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Human saliva can be a diagnostic tool for Zika virus detection

Zohaib Khurshid[a], Muhammad Zafar[b,c,*], Erum Khan[d], Maria Mali[e], Muhammad Latif[f]

[a] Department of Prosthodontics and Dental Implantology, College of Dentistry, King Faisal University, Al-Hofuf, Saudi Arabia
[b] Department of Dental Materials, College of Dentistry, Taibah University, Medina Munawwarah, Saudi Arabia
[c] Department of Dental Materials, Islamic International Dental College, Riphah International University, Islamabad, Pakistan
[d] Department of Oral Pathology, Liaquat University of Medical and Health Sciences, Jamshoro, Pakistan
[e] Department of Orthodontics, College of Dentistry, Islamic International Dental College, Riphah International University, Islamabad, Pakistan
[f] Centre for Genetics and Inherited Diseases (CGID), Taibah University, Medina Munawwarah, Saudi Arabia

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ABSTRACT

Zika virus (ZIKV), an *Aedes* mosquito-borne flavivirus, has captured public health attention worldwide. Initially, the virus was reported in Africa and Asia. However, the outbreak of ZIKV in Brazil and the United States of America demonstrated the global health risk. Symptoms of ZIKV infection vary from mild fever, rash, and joint pain to an apparent increase in microcephaly in infants and severe manifestations including Guillain–Barré syndrome in adults. Such consequences led to enormous health challenges, and consequently the World Health Organization declared a global health emergency. This review incorporates all aspects of ZIKV that could significantly impact human health, including epidemiology, clinical presentation, possible complications, cutting-edge therapeutic management of ZIKV infection, and latest developments in ZIKV diagnosis, particularly the value of human saliva as a diagnostic fluid.

Contents

Introduction

During the last few decades, human saliva has been identified as having significant characteristics to assist in evaluating systemic and oral health. Notably, human saliva has been called a "*mirror of the body's health*." [1] Human whole-mouth saliva (WMS) is a complex mixture of desquamated oral epithelial cells, salivary gland secretions (major/minor), gingival crevicular fluid (GCF), and microorganisms, including viruses, bacteria, and fungi [1]. Furthermore, it contains proteinases, proline-rich proteins (PRPs), amylase, IgA, lactoferrin, mucins, statherin, glycoproteins, matrix metalloproteases (MMPs), cathelicidins, histatins, and defensins [2]. Due to non-invasive sampling, ease of use, cost-effectiveness, and relatively minimal risk of cross-infection, saliva is an ideal diagnostic bio-fluid [3].

Saliva is an exocrine solution which communicates with the oral cavity through both intracellular and extracellular pathways. Interestingly, small molecules may enter the saliva from serum by passing through the capillary barriers, interstitial spaces, and the membranes of the acinar/ductal cells until the saliva is excreted through excretory tubules. These complex routes and the dynamic

* Corresponding author at: Department of Dental Materials, College of Dentistry, Taibah University, Medina Munawwarah, Saudi Arabia.
E-mail addresses: drzohaibkhurshid@gmail.com, zsultan@kfu.edu.sa (Z. Khurshid), MZAFAR@taibahu.edu.sa, sohail.zafar@riphah.edu.pk (M. Zafar), erumkhan_2010@icloud.com (E. Khan), drmariahmali@gmail.com (M. Mali), latifmayo@kaist.ac.kr (M. Latif).

