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Original article

TEGUMENT BASED IN-SILICO DRUG TARGETING OF HERPES SIMPLEX VIRUS-1

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АСПЕКТЫ МЕДИКАМЕНТОЗНОГО ЛЕЧЕНИЯ ВПГ-ТИПА 1

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Alpha Trans Inducing Factor (α-TIF) is a herpes simplex virus type 1 (HSV-1) virion tegument protein present in the tegument layer between the capsid and the envelope, in association with cellular proteins and trans-activated viral activities. α-TIF stimulates the transcription of HSV-1 Immediate Early genes during lytic virus replication. The presence or absence of functional α-TIF protein *in vivo* is always associated with viral activities. Its role behind latent HSV-1 infection is ambiguous. No drug is designed for HSV till now based on this protein and thus its conformational details can be very important for drug designing purpose. **Methods.** Docking studies on this protein becomes logical. Four different ligand molecules *viz.* Adenosine-3'-5'-Diphosphate, P1-(5'-Adenosyl) P4-(5'-(2'-Deoxy-Thymidyl)) Tetraphosphate, 9-Hydroxymethylguanine, 9-(4-Hydroxybutyl)-N2-Phenylguanine were screened for the study. **Results.** P1-(5'-Adenosyl) P4-(5'-(2'-Deoxy-Thymidyl) Tetraphophate was found to be the best ligand as it showed the lowest docking energy of – 9.238 kcal/mol. **Conclusions.** The best ligand was found to bind different sites of the α-TIF protein and hence can be utilized to combat HSV-1 successfully, with a synergistic effect of multiplicity of drug molecules on the target protein.

Keywords: α-TIF, tegument, HSV-1, Modeller, Deep viewer-Swiss Pdb viewer, Procheck, Rasmol, Docking, Arguslab, Chimera.

Ашиш Рунтала, Амит Кумар Синг. **Аспекты медикаментозного лечения ВПГ-типа 1 Саратовский научно-медицинский журнал, 2010, том 6, № 2, с. 353-357.**

Альфа-транспорт-индуцирующий фактор является вирионом вируса простого герпеса типа 1, покрытого протеином между капсидом и оболочкой, связанным с клеточными протеинами и обеспечивающий транспортную функцию. Альфа-транспорт-индуцирующий фактор стимулирует быструю расшифровку генетического аппарата ВПГ типа 1 в течение вирусной репликации. Наличие или отсутствие функционального альфа транспорт индуцирующего протеина в условиях эксперимента всегда связано с вирусной активностью. Его роль после перенесенной латентной вирусной инфекции ВПГ типа 1 – значительна. До настоящего времени не было разработано лекарственных препаратов на основе протеина для лечения ВПГ типа 1. Поэтому его структурные особенности важны для создания нового препарата. Сравнительный анализ белка заключался в его поэтапном изучении. Четыре различные молекулярные лиганды, т.е. Аденозин -3'-5'-Дифосфат, P1-(5'-Аденозил) P4-(5'-(2'-Ди-окситимидил)) Тетрафосфат, 9-Гидроксиметилгуанин, 9-(4-Гидроксибутил)-N2-Фенилгуанин – были взяты для изучения. В результате исследования P1-(5'-Аденозил) P4-(5'-(2'-Диокси-Тимидил) Тетрафосфат – оказался лучшей лигандой, т.к. показал наименьшую энергию – 9.238 ккал/моль. Доказано, что лучшая лиганда связывает различные части транспорт индуцирующего протеина и таким образом может быть выделен для успешной борьбы с ВПГ типа 1, с подобным эффектом множества лекарственных молекул в основе протеина.

Introduction. Proteins are the basis of functional machinery in a cell. Proteins are the building blocks of an organism as they are involved in regulating the various activities [1]. Structural representations are the most useful parameters for understanding protein function. So, the Structure Prediction aims at deriving detailed structural information of high resolution for understanding biological function qualitatively [2]. Understanding structure has potential applications in the various genome projects being undertaken, such as mapping the functions of proteins in metabolic pathways for whole genomes and deducing significant evolutionary relationships.

