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Research Article

**Sero-prevalence of Hepatitis B Virus among patients
attending Dental Clinics in Khartoum state-Sudan**

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ABSTRACT

Hepatitis B is an infectious disease caused by hepatitis B virus, it can cause both acute and chronic infections, there is dearth of consensus national prevalence data on Hepatitis B virus infection among patients attending dental clinics in Sudan, and no prevalence study is available. The aim of this study to detect the presence of HBsAg, and the relation of it's presence with other factors such as gender, age, social status, history of surgical operation and blood transfusion among patients attending dental clinics. HBsAg was tested by using ELISA in 90 (43 male and 47 female) apparently healthy individuals who attended dental clinics for extraction or other oral and maxillofacial surgery and other surgical procedure. The overall sero-positivity to HBs Ag among study population was 4.4 %. The sero-positivity was 2(4.7%) among males and 2(4.3%) among females. most positive result (8%)observed among 51-70 years age range. Statistical analysis showed that there was insignificant correlation between sero-positivity and factors mentioned above.

Keywords: Sero-Prevalence, Hepatitis B, surface antigen, ELISA, dental clinics, Khartoum – Sudan.

INTRODUCTION

Individual seeking dental care may be healthy or suffering from various infectious diseases or may be carriers of infectious diseases that cannot be easily identified¹.

Such patient may act as a source for spreading infection among dental health care workers and other patients in dental clinics. The major route of cross infection in dental surgery is via infection through intact skin or mucosa due to accidents involving sharps or direct inoculation onto cuts and abrasions in the skin^{2,3}.

Dental treatment procedures frequently cause bleeding and exposure to infected blood and saliva which are known means of infectious disease transmission. Routine use of barrier techniques such as gloves spectacles has been reported to be important in preventing the three routes of transmission (dentist to patient, patient to dentist and patient to patient) in dental clinics⁴.

Hepatitis B virus, abbreviated HBV is a species of the genus orthohepadnavirus which is likewise a part of the hepadnaviridae family of viruses⁵. In addition to causing hepatitis, infection with HBV can lead to cirrhosis and hepatocellular carcinoma⁶. It has also

been suggested that it may increase the risk of pancreatic cancer⁷ The virus is transmitted by exposure to infectious blood or body fluid, infection around time of birth, intravenous drug use, sexual intercourse (semen, vaginal fluid), blood transfusion ,dialysis , tattooing and acupuncture⁸

Sudan is classified among countries with high hepatitis B sero-prevalence exposure, to the virus varied from 47%-78%, with hepatitis B surface antigen sero-prevalence ranging from 6.8% in central Sudan to 26% in southern Sudan⁹. there is no published data on hepatitis B infections among patients attending dental clinics in Sudan and no prevalence study is available. This study is therefore attempt at finding out the prevalence of HBV infection (using HBsAg) among asymptomatic adult patients as a marker of infection in those attending the dental clinics in of Khartoum state .this is to highlight the potential hazards of HBV to the oral and maxillofacial surgeons and other associated health workers as well as to patients attending clinics.

MATERIAL AND METHODS

This was descriptive- cross sectional study which had been conducted in Khartoum state during period from January to March 2015, 90 patients who attended dental clinics for extraction and other surgical procedures such as diagnostic biopsy prior to oral and maxillofacial surgical procedures were enrolled, Data was collected by using direct interviewing questionnaire; ethical clearance was obtained from research ethical committee of faculty of graduate studies Al-Neelain University and ministry of health Khartoum state, written consent also was obtained from patients.

Experimental work

Samples collection:

Blood samples were collected from 90 patients, under direct medical supervision by medial vein puncture using 5 ml syringe into plain tube to obtain serum by centrifugation at 5000 rpm for 10 min. serum was kept in -20°C till serological study was performed. Specimens were processed by Enzyme linked immune sorbent assay (ELISA) (3rd generation ELISA) fortress (United Kingdom) for detection HBV surface Ag.