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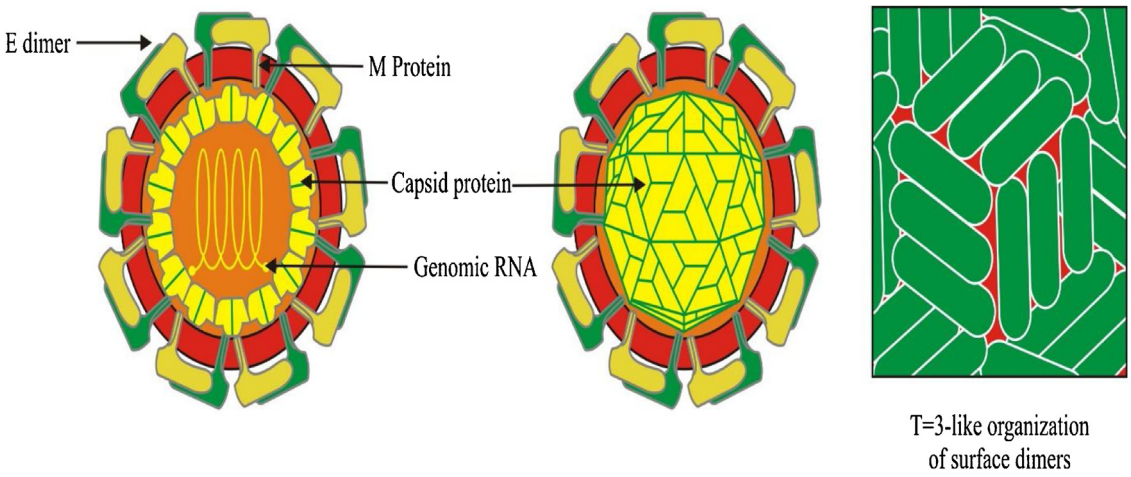

Fig. 1. Representation of the 3-D microscopic structure of Zika virus.

composition of saliva contribute to the value of WMS as an active diagnostic medium [4]. Human WMS contains numerous proteins and peptides of clinical relevance. Khan et al. [5] reported that up to 30% of blood proteins are also present in saliva. Human WMS is a significant factor in determining the prevalence of dental caries [6]. Other oral lesions, including periodontitis, implantitis, oral submucous fibrosis, oral cancer, and lichen planus, are detected in early stages through salivary biomarkers [7]. Speicher et al. [8] used OMNIgene™ (DNA Genotek) to identify human herpesvirus-8 (HHV-8) from human WMS. Likewise, TT virus (an un-enveloped single-stranded DNA virus), hepatitis G virus (blood-borne), Kaposi's sarcoma (KS)-associated herpesvirus (KSHV), and dengue virus have been found in WMS [9–11].

ZIKV is a flavivirus, one of the family of viruses that includes those for yellow fever, dengue fever, West Nile, and Japanese encephalitis. It was first isolated from rhesus monkeys and *Aedes africanus* mosquitoes from the Zika Forest of Uganda in 1947 [12]. Initially, ZIKV infections were limited to sporadic cases in Asia and Africa [13], but in 2007, after the first major ZIKV outbreak in Yap (Federated States of Micronesia, Pacific), the pattern of infection changed dramatically [12,14]. The most substantial outbreak took place in the French Polynesia and Pacific between October 2013 and March 2014 [15,16]. The World Health Organization (WHO) confirmed ZIKV transmission for the first time in the United States and Brazil in May 2015. Following this, the spread was recorded widely in 22 more areas, including Puerto Rico and the Virgin Islands in January 2016 [17]. More recently, travel-associated cases of ZIKV infection have been reported [18]. In October 2017, Brazil reported ĩ1.5 million cases of ZIKV infection, which included approximately 4000 suspected cases of microcephaly.

Considering these facts, WHO's International Health Regulations Emergency Committee declared ZIKV infection as a public health emergency of international concern and affirmed the likely associations of microcephaly and neurological disorders with ZIKV infections [19]. At 264 designated entry clearance points in China, procedures were implemented for monitoring travelers through verbal and temperature inspections; suspected ZIKV cases with fever, rashes, and conjunctivitis were further monitored [20]. Moreover, in August 2016, the Centers for Disease Control and Prevention (CDC) released travel notices for certain countries, including the Caribbean states, regarding the ZIKV infections.

ZIKV is a mosquito-borne flavivirus that is closely related to the Spondweni virus [21]. Zika virions are enveloped, 18–45 nm in diameter, icosahedral shaped, and their genome is ĩ1-kb single-positive-strand (sense) ribonucleic acid (RNA) enclosed in a capsid, surrounded by a 3-D membrane (Fig. 1) [22].