Herpes viruses are a leading cause of human viral disease, second only to influenza and cold viruses. They are capable of causing overt disease or remaining silent for many years only to be reactivated, like shingles. The name herpes comes from the Latin term *herpes* which, in turn, comes from the Greek word *herpein* which means to creep [3]. This reflects the creeping or spreading nature of the skin lesions caused by many herpes virus types. Once a patient has become infected by herpes virus, the infection remains for entire life. The initial infection may be followed by latency with subsequent reactivation. Herpes viruses infect most of the human population and persons living past middle age usually have antibodies to most of the herpes viruses except Human Herpes Virus Type 8 (HHV-8).

Herpes viruses are enveloped viruses. They bud from inner nuclear membrane, modified by the insertion of herpes glycol-proteins. In the mature virus, these glycoproteins determine the cell to be infected because of the availability of the appropriate receptors. The viral membrane is quite fragile and a virus with a damaged envelope is not infectious. This essentially means that the virus readily falls apart and so the virus can only be

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obtained by direct contact with mucosal surfaces or secretions of an infected person. Besides drying, the virus is also sensitive to acids, detergents and organic solvents as might be expected for a virus with a lipid envelope.

These viruses have a doughnut shaped capsomere of about 100-200 nm in diameter with an icosahedral nucleocapsid, containing 162 capsomeres. The space between the envelope and capsid is called tegument. It contains virally-encoded proteins and enzymes involved in the initiation of replication. α-TIF, a herpes simplex virus type 1 (HSV-1) tegument protein, in association with cellular proteins, transactivates viral immediate early genes. In order to examine the role of α-TIF during acute and latent infection in vivo, a mutant virus containing a 12-base-pair insertion in the α-TIF gene, lacking transactivating function of α-TIF was examined in mice. Following corneal inoculation, mutant parental virus (17+) and the revertant (1814R) strains replicated effectively in eyes and trigeminal ganglia with 30 - 60% mortality rates.

Neither equal Plaque Forming Units (PFU) nor particle numbers replicated in trigeminal ganglia and therefore don't kill any mice. Even low inoculated amounts are sufficient to establish latent infection in some animals. Since no infection is detected at all in mouse trigeminal ganglia, it poses a target regarding the commencement of latency soon after primary infection. Latency with the infection of reverent strain 1814 is detected right after the presence of virus in the sensory ganglia, during 24 to 48 hour tenure after infection. Thus, though α-TIF may be required for lytic infection *in vivo*, it is dispensable for the establishment of reactivation from latent infection. These details support the hypothesis that the latent and lytic pathways of HSV-1 are distinct and that latency is established soon after infection without any requirement for viral replication. However, α-TIF levels reaching neuronal nuclei may be a critical determinant of lytic or latent infection. α-TIF is a HSV-1 virion protein present in the tegument layer between the capsid and the envelope. α-TIF stimulates the transcription of HSV-1 immediate early genes during lysis and virus replication. This study examines the consequences of the presence and absence of functional α-TIF protein *in vivo* with respect to viral replication and latency. A hypothesis for the mechanism of latent HSV-1 infection that includes a role for this protein is also proposed.

Methods. Homology modeling was carried out, as we found a high homology between target sequence of α-TIF and the known structures. This process is conceptually very simple. The target sequence is aligned with known structures in Protein Data Bank (PDB) database through Basic Local Alignment Search Tool in the PDB dataset (PDB-BLAST) tool using Position-Specific Iterated BLAST algorithm (PSI-BLAST) [6, 7] available on National Centre for Biotechnology Information (NCBI) web server with the already modeled sequences, for which the structure has already been determined by experimental methods. FAST Alignment (FASTA) format of this α-TIF target protein can be accessed from GENOME BANK (GENBANK) with accession number AAA45862.1.

PSI-BLAST on the target sequence resulted in Virion Protein 16 (VP16) as the known template structure to model α-TIF protein sequence, as it showed highest 86% sequence similarity, with 2e-180 E-value, which is an excellent score for a template structure. This template is a Transcriptional Regulatory Protein [9]. Its chain A is the template for the target sequence, being the conserved core of the Herpes Simplex Virus Transcriptional Regulatory Protein. Its conformational details were taken from Protein Data Bank (PDB) databank [10].

Results.

Modeling by Modeller9v7

Modeller9v7 was used to generate 100 structural model decoys which were then checked for their DOPE (Discrete Optimized Potential Energy) scores along with the z-score for the statistical accuracy of the prediction. Through energy graph displayed in Figure 1, model 91 was found to have the reasonably low DOPE score of -47037.96484with the lowest z score of 0.18014 and was thus found to be the most stable model conformation.