Enzyme linked immune sorbent assay for detection HBVs Ag

All reagents and samples were allowed to reach room temperature for 15 minutes before use Washing buffer was prepared 1:20 from buffer concentrate with distilled water. 20µl of sample diluents was added into appropriate wells except the blank well and negative well. 100µl from each sample was added to the appropriate wells and mixed by pipette repeatedly until liquids turn blue. 100µl from negative and positive control was dispensed and added to the negative and positive wells separately without dispensing liquid into the blank control well. Microtiter wells was flicked for 30 seconds and mixed well, then plate was covered and incubated for 60 minutes at 37°C Plate was taken out and 100µl of HRP-Conjugate Reagent was added in to each well except the blank, the plate was mixed well and covered with the plate cover and incubated for 30 min at 37°C. The plate cover was removed and discarded. The liquid was aspirated and each well was rinsed in wash buffer. This step was repeated for 5 times until each well become dry. 50µl of substrate A and 50µl substrate B solution was added in to each well including the Blank and mixed by tapping the plate gently. The plate was incubated at 37°C for 15 min.

50 µl Stop solution was added into each well and mixed gently.

Measuring the absorbance: The plate reader was calibrated with blank well and the absorbance was read at 450nm. The results were calculated by relating each sample optical density (OD) value to the Cut off value of plate. Calculation Cut off (C.O) value.

$$C.O = *Nc*2.1$$

*Nc= the mean absorbance value for the three negative controls.

The absorbance was read with micro well reader at 450nm.

Interpretation of Results

Negative results: samples giving absorbance less than Cut-off value are negative for this assay. Positive result: sample giving absorbance equal to or greater than Cut-off considered initially reactive. Borderline: sample with absorbance to Cut-off value are considered borderline and retesting of these samples in duplicate is recommended.

Data analysis: Data was analyzed by SPSS (Statistical Package of Social Science) software program version 16 .

RESULT

A total of 90 patients who attended dental clinics during the period from January- March 2015, consented to the study were included, 43(47.8%) were males and 47(52.2%) were females with male: female ratio of 1:1.1. The overall sero-positivity to HBV infection was 4.4% **fig(1)**, the sero-positivity among males was 4.7% while it was 4.3% among females. The study group was divided into 4 age groups The average age of patients was 42.27 years (range from 12 to 80 years) a high sero-positivity was prevalent in the age group (51-70yaers) (2.2%) **fig(2)**. 59(65.6%) of patients were married and **31(34.4%)** were single, the sero positivity among them were **3.4%, 6.5%** respectively. Regarding residence most of patients were from Khartoum 28(31.1%), however most of positive result observed among those who were from Bahri 2(8%), table (1). Patients had eight occupation groups , most of positive result observed among free acts group 2(10%)table(2).regarding patient's history: most of sero positivity was observed among those who had history of hepatitis, had no history of surgical operation, had family history of hepatitis and had no history of tattooing. all positive results were observed among those who had no history of blood transfusion table(3). most patients who had history of hepatitis were did not know which type of hepatitis they got ,the sero-positivity among this group was **14.3%**. all patients had no history of haemodialysis and organ transplantation.

DISCUSSION

Hepatitis B Virus (HBV) infection is a global health problem, with an estimated 400 million being chronic carrier of the virus¹⁰, around 1 million die due to the consequences of the infection¹¹. Sudan is classified among the African countries with high prevalence of HBV, The detectable level of HBsAg is varied from region to region and ranged between **5 and 7%** in the general population^{12,13,14,15} and **26%** in hospital outpatients¹⁶. Establishment of vaccination program and well screening in blood banks during the past eight years is expected to reduce the rate of HBV infection and the carrier pool¹⁴. The present study revealed that the sero prevalence of HBsAg among dental patients was **4.4%**, Previous studies of HBsAg in Khartoum state were merely done in selected subjects such as pregnant women representing adult females in child bearing age and haemodialysis patients who have an increased risk to blood-borne infections due to frequent dialysis. When the present result has been compared with other studies in Sudan, it seem to be closely similar to study conducted by Emad-Aldin-etal(2013) in which a **4.91% of HBVsAg positivity-** in **3172** patients undergoing surgery at Al-shaab Teaching Hospital- was reported¹⁷ and it was slightly lower than results obtained by Elsheikh-etal(2007) and OsmanAMM-etal (2014) in which **5.6% and 5.1%** HBVsAg positivity in pregnant women in Khartoum state was reported respectively^{13,18}. It was also lower than prevalence of HBV among patients attended dental clinics in other countries such as a 9.4% reported by Adewole-etal(2013) in Nigeria¹⁹ and a **38.75%** reported by SultanM-etal(2008) in Turkey²⁰.