The RNA consists of 10,794 nucleotides encoding 3419 amino acids, and virus replication occurs in the cellular cytoplasm [23]. Cryo-electron microscopy (CEM) of ZIKV structure at 3.7 Å resolution revealed a small surface that helps the virus to survive in the harsh conditions of semen, saliva, and urine [22,24].

Transmission and clinical manifestations

Mosquito species *Aedes aegypti* and *Aedes albopictus* are well-known potential vectors for yellow fever, dengue, and chikungunya, and nations endemic with these fevers are at high risk for ZIKV as well [25]. In addition to the daytime urban dwelling spread of ZIKV by mosquito bites [26], it has recently been revealed that ZIKV can be rapidly transmitted through human-to-human intimate contact during kissing and sexual intercourse [27], through blood transfusions, and by vertical transmission, including the trans-placentally, during breastfeeding, while giving birth, and through close contact between the mother and the newborn child [28]. The occurrence of ZIKV outbreaks regardless of the presence of *Aedes species* mosquitos has contributed to the severe threat to the public health with serious consequences.

After an incubation period (3–7 days), ZIKV in up to 80% of cases seems to be asymptomatic or subclinical. These cases have mild symptoms—low grade fever (37.8°–38.5 °C), headache, non-purulent conjunctivitis, myalgia, arthralgia, peripheral edema, widespread maculopapular and pruritic rash, and gastro-intestinal disturbances [26,29], which usually resolve on their own. However, severe clinical sequelae of ZIKV include infants born with microcephaly due to infection during the mother's pregnancy, and central nervous system malformations including meningoencephalitis, meningitis, and Guillain–Barré syndrome (GBS) in adults [12]. The latest data from the Ministry of Health in Brazil provide evidence of 4180 cases of microcephaly, out of which 270 cases were positively correlated with ZIKV among only 732 classified cases altogether [30]. Moreover, the National IHR Focal Point of El Salvador reported an average of 169 cases per year of GBS, which increased 20-fold between December 01, 2015 and January 06, 2016, while ZIKV was epidemic in French Polynesia [31].

Although there are similarities between the symptoms of Chikungunya virus, dengue virus, and ZIKV infections [32], a number of differences (such as high-grade fever, shock, and hemorrhage) can differentiate ZIKV infection from others (Chikungunya and dengue). ZIKV can be differentially diagnosed from rubella, HIV seroconversion, scarlet fever, rickettsia infection, secondary syphilis, leptospirosis, measles, parvovirus, and enterovirus [26].

Hence, diagnostic guidelines are contingent on laboratory testing only as clinical evaluation alone remains unreliable.

Laboratory diagnosis with human whole mouth saliva (WMS)

Detection of ZIKV was initially limited to serum and cerebrospinal fluid. Later the focus turned to other fluids, including urine, saliva, and amniotic fluid, and tissue for diagnostic testing [33]. Serological analysis and detection of ZIKV IgM and IgG antibodies are compromised due to low viremia and cross-reactivity with other flaviviruses, which causes a higher likelihood of false positive results [34]. Viral culture has not been commonly pursued, and therefore detection of antigen is unavailable. There are currently a limited number of commercial tests available to detect ZIKV. Most of the available tests, such as prototype multiplex molecular assays, are still in the development phase [30]. At present, the reverse transcriptase polymerase chain reaction (RT-PCR) assay is considered a sensitive diagnostic assay with a high specificity for ZIKV [33]. One study [13] reported identifying ZIKV from a WMS sample from an infected one-year-old child by using the NucliSENS® easyMAG® system (BioMerieux). In this study, quantification of the ZIKV was greater from the WMS sample than from a blood sample from the same child. This demonstrated the ability to detect only acute phase ZIKV infection. Hence, saliva has the potential to act as a diagnostic medium for ZIKV in the first week of symptom onset. Corstjens et al. [35] also reviewed the use of saliva in virus detection and detailed a comprehensive literature that described the systematic correlation of saliva with virus activity. They concluded that saliva may be useful for the detection of viruses. Shedding of ZIKV RNA in saliva and urine was reported in Italy in January 2016 from a female patient until 29 days after the onset of symptoms [36]. This study also noted that, based on a phylogenetic analysis, the virus belonged to the Asian pedigree and clustered to the ZIKV strains of Latin American origin. The high viral load was identified through isolated saliva instead of from the emergence of antibodies. The saliva emerged as a significant diagnostic tool for the nucleic acid detection.