Assessment

The model was obtained by homology modeling. But, there is a need to access this resultant model for the errors. The resultant structure was assessed by various

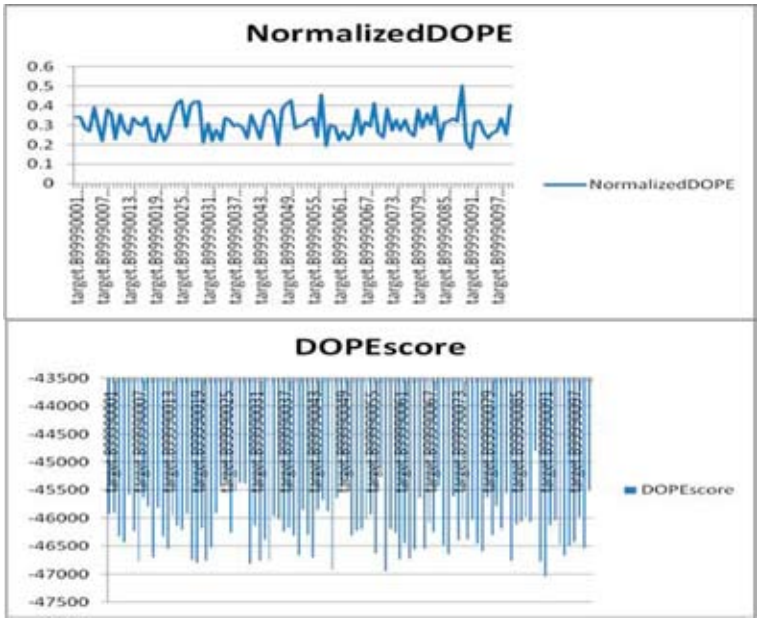

Figure1: Normalized DOPE or z score of analysis for selecting the best model decoy among generated models, shows that model 91 has the reasonably low DOPE score of-47037.96484 forming the best set with the lowest z score of 0.18014 and was thus selected for later analysis

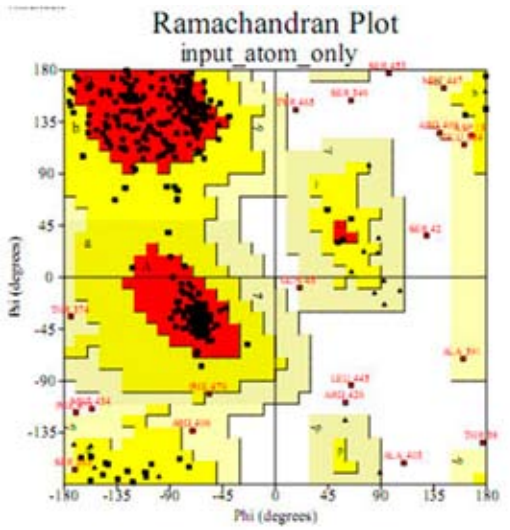

Figure 2: Ramachandran Plot for the best model highlights the structural details, showing the native fold for the protein

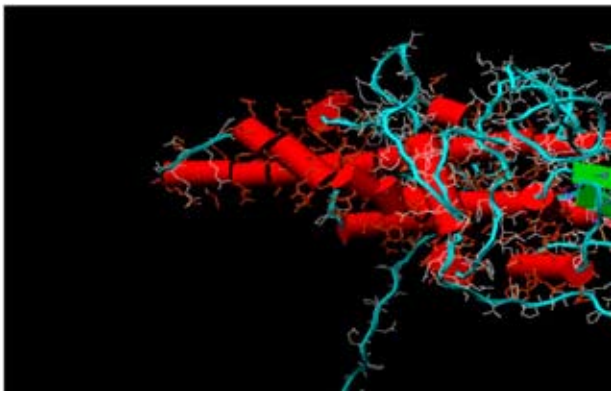

Figure 3: α-TIF protein of HSV-1. Its secondary structure is highlighted, Helices – Red
Strands-Green, Turns – Cyan. Figure clearly displays high proportion of turns as compared to strands. It also depicts that helix forms the Hydrophobic core of the protein α-TIF