The present study revealed high sero-positivity among age group (51-70) years, Males and females

had approximately equal sero-positivity. But was disagree with results of and Nigeria and Alexandria in which the presence of HBV was found to be more in males and within age groups of 30-39 and 20-29 years^{19, 21}. Present findings were different to result obtained by SHITTUA.O.-etal (2014) among blood donors in Nigeria, The present sero positivity to HBV was lower than a 10.9% reported in his study, 25%(1/4) of our positive result were among non working while he reported a 36.8%, 25%(1/4) of positive result were among patients who had history of surgical operation while he reported a 2.6%, 100% of positive results were among patients who had no history of blood transfusion while he reported a 5.2% sero-positivity among those who had history of blood transfusion, 25%(1/4) of positive result were among patients who had history of tattooing while he reported a 10.5%²². The present prevalence of HBsAg was higher than the prevalence of 2.5% obtained by Solang Zacalusni Freitas-etal (2014) among HIV- infected patients in the State of MS, Central Brazil, The present sero-positivity 7.1%(1/13) among patients who had family history of hepatitis was extremely lower than a 42.7% reported in the same study²³. In the present study a high sero-positivity 12.5% (2/16) of HBV were observed among patients who had history of hepatitis opposite result reported by Abdelsalam – e tal(2013) among tuberculosis patients in Sudan in which the majority(84.2%) of patients had no history of hepatitis, None of HBV positive patients participated in the present study had history of blood transfusion similar observation reported in the same study²⁴.

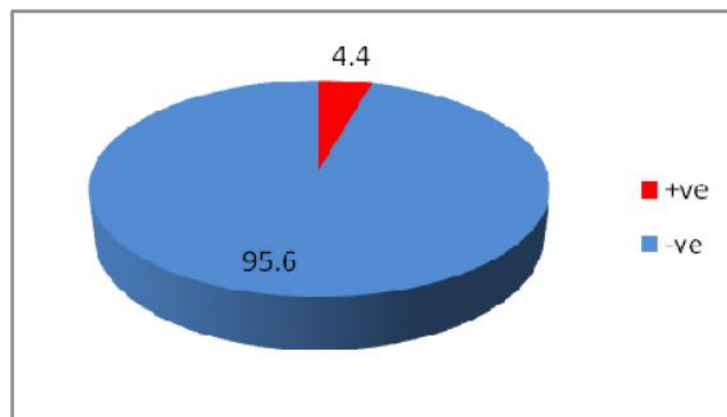


Fig 1
Sero-prevalence of HBV among patients attending dental clinics (n=90)

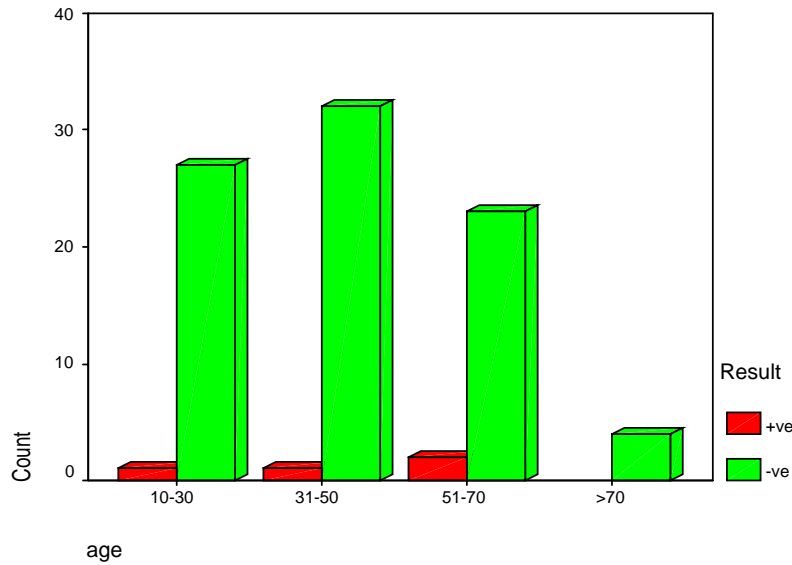


Fig 2
Distribution of HBV seropositivity among patients according to their age

Table 1
Distribution of HBV seropositivity among patients according to their residence