To confirm that an illness is caused by ZIKV and not by another flavivirus, RNA of ZIKV in a clinical specimen is essential. Bingham et al. [37] conducted an experiment for validation of diagnostic approaches using serum, urine, and saliva among travel-associated ZIKV cases in Florida in 2016. Samples were collected initially from 66 individuals and later from 53 other individuals for comparison of urine, serum, and saliva collected from each individual on the same day. This study revealed a higher rate of ZIKV RNA detection in saliva and urine (ranging from 81% to 92%, respectively) compared to the RNA level detected in the serum (51%).

Similarly, Nicastrl et al. [38] published a case report in January 2016 of a man with non-vector-borne ZIKV infection, probably sexually transmitted, on his return to Italy from Haiti. ZIKV RNA was detected in the patient's urine and saliva 91 days after the first onset of his symptoms, and in his semen six months later [38]. A groundbreaking discovery in the diagnosis of the ZIKV was reported by the University of Western Ontario (UWO) [39]. The protein and peptide fingerprints from the WMS of a ZIKV-infected pregnant mother and her twins (one born with microcephaly and one without) were investigated, and nine significant peptides were identified from their saliva.

To detect ZIKV, the real-time RT-PCR Viral RNA Mini Kit (QIAamp) and One-Step RT-PCR Kit (Qiagen, Hilden, Germany) are available. They work by targeting partial sequences of the envelope genes, ZIKVENF and ZIKVENVR, from saliva, urine, and serum samples [20]. Recently, one group from the USA [40] performed experiments on the detection of ZIKV RNA in saliva samples by optimizing a serological assay using a microfluidic device and point-of-care technology. An optimized reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay and an isothermal amplification device were used to detect ZIKV RNA. This study evidenced a protocol to modify the current loop-mediated isothermal amplification (LAMP) assay to be useful in detecting ZIKV RNA.

Conclusion

Overall, molecular biologists, biomedical engineers, protein chemists, immunologists, oral practitioners, and health policy specialists must work as a team to control the medical emergency of ZIKV infections. Mass gatherings, for example, the Umrah and Hajj in the Kingdom of Saudi Arabia, the FIFA football world cup 2022 in Qatar, the ICC World Twenty20 in Australia, and EXPO 2020 in Dubai, are open occasions to explore the potential of ZIKV transmission and its prevention [19]. The use of human body glandular secretions, particularly saliva, as diagnostic tools provides us with an opportunity for simpler and more efficient molecular and proteomic analysis/diagnosis of ZIKV. These outcomes will be beneficial for studying point-of-care (POC) technology and biosensor development.

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Competing interests

None declared.