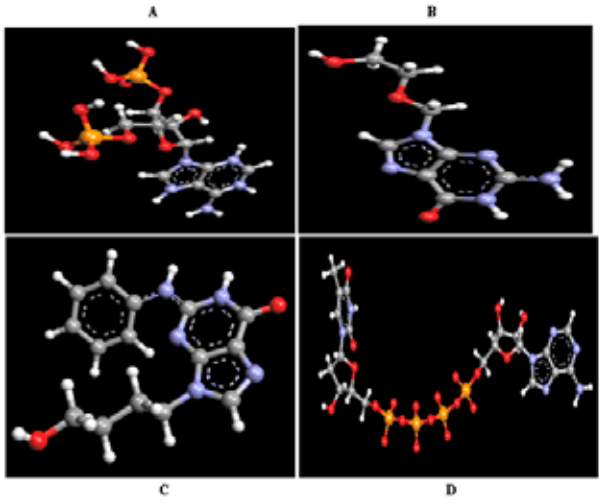

Figure 4: Structural details of different ligands screened for docking α-TIF protein. **A.** Adenosine-3'-5'-Diphosphate, **B.** P1-(5'-Adenosyl) P4-(5'-(2'-Deoxy-Thymidyl)) Tetra phosphate, **C** 9-Hydroxymethylguanine, **D** 9-(4-Hydroxybutyl)-N2-Phenylguanine

Table 1

α-TIF protein conformation prediction was found to be the native state considering the following Ramachandran analytical statistics

	Number of residues	Percentage
Most favoured regions	356	85.2
Additional allowed regions	42	10
Generously allowed regions	9	2.2
Disallowed regions	11	2.6
Non-glycine and non-proline residues	418	100
End residues (excluding glycine & proline)	1	
Glycine residues	31	
Proline residues	40	
Total number of residues	490	

tools, described below. This was all needed for the structural validation of the resultant structure of the modeling process.

RMSD (Root Mean Square Deviation): First it is logical to confirm that the designed α-TIF conformation is not digressing from the template and the possible native state [18]. It can be easily studied by determining the RMSD of α-TIF conformation against VP16 template structure in most of the protein structural visualization programs like DeepView, Swiss PDB Viewer etc. The RMSD value, considering the alpha carbons, was determined to be 1.17A° and 1.16A° when backbone atoms and side chain atoms were considered.

Whatcheck: Through Protein Motif (Promotif) then, it was revealed that this structure has a variety of structural details [12]. The α-TIF has 1 chain with 3 strands having the number and percentage of alpha helices and 3, 10 helices respectively equal to 10, 34.7% and 2, 1%. The 3 strands of this structure lies in the locations of 255-258, 277-280 and 283-284 as displayed in Figure 2.

Procheck: Protein Structure Checks (PROCHECK) showed that the structure having 490 residues has 82.5% residues lying in the core, 10% in allowed region, 2.2 % in generously allowed region and 2.6% in disallowed region [15]. Overall Ramachandran plot showed that 85.2% residues lie in the most favorable region. No main chain or side chain parameters were found worse in the depicted structure. All residues showed a maximum deviation of 4.0 A° with 12 bad contacts throughout the resultant conformation. Bond lengths and Bond angles were also found to be 96.7 % and 87.5% in the structurally stable region.

The procheck .prm file was set according to our desired parameters. Procheck v. 3.3 was used for the analysis. This tool resulted in 10 different plots. These were the Ramachandran plot, Glycine & Proline Ramachandran plot, side chain torsion angles Chi1-Chi2 plots, Main-chain parameters, Side-chain parameters, Residue properties, Main-chain bond length distributions, Main-chain bond angle distributions, Root Mean Square distances from planarity, Distorted geometry plots; overall G-value is less than -0.5 which is considered as a good value. Procheck result is summed up below (Table 1 and Table 2).

Model Visualization: Structural models were visualized by University of California San-Francisco (UCSF)

Table 2

Different parameters important for the structural assessment, including chi and Phi-Psi angle distributions is scored, showing that the predicted conformation is the native state

Parameter	Score	Average Score
Dihedral Angles		
Phi-Psi Distribution	.01	
Chi1-Chi2 Distribution	-0.14	
Chi1 only	0.05	-0.09
Chi3 and Chi4	-0.01	
Omega	-0.21	
Main Chain Covalent Forces		
Main-Chain Bond Lengths	-0.33	-0.60
Main-Chain Bond Angles	-0.79	
Overall Average		-0.27

Chimera [5]. Model is shown in ribbon structure in figure 3, where secondary structures are denoted by different colors like; helix-red, strand-green, light blue-turns [17].