			Result		Total
			+ve	-ve of	
Residence	Khartoum	Count	1	27	28
		% within Residence	3.6%	96.4%	100.0%
		% within Result	25.0%	31.4%	31.1%
		% of Total	1.1%	30.0%	31.1%
	Omdurmn	Count	1	15	16
		% within Residence	6.3%	93.8%	100.0%
		% within Result	25.0%	17.4%	17.8%
		% of Total	1.1%	16.7%	17.8%
	Bahri	Count	2	23	25
		% within Residence	8.0%	92.0%	100.0%
		% within Result	50.0%	26.7%	27.8%
		% of Total	2.2%	25.6%	27.8%
Out of khartoum	Count	0	21	21	
	% within Residence	.0%	100.0%	100.0%	
	% within Result	.0%	24.4%	23.3%	
	% of Total	.0%	23.3%	23.3%	
Total	Count	4	86	90	
	% within Residence	4.4%	95.6%	100.0%	
	% within Result	100.0%	100.0%	100.0%	
	% of Total	4.4%	95.6%	100.0%	

Table 2
Distribution of HBV seropositivity among patients according to their Occupations

Occupation			Result		Total
			+ve	-ve	
	Free acts	Count	2	18	20
		% within Occupation	10.0%	90.0%	100.0%
		% within Result	50.0%	20.9%	22.2%
		% of Total	2.2%	20.0%	22.2%
	House wife	Count	0	26	26
		% within Occupation	.0%	100.0%	100.0%
		% within Result	.0%	30.2%	28.9%
		% of Total	.0%	28.9%	28.9%
	Tea maker	Count	1	2	3
		% within Occupation	33.3%	66.7%	100.0%
		% within Result	25.0%	2.3%	3.3%
		% of Total	1.1%	2.2%	3.3%
Student	Count	0	10	10	
	% within Occupation	.0%	100.0%	100.0%	
	% within Result	.0%	11.6%	11.1%	
	% of Total	.0%	11.1%	11.1%	
Employee	Count	0	13	13	
	% within Occupation	.0%	100.0%	100.0%	
	% within Result	.0%	15.1%	14.4%	
	% of Total	.0%	14.4%	14.4%	
Worker	Count	0	3	3	
	% within Occupation	.0%	100.0%	100.0%	
	% within Result	.0%	3.5%	3.3%	
	% of Total	.0%	3.3%	3.3%	
Health care worker	Count	0	4	4	
	% within Occupation	.0%	100.0%	100.0%	
	% within Result	.0%	4.7%	4.4%	
	% of Total	.0%	4.4%	4.4%	
Nonworking	Count	1	10	11	
	% within Occupation	9.1%	90.9%	100.0%	
	% within Result	25.0%	11.6%	12.2%	
	% of Total	1.1%	11.1%	12.2%	
Total	Count	4	86	90	
	% within Occupation	4.4%	95.6%	100.0%	
	% within Result	100.0%	100.0%	100.0%	
	% of Total	4.4%	95.6%	100.0%	

Table 3
Distribution of HBV seropositivity among patients according to their history

Risk factors			Result		Total
			+ve	-ve	
History of hepatitis	Yes	Count	2	14	16
		percent	12.5%	87.5%	100.0%
	No	Count	2	72	74
		percent	2.7%	97.3%	100.0%
	Total	count	4	86	90
History of surgical operation	Yes	Count	1	32	33
		percent	3.0%	97.0%	100.0%
	No	Count	3	54	57
		percent	5.3%	94.7%	100.0%
	Total	count	4	86	90
Family history of hepatitis	Yes	Count	1	13	14
		percent	7.1%	92.9%	100.0%
	No	Count	3	73	76
		percent	3.9%	96.1%	100.0%
	Total	count	4	86	90
History of blood transfusion	Yes	Count	0	21	21
		percent	.0%	100.0%	100.0%
	No	Count	4	65	69
		percent	5.8%	94.2%	100.0%
	Total	count	4	86	90
History of tattooing	Yes	Count	1	20	21
		percent	4.8%	95.2%	100.0%
	No	Count	3	66	69
		percent	4.3%	95.7%	100.0%
	Total	count	4	86	90

The risk of transmission of HBV to health care workers, nurses, operative theatre staff and surgeons have been stressed²⁵. The first case of patient to patient transmission of HBV in oral surgery was reported in the United State in 2007, attributed to blood with high viral load inadvertently on a surface/clothing missed during cleaning up / disinfection of operatory, suggested as source of infection from the source patient to the succeeding patient treated 161 minutes later²⁶. The infection occurred despite adherence to standard precautions and all recommended infection control

practices, The explanation was that most oral surgery procedures result in bleeding hence HBV present a risk of transmission of both dental staff and patients. it was therefore stressed that appropriate infection control measures must be followed on routine basis^{27,28,29}. Also all dental practices should adhere to the guidelines for infection control in dental health care setting published in 2003 by CDC. These guidelines are advocated to be followed for each and every patient in addition to standard precaution such as hand hygiene, surface disinfectant and sterilization²⁷.