Ethical approval

Not required

References

[1] Hofman LF. Human saliva as a diagnostic specimen. J Nutr 2001;131, 1621S–5S.
[2] Khurshid Z, Naseem M, Sheikh Z, Najeeb S, Shahab S, Zafar MS. Oral antimicrobial peptides: types and role in the oral cavity. Saudi Pharm J 2016;24:515–24.
[3] Khurshid Z, Zohaib S, Najeeb S, Zafar M, Slowey P, Almas K. Human saliva collection devices for proteomics: an update. Int J Mol Sci 2016;17:846.
[4] Siqueira WL, Salih E, Wan DL, Helmerhorst EJ, Oppenheim FG. Proteome of human minor salivary gland secretion. J Dent Res 2008;87:445–50.
[5] Khan R, Khurshid Z, Yahya Ibrahim Asiri F. Advancing point-of-Care (PoC) testing using human saliva as liquid biopsy. Diagnostics 2017;7:39.
[6] Singh S, Sharma A, Sood PB, Sood A, Zaidi I, Sinha A. Saliva as a prediction tool for dental caries: an in vivo study. J Oral Biol Craniofacial Res 2015;5:59–64.
[7] Amado FML, Ferreira RP, Vitorino R. One decade of salivary proteomics: current approaches and outstanding challenges. Clin Biochem 2013;46:506–17.
[8] Speicher DJ, Wanzala P, D'Lima M, Johnson KE, Johnson NW. Detecting DNA viruses in oral fluids: evaluation of collection and storage methods. Diagn Microbiol Infect Dis 2015;82:120–7.
[9] Chen M, Sonnerborg A, Johansson B, Sallberg M. Detection of hepatitis G virus (GB virus C) RNA in human saliva. J Clin Microbiol 1997;35:973–5.
[10] Inami T, Konomi N, Arakawa Y, Abe K. High prevalence of TT virus DNA in human saliva and semen. J Clin Microbiol 2000;38:2407–8.
[11] Koelle DM, Huang ML, Chandran B, Vieira J, Piepkorn M, Corey L. Frequent detection of Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) DNA in saliva of human immunodeficiency virus-infected men: clinical and immunologic correlates. J Infect Dis 1997;176:94–102.
[12] Plourde AR, Bloch EM. A literature review of Zika virus. Emerg Infect Dis 2016;22:1185–92.
[13] Musso D, Roche C, Nhan T-X, Robin E, Teissier A, Cao-Lormeau V-M. Detection of Zika virus in saliva. J Clin Virol 2015;68:53–5.
[14] Gourinat A-C, O'Connor O, Calvez E, Goarant C, Dupont-Rouzeyrol M. Detection of Zika virus in urine. Emerg Infect Dis 2015;21:84–6.
[15] Duffy MR, Chen T-H, Hancock WT, Powers AM, Kool JL, Lanciotti RS, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. N Engl J Med 2009;360:2536–43.
[16] Musso D. Zika virus transmission from French polynesia to Brazil. Emerg Infect Dis 2015;21:1887–9.