Docking

To trace the cure for HSV-1, different ligands were screened for herpes virus considering the PDB as a lignad depository source [19]. These ligands were then screened out on different parameters. First, ligands binding to herpes capsid protein were screened and all the ligands sharing similar properties were sorted out. Common ions, which are already reported as ligands in pdb were then rejected. Then, the flexible docking studies of the protein with the selected ligands were carried out using the ArgusLab docking software [13]. All residues of ligands were considered for doing docking studies. It was done by searching 106 to 150 different poses of the ligands, showing that these sites could be used for further analysis [16]. Docking time utilized was from 145 to 290 seconds, showing that these ligands can effectively act on target protein in a very limited time period. Docking energy parameter for different ligands is tabulated below in table 3 and the structural conformation of these ligands is shown in figure 4. Structural detail of best docked protein with ligand is shown in Figure 5.

Table 3

Docking energy of different screened ligands with the predicted protein conformation is tabulated, showing that P1-(5'-Adenosyl) P4-(5'-(2'-Deoxy-Thymidyl) Tetra phosphate was best screened ligand as it showed the lowest Docking Energy of -9.238 Kcal/mol

Ligands	Docking energy
P1-(5'-Adenosyl)-P4-(5'-(2'-Deoxythymidyl))-Tetraphosphate	– 9.238 kcal/mol
Adenosine-3'-5'-Diphosphate	– 7.955kcal/mol
9-Hyroxyethoxymethylguanine	– 7.26kcal/mol
9-(4-Hydroxybutyl)-N2-Phenylguanine	– 8.54kcal/mol

Discussion. NCBI server was first used to get fasta format of α-TIF, then by blast against PDB we got template for our protein, and verified the result with the help of PDBsum server [4]. Then after template identification, alignment was done between target and template by using align2d.py file of modeller9v5. After that by using model-single.py file of modeller9v5 we generated 100 models to verify the quality of generated models procheck was done. By procheck 10 plots were generated for every model, by comparing these plots, it was concluded that model 13 is the best structural conformation. Then energy minimization of model 13 was attempted by swiss pdb viewer, and finally best model structure was accessed by using various other servers like Whatcheck, etc. to check the compactness of structure. Flexible docking was then attempted by arguslab software because of its various interactive features like retrieving all possible poses where ligand can bind to a