The obtained sero-positivity to HBV is important and there is a chance of transmission of infection to oral and maxillofacial surgeons, patients and other health care workers. Dental health care workers have been found to be 10 times more at risk of being a chronic carrier of HBV than in the general population³⁰. Statistical analysis showed that there was insignificant correlation between risk factors and sero-positivity of HBsAg.

CONCLUSION

The sero frequency of hepatitis B is high among patients attending dental clinics so a pre-operative screening (of all patients being prepared for surgery) for HBV is recommended as a routine, this is not for stigmatization, but to enable the healthcare givers make adequate preparations and take appropriate preventive measures when managing such patients. To reduce the chances of infection of healthcare givers therefore, all of doctors, dentists, surgeons should be vaccinated against HBV preferably at the start of their careers³¹.

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REFERENCES

1. samaranayake L. Rule of infection control. *Int Den J*, 1993;43(6):578-84
2. Girdler NM, Mattherws RW and Scully C. Use and acceptability of rubber gloves for outpatient dental treatment. *J Dent* 1987; 15(5):209-12.
3. Verrusio AC, Neidle EA, Nas KD, Silverman S, Jr, Horowitz AM and Wager KS. The dentists and infectious diseases: a national survey behavior and attitudes.1989.
4. Adel AM, Nadia MM, Azza MT. Knowledge and attitudes of dental patients towards cross infection control measures in dental practice 1997.
5. Hunt, Richard,"hepatitis viruses". University of southern California, department of pathology and microbiology. Retrieved 2008-03-13.
6. Hassan MM, Li D, El-Deeb AS, Wolff RA, Bondy ML, Davila M and Abbruzzese JL.

- Association between hepatitis B virus and pancreatic cancer. *J. Clin. Oncol.*26 (28):4557-62.
7. Schwalbe M, Ohlenschlager O, Marchanka A, et al. solution structure of stem-loop alpha of the hepatitis B virus posttranscriptional regulatory element". *Nucleic acid Res.* 2008; 36 (5):1681-9.
 8. Hepatitis B FAQs for the public _transmission. U.S. Centers for Disease Control and Prevention (CDC).Retrieved 2011-11-29.
 9. Expanded program on immunization, hepatitis B vaccine: WHO 1996.
 10. Lee WM, Hepatitis B virus infection. *N Engl J Med*, 1997; 337:1733-45.
 11. Mamun AIM ,Fasle Akbar SM, HBeAg negative chronic Hepatitis B an overview .*Hepatitis B Annual*, 2009; 6(1):131-140.
 12. Abou MA, Eltahir YM and Ali AS. seroprevalence of hepatitis B virus and hepatitis C virus among blood donors in Nyala,South DarFur,Sudan. *Virology J*, 2009; 6:146.
 13. Elsheikh RM, Daak AA, Elsheikh MA, Karsany MS and Adam I. Hepatitis B virus and Hepatitis C virus in pregnant Sudanese women *Virol. J*, 2007.4:104.
 14. Mudawi HM, Smith HM, Rahoud SA, Fletcher IA, Saeed OK and Fedail SS. Prevalence of Hepatitis B virus infection in the Gezira state of central Sudan. *Saudi J.Gastroentrol*, 2007; 13(2):81-3.
 15. Omer EE, P.Van't Veer A, Kadaru M, Kampman E, El khidir IM, Fedail SS and kok FJ, the role of Hepatitis B and Hepatitis C viral infections in the incidence of Hepatocellular carcinoma in Sudan. *Trans. R.Soc. Trop.Med.Hyg*, 2001;95(5):487-491.
 16. McCarthy MC, A.el-Tigani, Khalid IO and Hyams KC. Hepatitis B and C in Juba Southern Sudan: Result of a serosurvey .*Trans. R. Soc. Trop. Med. Hyg*, 1994; 88(5):534-536
 17. Emad-Aldin Ibrahim Osman, Nagwa Ahmed Abdulrahman, Osman Abbass, Waleed Hussein Omer, Hafi Anwer Saad and Muzamil Mahdi Abdel Hamid. Prevalence of Hepatitis B surface antigen and Hepatitis C virus antibodies among pre-surgery screened patients in Khartoum, Central Sudan. *Global J of virology and immunology*, 2013; 1(1):22-25.
 18. OsmanAMM, MirghaniOA, Gasim GI, Adam I. Hepatitis B Virus, Hepatitis C Virus and Human Immunodeficiency Virus

