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Effect of Chemical Composition of Wastewater on Herpes Simplex Virus Type 1 Infectivity and Seroprevalence of The Virus Among Wastewater Treatment Plant Workers

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Abstract

Herpes simplex virus type 1 (HSV-1) infection appears in the form of lesions containing infectious virus that if ruptured, virus can reach wastewater while washing or showering. We aimed to test infectivity of (HSV-1) in wastewater of different chemical composition and if it represents an additional risk factor for infection among wastewater treatment plant workers (WWTPW). Virus with known viral count was inoculated in three autoclaved wastewater (WW) samples collected from Balas, Berka and Arab abou-said WWTPs. Infectious viral particles were counted by Plaque count assay at different time intervals (day 1 to 21). Heavy metals concentrations were detected in WW samples by using atomic absorbtion spectrometer. Finally enzyme linked immunosorbent assay (ELISA) was used to detect anti-HSV-1-antibodies among 52 WWTPW, compared to control group (52 samples). Results showed sharp decrease in viral count starting from day 1 and reached 100 % by day 17. Berka wastewater sample gave the highest % reduction in all tested time points that might be due to the high Fe and Zn concentrations compared to the other two stations,. Prevalence of anti-HSV-1-IgG among WWTPW (35%) was nearly equal to control group (42%) showing that working in contact with wastewater wasn't an additional risk factor for infection with HSV-1 among WWTPW.
Key words: Herpes simplex Virus type 1; Heavy metals; Waste water; Seroprevalence; Viral reduction; risk factor.

1. Introduction

Herpes simplex virus type 1 (HSV-1) is an enveloped DNA virus **[1].** It was reported that 53 % of the human population were found to be seropositive to the virus **[2].**It is usually spread by direct contact with mucous membranes **[3]** and wounds in skin. HSV-1 initially infects epithelial cells as a lytic infection, and then enters peripheral neurons where it establishes latency **[4, 5].** In case of ocular infections, the virus is imparted through sensory neurons to set up latency in trigeminal ganglia where it remains asymptomatic until reactivation of the virus leads to secondary or recurrent lytic outbreaks **[6,7].** HSV-1 causes number of complications including blindness, encephalitis or aseptic meningitis [2]. Acyclovir is used for its treatment but resistance to the drug was reported **[8],** resistance also occurred to 3.5–10% of immune compromised patients **[9].**

Wastewater acts as a reservoir for a large number of viruses, although the enteric viruses excreted in human or animal waste are the major group of viruses present in it **[10].** Reports detected other kinds of viruses in waste water such as avian influenza A virus **[11]** and different types of coronavirus including the novel coronavirus **[12].** HSV-1 is not one of the popular waterborne viruses but like all other pathogens can reach water through

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infected persons. Survival of HSV-1in water was studied and results showed that the virus remains infectious up to 21 days in distilled water **[13]** and up to one week in river water and sea water **[14].** On the other hand, the virus can only remain infectious for 1 day in pool water due to presence of chlorine **[14].**

Infection with HSV-1 appears in the form of lesions in skin, tongue and lips filled with body fluids containing infectious viral particles **[15]** so any rupture in those lesions can make the virus reach waste water during washing face or showering of infected persons. Reports said that enveloped viruses are less stable in water than non-enveloped viruses that can persist in water up to three months.

Actually there isn't much available data about survival of HSV-1 in waste water. Also there is no available data showing if this can increase risk of infection among waste water treatment plant workers (WWTPW) who are in direct contact with waste water.so far single report showed presence of HSV-1 in aerosol of WWTP and showed that it might reach WWTPW via inhalation **[16].**

Exactly as wastewater represent a reservoir for pathogens, it also contains groups of chemical compounds including heavy metals where antimicrobial effect of some of them as zinc **[17]** and Iron **[18]** were reported, so their presence in wastewater might have inhibitory effect on infectivity of virus present in wastewater.