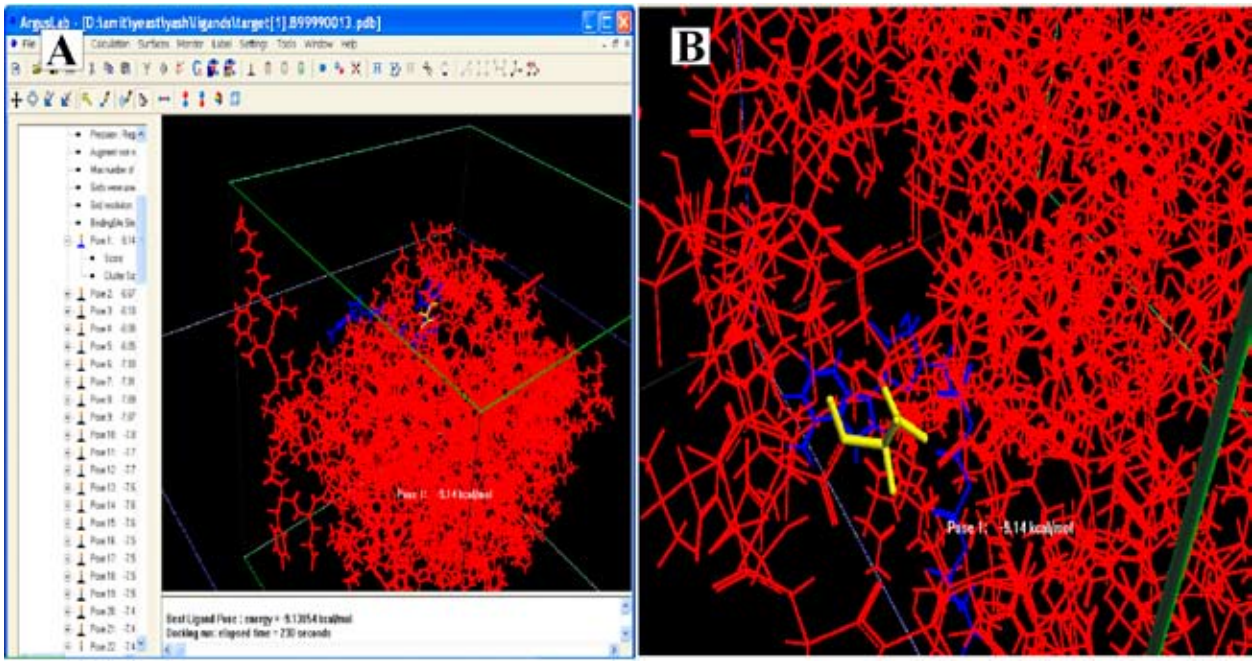

Figure 5: (A) Illustration of the docking result of best fit ligand P1-(5'-Adenosyl) P4-(5'-(2'-Deoxy- Thymidyl)) Tetraphosphate (shown in blue and yellow) with protein α-TIF (shown red). It is clearly visible that ligand binds at outermost portion of protein making it a better future drug. **(B)** Closer view of docking confirming that the ligand is better entangled in outer region of protein efficiently

protein. Thus, one can carefully select different residues from the protein to look for specific binding site. After getting docking result in form of dock energy, it can be easily concluded that adenosyl terta-phosphate is the best screened ligand for the desired protein conformation.

Conclusion. α-TIF, a herpes simplex virus type 1 (HSV-1) tegument protein, in association with cellular proteins, trans-activates viral immediate early genes. In order to examine the role of α-TIF during acute and latent infection, the structure of α-TIF protein is too important [20]. This structure can be used further for drug, vaccine or antibody designing. So predicted structure of α-TIF can be very useful in future in controlling infection of HSV-1. Docking energy accomplished in this work has many applications like ligands screened out here can be used for drug trials as they are already in use so they can be easily used against HSV-1.

Conflict of Interest.

This article does not implies consideration of any significant financial interest in a company (or its competitor) producing any of the software or server used in the article. Amit has the credit to collect the data initially.

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Original article

OCCUPATIONAL RISK OF HIV INFECTION AMONG DENTAL SURGEONS IN NIGERIAN

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РИСК ЗАРАЖЕНИЯ ВИЧ – ИНФЕКЦИЕЙ У ХИРУРГОВ-СТОМАТОЛОГОВ НИГЕРИИ В ПРОФЕССИОНАЛЬНОЙ СРЕДЕ

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C.Ch. Azodo. **Occupational risk of HIV infection among dental surgeons in Nigerian. Saratov Journal of Medical Scientific Research, 2010, vol. 6, iss. 2, p. 357-360.**

Background: Prevention of accidents and management of exposures in the work environment is an important occupation health issue. This study objective was to investigate the occupational risk of HIV among Nigerian dental surgeons. **Methods:** This descriptive cross sectional survey of 300 dental surgeons practicing in private and government owned dental centers in Nigeria was conducted from June 2006 to January 2007. **Results:** Percutaneous injury was recorded among 69.3% of respondents and only 1.2% had Post exposure prophylaxis. Those with abraded skin that will treat patient without additional barrier were 8.6%. Percutaneous injury was positively related to gender, position, additional qualifications ($p<0.05$). **Conclusion:** Percutaneous injury is significantly high and low preventive measure at such exposure. Policies, practices, and trainings geared towards protecting and reducing the prevalence of percutaneous injury among dental surgeons, and improving post exposure prophylaxis uptake in the event of exposure is a necessity

Keywords:, dentist, infection, HIV, occupational risk.

К.Ч. Азодо. **Риск заражения ВИЧ-инфекцией у хирургов-стоматологов Нигерии в профессиональной среде. Саратовский научно-медицинский журнал, 2010, том 6, № 2, с. 357-360.**

Сокращение количества инцидентов в профессиональной среде является важной проблемой современного здравоохранения. Целью исследования представляется изучение фактора риска заражения ВИЧ-инфекцией среди хирургов–стоматологов Нигерии. Данный дискриптивно-профильный анализ включал в исследование 300 хирургов-стоматологов, работающих в государственных и частных стоматологических клиниках Нигерии. Научная работа проводилась с июня 2006 года по январь 2007 года. Подкожные повреждения были выявлены у