table 3).

Findings from the Qualitative Study

Most of the caregivers said that children's HIV positive status disclosure is necessary for treatment effectiveness. Hence, after HIV positive status disclosure, children have good ART adherence level; improve their awareness to live with HIV, decrease worries and confusions. All of the study participants agreed upon the important of child's HIV positive status disclosure. However, it should be done when the child becomes matured and avoid for too young children since they cannot understand whenever we are talking about HIV/AIDS. We had asked both care givers and health care workers regarding to their experience about child's HIV positive status disclosure. One of the care givers (mother) talked her disclosure experience as follows: "To disclose HIV positive status of our child, first I had discussed with my husband for a week. Then I had open discussion with my child about mode of transmission, treatment, and prevention of HIV/AIDS. I am HIV- infected and taking drug which helps me to carry out my activity of daily living. You are also infected with this disease which is acquired during labor when I gave birth. You are taking drug to keep you strong and healthy. If you take your drug properly, you can do everything effectively without any limitations what other healthy individuals can do." The other care giver

mentioned her experience as follows: "I had lost my strength to disclose my child's HIV positive status. I fear my child worry following disclosure and I feel that I do not have enough information about HIV/AIDS to convince the child. Finally, I decided to go to the health facility with my husband in order to get assistance from health care workers. Then we (mother, father and health care workers) had given information about the disease. However, the child feels confused and crying. After disclosure the child refused us to take drugs for a few days. After a few days she starts to take care herself and take drug properly."

The most common feelings experienced by children during disclosure of their HIV positive status are: Some of the children are crying on their care givers, refuse to go to school, and feel anxiety for a week. Despite it is rare; children have got difficulty to take sleep for a few weeks and attempt to harm themselves following their HIV positive status disclosure. Conversely, they withdraw from such unusual feeling with the help of their care givers and health care workers. On the other hand, some children (both genders) at the age of fourteen years and above try to experience unsafe sex and some others try to practice safe sexual practice. (ART nurses)

Disclosing the child's HIV positive status is also carried out through other already disclosed children living with HIV/AIDS. But, peer disclosing strategy is rarely implemented. A child who was disclosed her/his HIV positive status says I am living with HIV and attending my school effectively. At this time those previously disclosed children will share their experience to the newly disclosed child. The child will take others as a model and seeks to achieve his/her vision by taking drugs properly. (ART nurses)

Reasons for HIV positive status disclosing or not disclosing

Majority of the care givers were stated the following reasons to clarify for their children why they are taking medications (i.e. for treatment of pneumonia, common-cold, parasite, and germs). Frequent questions by the child why they are taking medications were the commonest reason of care givers to make decision for disclosing their children's HIV positive status. Additionally, if the child knows their HIV positive status they will take medications properly and take care of themselves. This will make life easy for both the care givers and child. Some of the care givers decided to disclose the child's HIV positive status to prevent accidental disclosure.

The caregivers were also asked why they did not disclose the child's HIV positive status, and mentioned the following reasons: The child's thought is immature so that they cannot understand talking about HIV/AIDS. Fear of social stigma and discrimination was also another reason for care givers why they did not disclose child's HIV positive status. As the children are too young, disclosing their HIV positive status will make the child to worry, and distressed. Moreover, lack of separated room, lack of specific short term training, time, and challenges to assure care givers were challenge mentioned by health care workers to conduct disclosure.

Discussion

In this study, the prevalence of disclosure among HIV-infected children was 46.8%. This is in line with the finding of studies done in Southwest Ethiopia 45.6% [19], Eastern Ethiopia (49%) [14], Western Ethiopia (43.6%) [16], and Northern parts of Ethiopia (44%) [20]. On the contrary, this finding is lower than studies conducted in Uganda (65%) and Rwanda (64%) [21, 22]. The possible sources of discrepancy might be due to the differences in sample size, study time, and socio-cultural factors across the study area. Moreover, the finding of this study was higher than studies done in Zambia (29.8%) [10], Ghana (23.3%) [11], Nigeria (30.9%) [23], and Namibia (33%) [7]. The possible source of variation could be due to age of the child, psychosocial factors (social stigma and discrimination), and care givers awareness variation towards the important of disclosure.

We found that different factors were significantly associated with children HIV positive status disclosure. In this regard, children with the age of 12 years and above were more likely to be disclosed their HIV positive status. This finding is in line with previous studies done in Nigeria [23], Uganda [21], Tanzania [24], Ghana [25], and Ethiopia [26]. This is due to the fact that children at the age of 12 years and above are matured enough to understand about HIV /AIDS as they can get some information regarding to this at their school. Additionally, majority of care givers agreed up on the important of HIV positive status disclosure when the child is matured.