So this study aimed to test survival of HSV-1 inoculated in wastewater samples collected from three different wastewater treatment plants to detect duration time that HSV-1 can remain infectious in wastewater and if the presence of heavy metals have an inhibitory effect on them, also we aimed to record if this can be a factor of increasing infection risk among WWTPW with that virus.

2.Experimental

2.1.Virus and cells:

Herpes simplex virus type 1: virus was propagated and titrated to give final count of $1.5x10^7$ PFU/ml. Vero cell line was used. The cells were propagated in DMEM medium (Lonza,USA) supplemented with 10% fetal bovine serum (Gibco,USA), 1% antibiotic–antimycotic mixture (Lonza,USA).

2.2.Wastewater samples

Samples were collected on February 2020 from inlet of three wastewater treatment plants (WWTP): El-Berka, Balas and Arab Abou Said. Three different WWTPs were chosen to ensure difference of chemical composition among the three samples. Samples were autoclaved and stored at 4ºC till use.

2.3. Human samples

A total of 52 blood samples were collected from WWTPWs whose work was in direct contact with wastewater in February 2020 as follows: Berka station (16 samples), Arab abou said station (24 samples) and Balas station (12 samples), this in addition to 52 blood samples collected from healthy persons attending the outpatient clinic of National Research Centre in period between May 2019 to December 2019 for regular medical check-up who have never been working in direct contact with waste water and considered as non-WWTPW controls.

Blood collection was performed in compliance with relevant laws and institutional guidelines in accordance with the ethical standards of the Declaration of Helsinki and after taking the approval of the Ethical Committee of the National Research Centre. Serum was separated from both groups in the same day of sample collection and stored at -80ºC till use in the following assays.

2.4.Testing infectivity of HSV-1 in waste water samples:

A volume of 10 ml of waste water samples (each in triplicate) were inoculated with $2x10^6$ viral particles and incubated at room temperature,10 ml of distilled water (DW) was treated similarly as control. A volume of 100 µl were taken at different time intervals from day 1 up to week three.

Plaque infectivity assay [19] was carried out to detect viable viral count in a given inoculums. It was performed using 12 well plate where Vero cells (10^5 cell/ml) were cultivated for 1 day at 37ºC.Growth medium was removed from the cell culture plates and were washed once with PBS. Then 100µl of each waste water samples as well as distilled water were inoculated onto cells. After 1 h contact time for virus adsorption, 1 mL of Dulbecco's Modified Eagles Media (DMEM) supplemented with 2% agarose was added onto the cell monolayer, plates were left to solidify and incubated at 37 ºC till formation of viral plaques. Formalin (10%) was added for 2 h then plates were stained with crystal violet. Control wells were included where untreated virus was incubated with Vero cells and finally plaques were counted and percentage reduction in plaques formation in comparison to control wells was recorded as following where each reading represent them mean of three readings:

% reduction in viral count=viral count (control) - viral count (waste water samples or distilled water)/viral count (control) X100.

2.5.Heavy metals composition of waste water treatment plants

Presence of heavy metals was recorded in the three waste water samples under test, they are Na, Zn, Pb, Ni, Cu, Mn, Fe, Cr, Cd and As . Atomic absorption spectrometer varianspectra (AA 220) with graphite furnace spectra AA GTA (110) was used for the determination of the trace levels of environmentally sensitive element including metals.

Samples were digested with the analytical method [20]. For each series of measurements intensity calibration curve was constructed composed of a blank and three or more standards from Merck (Germany). Accuracy and precision of metal measurements were confirmed using external reference standards from Merck, a standard reference material and quality control sample from the National institute of standards and technology (NIST) to confirm the measurement reading.

2.6.Detection of HSV-1 IgG among WWTPW compared to controls from normal population:

Serum samples of 52 WWTPW and 52 control groups were tested for sero-positivity using HSV-1 IgG ELISA test kit (Beijing kewei clinical diagnostic reagents inc, China) according to kit instructions.