[17] Meaney-Delman D, Rasmussen SA, Staples JE, Oduyebo T, Ellington SR, Petersen EE, et al. Zika virus and pregnancy: what obstetric health care providers need to know. Obstet Gynecol 2016;127:642–8.
[18] Meaney-Delman D, Hills SL, Williams C, Galang RR, Iyengar P, Hennenfent AK, et al. Zika virus infection among U.S. pregnant travelers — August 2015-February 2016. MMWR Morb Mortal Wkly Rep 2016;65:211–4.
[19] Elachola H, Gozzer E, Zhuo J, Memish ZA. A crucial time for public health preparedness: Zika virus and the 2016 Olympics, Umrah, and Hajj. Lancet 2016;387:630–2.
[20] Zhang J, Jin X, Zhu Z, Huang L, Liang S, Xu Y, et al. Early detection of Zika virus infection among travellers from areas of ongoing transmission in China. J Travel Med 2016;23:606–12.
[21] Buathong R, Hermann L, Thaisomboonsuk B, Rutvisuttinunt W, Klungthong C, Chinnawirotpisan P, et al. Detection of Zika virus infection in Thailand, 2012-2014. Am J Trop Med Hyg 2015;93:380–3.
[22] Sirohi D, Chen Z, Sun L, Klose T, Pierson TC, Rossmann MG, et al. The 3.8 A resolution cryo-EM structure of Zika virus. Science 2016;352(80-):467–70.
[23] Hamel R, Dejarnac O, Wichit S, Ekchariyawat P, Neyret A, Luplertlop N, et al. Biology of Zika virus infection in human skin cells. J Virol 2015;89:8880–96.
[24] Kostyuchenko VA, Lim EXY, Zhang S, Fibriansah G, Ng T-S, Ooi JSG, et al. Structure of the thermally stable Zika virus. Nature 2016;533(7603):425.
[25] Diagne CT, Diallo D, Faye O, Ba Y, Faye O, Gaye A, et al. Potential of selected *Senegalese Aedes* spp. Mosquitoes (Diptera: culicidae) to transmit Zika virus. BMC Infect Dis 2015;15:492.
[26] Basarab M, Bowman C, Aarons EJ, Cropley I. Zika virus. BMJ 2016;1049:i1049.
[27] Gyawali N, Bradbury RS, Taylor-Robinson AW. The global spread of Zika virus: is public and media concern justified in regions currently unaffected? Infect Dis Poverty 2016;5:37.
[28] Besnard M, Lastère S, Teissier A, Cao-Lormeau V, Musso D. Evidence of perinatal transmission of Zika virus, French Polynesia, December 2013 and February 2014. Eurosurveillance 2014;19:20751.
[29] Faye O, Freire CCM, Iamarino A, Faye O, de Oliveira JVC, Diallo M, et al. Molecular evolution of Zika virus during its emergence in the 20th century. PLoS Negl Trop Dis 2014;8:36.
[30] Chang C, Ortiz K, Ansari A, Gershwin ME. The Zika outbreak of the 21st century. J Autoimmun 2016;68:1–13.
[31] Tognarelli J, Ulloa S, Villagra E, Lagos J, Aguayo C, Fasce R, et al. A report on the outbreak of Zika virus on Easter Island, South Pacific, 2014. Arch Virol 2016;161:665–8.
[32] Moulin E, Selby K, Cherpillod P, Kaiser L, Boillat-Blanco N. Simultaneous outbreaks of dengue, chikungunya and Zika virus infections: diagnosis challenge in a returning traveller with nonspecific febrile illness. New Microbes New Infect 2016;11:6–7.
[33] Rabe IB, Staples JE, Villanueva J, Hummel KB, Johnson JA, Rose L, et al. Interim guidance for interpretation of Zika virus antibody test results. MMWR Morb Mortal Wkly Rep 2016;65:543–6.
[34] Charrel RN, Leparc-Goffart I, Pas S, de Lamballerie X, Koopmans Marion, Reusken Chantal BEM. State of knowledge on Zika virus for an adequate laboratory response. Publ Bull World Heal Organ Type Res Emerg 2016:1–29. Artic ID BLT16171207.
[35] Corstjens PLAM, Abrams WR, Malamud D. Saliva and viral infections. Periodontol 2000 2016;70:93–110.
[36] Barzon L, Pacenti M, Berto A, Sinigaglia A, Franchin E, Lavezzo E, et al. Isolation of infectious Zika virus from saliva and prolonged viral RNA shedding in a traveller returning from the Dominican Republic to Italy, January 2016. Eurosurveillance 2016;21:30159.
[37] Bingham AM, Cone M, Mock V, Heberlein-Larson L, Stanek D, Blackmore C, et al. Comparison of test results for Zika virus RNA in Urine, serum, and saliva specimens from persons with travel-associated Zika virus disease — Florida, 2016. MMWR Morb Mortal Wkly Rep 2016;65:475–8.
[38] Nicastri E, Castilletti C, Liuzzi G, Iannetta M, Capobianchi MR, Ippolito G. Persistent detection of Zika virus RNA in semen for six months after symptom onset in a traveller returning from Haiti to Italy, February 2016. Eurosurveillance 2016;21.
[39] Zuanazzi D, Arts EJ, Jorge PK, Mulyar Y, Gibson R, Xiao Y, et al. Postnatal identification of Zika virus peptides from saliva. J Dent Res 2017;96:1078–84.
[40] Sabalza M, Yasmin R, Barber CA, Castro T, Malamud D, Kim BJ, et al. Detection of Zika virus using reverse-transcription LAMP coupled with reverse dot blot analysis in saliva. PLoS One 2018;13:e0192398, http://dx.doi.org/10.1371/journal.pone.0192398.