This study showed that children who had taking ART for ≥ 72 months were more prone to be disclosed their HIV positive status. This result is concordant with other reports from Uganda [27] and Ethiopia [26]. This is well known that children and care givers having long period of ART follow up visits can get ongoing information regarding to HIV/AIDS which facilitates children HIV positive status disclosure. Lost any of his/her parents was also significantly associated with children HIV positive status disclosure. This is because children who were lost any of his/her parents ask to their family members the reason why their parent passed away. Majority of the parent could be dead due to HIV/AIDS related factors. Following this the children may know their status.

Lastly, children who have ART follow up in the hospital level were more likely to be disclosed their HIV positive status. This finding is in agreement with studies reported from Southwest and Northwest Ethiopia [19, 20]. This might be due to the fact that, even though both hospitals and health centers are using the same guidelines, there is a difference in the diversity of health care workers which result a variation on detecting, counseling, and disclosure.

The results from qualitative data of this study also revealed that there were different justifications related with children's HIV positive status disclosure. Majority of caregivers approved that children's HIV positive status disclosure is essential for treatment effectiveness. After HIV positive status disclosure, children have good ART adherence level; improve their awareness to live with HIV, decrease worries and confusions. Conversely, the child age (too young) was the most common intention of care givers for not to disclose HIV positive status of their child. This finding is supported by studies conducted in Tanzania [24], and Ethiopia [14, 16, 20].

Moreover, fear of social stigma, fear of emotional and psychological disturbance, fear of child to keep secret, and ages (too young) of child were hindering factors mentioned by care givers to disclose children HIV positive status. This is in line with previous studies done in Zambia [28], and Ethiopia [16, 26]. Likewise, the child's frequent questions towards the reason why they are taking drug every day was also care givers reason to disclose. This finding is in agreement with studies reported from Zambia [28], Nigeria [29], and Ethiopia [16, 26]. Lastly, lack of separated room, lack of specific short term training for HIV status disclosure, time, and challenges to assure care givers were challenge mentioned by health care workers to conduct disclosure.

Limitations

Before interpreting the results, this study has its own limitation that must be considered. Since the study was based on respondents answer for questions related to events happening in the previous time, there might be potential recall bias. Similarly, there might be social desirability bias as the study was based on care givers information. To cross check some data and minimize these problems secondary information was used. Additionally, this cross-sectional study could not establish the cause and effect relationship.

Conclusion

The results of this study established that, there was low level of children's HIV positive status disclosure. In the study area there was no published articles, this study will be used as baseline evidence regarding to children's HIV positive status disclosure. Types of health facilities, age of the child, death of any of his/her parent, and child's duration on ART were found factors significantly associated with children HIV positive status disclosure. Caregivers did not disclose their children because their child was too immature to understand the HIV/AIDS, fear of stigma and discrimination from the community. Additionally, children's HIV positive status disclosure was delayed due to health care workers believed that they lack short term training, time, separated room, and challenges to reassure caregivers. As result, in both the hospitals and health centers the ART case managers and multidisciplinary team should focus on implementing HIV positive status disclosure counseling service for caregivers and the children in order to improve the children treatment outcome. Furthermore, working on caregiver's knowledge and attitude, updating health care workers skills are important to manage challenges and increase the rate of disclosure.

Abbreviations

AHC: Areka Health Center; **AIDS:** Acquired Immune Deficiency Syndrome; **AOR:** Adjusted odd ratio; **ART:** Antiretroviral Therapy; **BPH:** Bitena Primary Hospital; **BHC:** Boditi Health Center; **CI:** Confidence Interval; **COR:** Crud odd ratio; **HIV:** Human immunodeficiency Virus; **HPH:** Humbo Primary Hospital; **IDI:** In-depth interview; **SHC:** Sodo Health Center; **WSUCSH:** Wolaita Sodo University Comprehensive Specialized Hospital; **UNAIDS:** Joint United Nations Programme on HIV/ AIDS; **WHO:** World Health Organization.

Declarations

Consent for publication

Not applicable

Authors' contributions

BGW: conceived and designed the study, did the literature search, wrote the proposal, coordinate the data collection process, perform statistical analysis, interpret the data and prepare the first draft of the manuscript. CMT, AK, MW, and BC approve the proposal with revision; participate in data analysis, interpretation, and manuscript write-up. TL, WAB, NS, and TD reinterpret, reanalysis, and revised the manuscript critically. All authors have read and approved the final version of the manuscript.

Availability of data and materials

All the data supporting the study findings are within the manuscript. The additional detailed raw data sets used and/or analyzed during this study are available from the corresponding author up on reasonable request.

Ethics approval and consent to participate

The study was done after attaining supportive letter from Wolaita Sodo University, College of health science and medicine with protocol number SON/209/2021, written on August 24, 2021. The study was also conducted as per the declaration of Helsinki. An informed written consent was obtained from the care givers or parents after the objective, purpose and procedures of study was fully informed. To keep privacy participants name and unique ART number were excluded from the data collection tool. Additionally, confidentiality of data was kept at all levels of the study and not used for any other purposes than the stated objectives.