2.7.Statistical analysis

Statistical analysis and plots were done using the analysis toolkit on Microsoft office excel 2016. Statistical significance was calculated by comparing the differences between medians of different studied groups using the Student's t-test. Differences were considered significant when the P-value was <0.05.
In addition, simple linear regression analysis was made to record if number of working years in contact with wastewater can significantly predicts the probability of being seropositive.

3.Results:

3.1.Persistence of HSV-1 in waste water samples

HSV-1 with known viral count was inoculated in 10ml of autoclaved wastewater samples collected from three different treatment plants namely Berka, Balas, Arab Abou Said and its infectivity was tested at different time intervals starting from 1 day to three weeks by inoculating 100 µl of each sample on vero cells, Results in Figure (1) showed that virus inoculated in DW was not affected after day1 and 4 post inoculation as we observed no change in the viral count from the viral control, loss of infectivity starts at day 7 and 10 with 20 and 30 % reduction in viral count respectively and increased to be 60% at day 14. By day17 and 21 loss of infectivity was 89 and 95 % respectively.

Reduction in viral infectivity was different when HSV-1 was inoculated in the three wastewater samples tested to be 64 (Balas) to 70 % (Berka) reduction in day 1 and increased to range from 82 to 86% in day 4. This was followed by a gradual increase to day 17 to reach 100% reduction at day 21 in the three wastewater treatment plant. Although sharp loss of infectivity was shown at day 1 and 4, we cannot deny the small count of viral particles that can remain infectious up to 17 days in wastewater. We also observed that at all tested time points, wastewater of Berka station showed slight increase in % reduction at all tested time points compared to the other two waste water samples.

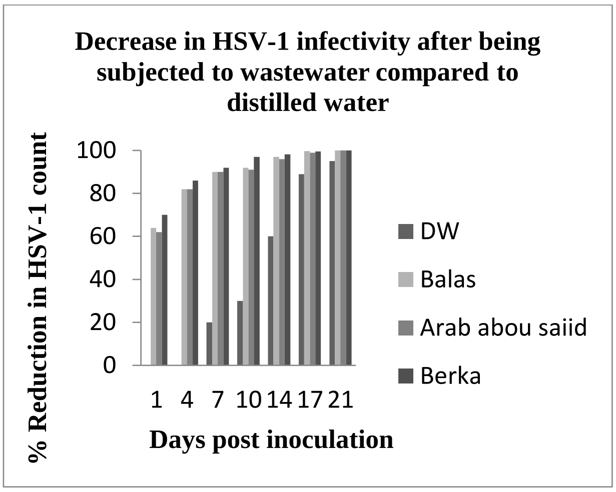

Fig.1. Effect of wastewater from different sources on HSV-1 infectivity compared to DW. Results showed that wastewater cause decrease in viral infectivity from first day but still un-neglected viral count stayed infectious up to 17 days post inoculation, also Berka station wastewater showed slight increase in % reduction at all tested time points than wastewater samples from the other two stations.

3.2.Heavy metal composition of waste water samples from three tested stations

Atomic absorption spectrometer was used to detect different heavy metals in three tested waste water and results in Table (1) showed that Berka station contained the highest amount of Zn, Ni, Fe and Cr, Balas was the highest in Pb and Mn and finally Arab abou said contained the highest amount of Na.

Table (1): Detection of kinds and amounts of heavy metal in waste water samples from Berka, Balas and Arab abou said stations.

Metal (µg/L)	Berka	Balas	Arab Abou Saiid
Na	85	120	140
Zn	0.23	0.15	0.14
Pb	<0.001	0.48	<0.001
Ni	0.04	0.03	0.03
Cu	0.03	0.03	<0.01
Mn	0.13	0.2	0.08
Fe	10.6	2.3	1.7
Cr	0.05	<0.001	0.01
Cd	<0.001	<0.001	<0.001
As	<0.001	<0.001	<0.001

3.3.Sero prevalence of HSV-1 IgG in serum samples of WWTPW and control group.