- Infections among Pregnant Women in Central Sudan. Sudan journal of medical sciences, June 2014; Vol 9(2).
19. Adewole RA, Omilabu SA, Ayodele AOS, Gbotolorun OM and Anorue El. Prevalence of Hepatitis B surface antigen (HBsAg) in patients attending Dental Centre of a Tertiary Hospital (a pilot study). J of medicine and medical sciences. 2013; 4(7):275-279.
 20. Sultan. M, Bhatti.M.S, Fakhar – US-Nisa and Nagi AH. Audit of Hepatitis B and C Viruses in Patients attending Dental Clinics. E:/Biomedica, 2008; 24(1):50-53.
 21. Wasfi OAS, Sadek NA. Prevalence of Hepatitis B surface Antigen and Hepatitis C virus Antibodies among Blood Donors in Alexandria, Egypt, Eastern Mediterranean Health J, 2011; 17(3): 238-42.
 22. Shittu.A.O, Olawumi.H.O, Issa.B.A, Nwabuisi.C, Durotoye.I.A, Yussuf.A.D, Ajiboye.P.O, Adegunloye.O.A, Sulyman.D, and Salami.A.K, Risk factors and seroprevalence of Hepatitis B Surface Antigen among blood donors in university of Ilorin teaching Hospital, Ilorin, Nigeria. East African Medical J, March 2014; 91(3): 1-5.
 23. SolangZacalusniFreitas,CarolineCorderioSoares,AnaRitaCoimbraMotta-Castro, Prevalence, risk factors and genotyping of hepatitis B infection among HIV- infected patients in the State of MS, Central Brazil.Barzilian J of infectious diseases, sept-oct 2014;18(5) :473-48.
 24. AbdelsalamM.Nail,NazarE.Ahmed,MohammedO.E.Gaddour, Seroprevalence of hepatitis B and C Viruses among tuberculosis patient. Sudan journal of medical sciences, Marsh 2013; 8(1) 17-22.
 25. Emeka Bk, Kenechi U, Eshiobo I, Andrew D, Kefas John B, Bamidele A. A knowledge of Hepatitis B vaccine among operating room personnel in Nigeria and vaccination status Hepatitis research and treatment 1 D157089 Doi:10.1155/2011/157089.
 26. Depaola LG. Patient to Patient transmission of Hepatitis B virus associated with oral surgery: How did it happen? The Richmond institute (2007), V Issue (III): 1-6. newsletter@biotrol.com.
 27. Center for Diseases Control and Prevention (CDC)(2003).Guidelines for infection control in dental health-care settings , 2003.MMWR Morb Mortal Wkly Rep.2003 , Dec 19,2003;152(17):1-68.
 28. Center for Diseases Control and Prevention (CDC)(1990).possible transmission of human immunodeficiency virus to a patient during an invasive dental procedure, MMWR;39:489-93.
 29. AllosB, SchaffnerW, Transmission of hepatitis B in the healthcare setting:The elephant in the room and mouse ?. J.Infect Dis, 2007; 195(9):1245-47.
 30. Wisnom CJ, Lee RJ. Increased seroprevalence of hepatitis B in dental personnel necessitates awareness of revised paediatric hepatitis B vaccine recommendations J.Public Health Dentistry, (1993); 53:231-234.
 31. Odaibo GN, Arotiba JT, Fasola AO, Obiechina AE, Olaleye OD, Ajagbe HA. Prevalence of Hepatitis B virus antigen (HBsAg) in patients undergoing extraction at the University College Hospital, Ibadan, Afr. J.Med.Med.Sci, 2003; 32(3):243-245.