Competing interests

The authors declared that they have no competing interests.

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Tables

Table 1:- Socio-demographic and clinical characteristics of caregivers in Wolaita Zone, Southern Ethiopia, 2021 (n=203).

Variables	Category	Frequency (n)	Percent (%)
Age	<30 Year	29	14.3
	30-44 Year	113	55.7
	≥45 Year	61	30.0
Sex	Female	172	84.7
	Male	31	15.3
Marital Status	Married	98	48.3
	Unmarried	105	51.7
Educational status	No formal education	78	38.4
	Elementary (1-8)	57	28.1
	High school (9-12)	50	24.6
	College & above	18	8.9
Residency	Urban	188	92.6
	Rural	15	7.4
Marital Status	Married	99	48.8
	Unmarried	104	51.2
Relationship with children	First degree relatives	176	86.7
	Second degree relatives	27	13.3
HIV status	Positive	144	70.9
	Negative	59	29.1
Duration on ART	< 24 months	124	61.1
	≥ 24 months	79	38.9

Table 2:- Socio-demographic and clinical characteristics of children in Wolaita Zone, Southern Ethiopia, 2021 (n=203).

Variables	Category	Frequency (n)	Percent (%)
Age	<12	112	55.2
	≥12	91	44.8
Sex	Male	93	45.8
	Female	110	54.2
Educational status	Not started education	23	11.3
	Start education	180	88.7
Death of any of his/her parent	Yes	70	34.5
	No	133	65.5
Age at diagnosis of HIV	<4 year	109	53.7
	≥4 year	94	46.3
Duration on ART	<72 months	116	57.1
	≥72 months	87	42.9
History of ART interruption and restart	No	168	82.8
	Yes	35	17.2
WHO clinical stage	Stage I/II	192	94.6
	Stage III/IV	11	5.4
History of opportunistic infection	Yes	70	34.5
	No	133	65.5
History of Hospitalization	Yes	77	37.9
	No	126	62.1
ART adherence level	Good	181	89.2
	Fair/poor	22	10.8
With whom currently living	Biological parents	178	87.7
	Other relatives	25	12.3
Types of health facility	Health Center	78	38.4
	Hospital	125	61.6

Table 3:- Bivariable and multivariable logistic regression analysis of factors associated with HIV positive status disclosure among HIV-infected children in Wolaita Zone, Sothern Ethiopia, 2021 (n=203).

Factors	Disclosure status n (%)				
	Not disclosed	Disclosed	COR (95% CI)	AOR (95% CI)	P-value
Age of child					
<12	34 (30.4)	78 (69.6)	1	1	
≥12	7 (7.7)	84 (92.3)	6.21 (3.03-9.73)	7.54 (2.87-15.62) **	<0.001
Child's educational Status					
Not started education	11 (47.8)	12 (52.2)	1	1	
Start education	55 (30.6)	125 (69.4)	1.79 (0.89-3.87)	1.75 (0.45-2.83)	0.61
Residency					
Urban	60 (31.9)	128 (68.1)	1.28 (0.61-3.95)	1.19 (0.47-3.62)	0.42
Rural	3 (20.0)	12 (80.0)	1	1	
Death of any of his/her parent					
Yes	18 (25.7)	52 (74.3)	1.48 (1.17-2.857)	1.96 (1.05-3.84) **	0.02
No	107	171	1	1	
HIV status care givers					
Positive	54 (37.5)	90 (62.5)	2.47 (1.56-4.77)	2.44 (0.63-2.05)	0.16
Negative	14 (23.7)	45 (76.3)	1	1	
Unknown					
Child's duration on ART					
< 72 months	54 (46.6)	62 (53.4)	1	1	
≥ 72 months	17 (19.5)	70 (80.5)	3.43 (2.59-5.79)	3.84 (1.65-6.72) **	0.01
Age of child at HIV diagnosis					
< 4 year	47 (43.1)	62 (56.9)			0.85

			3.18(1.79-4.37)	1.26 (0.86-3.72)	
≥ 4 year	24 (25.5)	70 (74.5)	1	1	
Current care giver					
Biological parent	65 (36.5)	113 (63.5)	1	1	
Other relatives	4 (13.0)	21 (84.0)	3.9 (1.45-9.63)	4.59 (0.93-11.76)	0.52
Relationship with children					
First degree relative	64 (36.4)	112 (63.6)	0.47 (0.35-1.13)	2.08 (0.76-7.91)	0.29
Second degree relative	7 (25.9)	20 (74.1)	1	1	
Types of health facility					
Health Center	61(78.2)	17(21.8)	1	1	
Hospital	59(47.2)	66(52.8)	0.48(0.27-0.81)	2.13 (1.33-4.67)**	<0.001

Notes: **p-value < 0.05(statistically significant association), 1: reference category