Serum samples of 52 WWTPW, (16, Berka station, 24,Arab abou said station and 12, Balas station) workers who shared in the study are those having direct contact with wastewater and 52 control serum samples (people from common population who did not work in WWTP or have any contact with wastewater) were subjected to ELISA test to detect HSV-1 IgG as an indicator to previous exposure to HSV-1 infection. On using Optical density values of ELISA readings among WWTPW's and control groups to compare seropositive and seronegative values via an independent two-sample student's t-test (two-tailed and p-value ≤ .05). Results were t(18)= 2.10, p= 1.3 x 10^{-5} (<0.000) for WWTPW and t(24)= 2.06, p= 4.06 x 10^{-7} (<0.000) among control group where the difference was truly significant among seropositive and seronegative readings in both groups. Also Figure (2) showed that 35 % (18/52 samples) were IgG positive in case of WWTPW and 42 % (22/52 samples) in control group.

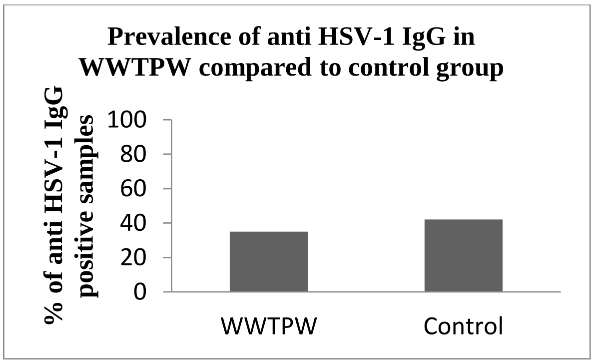

Fig.2. Prevalence of anti HSV-1 IgG as an indicator to previous exposure to HSV-1 infection was detected by ELISA test. Result showed that 34% of WWTPW were previously infected with the virus and 42 % in case of control group which is not a significant difference.

3.4.Comparing WWTPW spending ≤10 and >10 years working in contact with waste water among seropositive (SP) and seronegative (SN) samples.

Presence of antiHSV-1 IgG in WWTPW compared to control group showed that infection rate between two groups were nearly the same so to make sure that WWTPW's infection was due to familiar reasons in our daily life and not resulting from their working nature, a comparison was made between seropositive workers (18 samples) and seronegative ones (34 samples) considering number of working years that they spend in contact with wastewater. Workers were divided onto two groups, first group was for those spending less than 10 years working in contact with wastewater (≤ 10) and the second group was for workers spending more than 10 years working (>10) years in contact with wastewater to record if their infection rate was due to familiar ways of viral transmission or through wastewater.

Figure (3) showed that in ≤10 working years group 22% were seropositive (4/18) and 23 % were seronegative (8/34) and in >10 working years 77% were seropositive (14/18) and 76 % were seronegative (26/34).

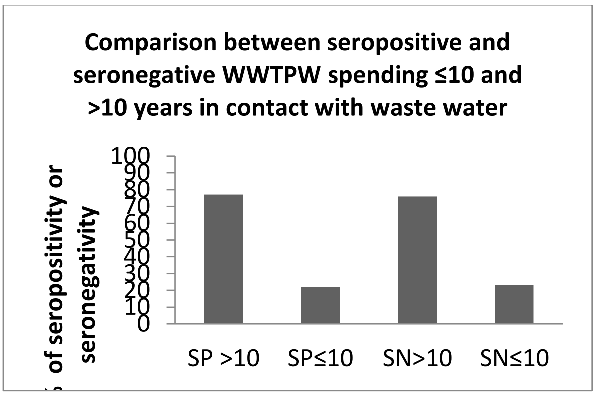

Fig.3. Comparison between seropositive and seronegative workers spending ≤10 years and >10 years in contact with waste water, results showed that there was no significant difference in seropositivity or seronegativity between two groups showing that HSV-1 in waste water doesn't represent risk factor among WWTPW. SP: seropositive, SN: seronegative.

In addition, we performed a simple linear regression analysis to assess whether number of working years in contact with wastewater can significantly predicts the probability of being seropositive. Results showed that the overall regression was statistically insignificant (R2 = 0.000284, F-value= 0.014, p-value= 0.905).

4.Discussion

HSV-1 is an enveloped virus and infection appears in the form of lesions filled with infectious viral particles around or inside the mouth and eyes. It usually reaches uninfected persons by direct contact between them and the virus through wounds in skin or mucous membrane.

HSV-1 is not a waterborne virus i.e. its presence in water is not common but it is like many other viruses that are not waterborne and reports confirm their presence in water and wastewater. But it is logic that any rupture in infected lesions can cause viral particles to come out and reach waste water while washing or showering. Single report included HSV-1 among detected viruses in aerosols of wastewater and that this might increase risk of infection among WWTPW [16].

Few reports spoke about HSV-1 persistence in different water types and showed that it can remain infectious up to three weeks in distilled water and only one week in River and sea water, although one can expect less persistent duration in waste water as HSV-1 is an enveloped virus which are not as stable as non-enveloped viruses in wastewater [21].

To our knowledge this is the first time that effect of wastewater on HSV-1 infectivity is tested showing that 30-36 % of HSV-1 inoculum can remain infectious up to 4 days in wastewater. We observed that at all time points tested % reduction in viral infectivity in Berka station was a bit higher, to understand this, the three tested wastewater samples were analyzed for presence of heavy metals, results showed that Berka station contained the higher amounts of Zn, Ni, Fe and Cr than the other two stations.

On researching of antimicrobial effect of heavy metals in wastewater we found that when heavy metals are changed to positively charged ions in water they became of ability to bind with negatively charged proteins in outer surface bacteria causing them to lose infectivity [22], but as all reports focus on bacteria one can expect the same for viruses as both viruses and bacteria present in wastewater have negatively charged proteins in their outer surface. Antimicrobial activity and its use in disinfection for this particular reason were reported for Zinc [17] and Iron as well [18]. This might explain the higher % reduction caused by Berka station wastewater.

Although high % reduction in viral infectivity appeared starting from day 4 post inoculation, one can't ignore the few viral particles that stay infectious up to day 17 post inoculation, as WWTPW works in contact with waste water so the virus, if present, can reach them through wounds in their skin or through mucous membranes so we tested presence of HSV-1 IgG, as an indicator to previous infection with the virus, in serum samples collected from workers and compare them to control group which include serum samples collected from people work away from waste water, results showed presence of HSV-1 IgG in 35% (18/52) in WWTPW and in 42% (22/25) in control group which actually don't represent a significant difference to show that contact with wastewater can increase infection rate with HSV-1.

As confirmation we compared % of seropositive and seronegative WWTPW who spent more than 10 years working in direct contact with wastewater with those who spent less than or equal 10 years, results showed that % of seropositivity and seronegativity was so close in both groups which was also shown by regression test confirming that direct contact with wastewater is not an additional factor for HSV-1 infection.

5.Conclusion

HSV-1 is one of the enveloped viruses that can reach waste water, most of viral particles lose their infectivity within 4 days and few remaining viral particles remain infectious up to 17 days. Heavy metal contents of wastewater can help in reducing viral infectivity. Few viral particles that remain infectious don't represent a real risk for increasing rate of HSV-1 infection among WWTPW.

List of abbreviations

HSV-1: Herpe0s simplex virus -1, WW: waste water, WWTPW: waste water treatment plant workers, DW: Distilled water, PFU: Plaque forming unit, SP: seropositive, SN: seronegative.

6. Conflict of interest

All authors declare that they have no conflict of interest.

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