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Pattern of Viral Infections among Kurdish Chemical Attack Survivors: An Observational Study

Gasha S. Ahmed[1], Hawbash M. Rahim[1]*, Salih A. Hama[2]

[1]Department of Medical Laboratory Science, College of Health Sciences, University of Human Development, Sulaymaniyah, Kurdistan Region, Iraq, [2]Department of Biology, College of Science, University of Sulaimani, Kurdistan Region, Iraq

ABSTRACT

Background: Survivors of chemical attacks can experience permanent immune-related changes that can affect their vulnerability to viral infections. The paper compared seroprevalence rates of hepatotropic viruses and herpesviruses in Kurdish survivors of chemical attacks and those who were not exposed to the chemical. **Methods:** This observational study will involve 321 participants (a sample size of 221 survivors and 100 controls), who will be recruited in Iraqi Kurdistan, between November 2023 and May 2024. Enzyme-linked immunosorbent assay was used to test serum samples using Hepatitis B virus (HBV), Hepatitis C virus (HCV), Hepatitis D virus (HDV), Cytomegalovirus (CMV), Epstein–Barr virus (EBV), and Varicella –Zoster virus (VZV). **Results:** HBV positivity was detected in 5.4% of survivors and 2.0% of controls, and no significant difference was detected (P = 0.240). The seropositivity of HCV was also significantly higher among survivors (4.5% vs. 0.0%, P = 0.034). Only survivors were found to have HDV (1.4% vs. 0.0%), but this was not significant. CMV immunoglobulin G (IgG) was common in both groups (56.6% vs. 61.0%, P = 0.467), but CMV IgM was identified exclusively in survivors (7.7% vs. 0.0%, P = 0.002). The EBV IgG was very high and similar in the two groups (91.4 and 92.0, P = 1.000), but EBV IgM was uncommon and only observed in survivors (1.8 and 0.0). VZV IgG was also found to be significantly lower in survivors compared to controls (81.4% vs. 91.0% P = 0.031), whereas VZV IgM was only detected in survivors (2.7% vs. 0.0%). **Conclusion:** Survivors showed higher HCV seropositivity and exclusive IgM positivity in herpesviruses, which can indicate more opportunities to be exposed to the virus or altered immune regulation in chemical-attack victims.

Index Terms: Chemical Weapons, Iraqi Kurds, Halabja, Seroprevalence, Viral Infection

1. INTRODUCTION

Although the use of chemical weapons is banned by international agreements, it is still being used. continued to wage war, sometimes against civilians. During the late 1980s, the Iraqi As part of it, Baathist regime launched a series of chemical attacks against Kurdish communities. Of the Anfal campaign [1]. Human Rights Watch documented that at least sixty villages in Iraqi Kurdistan, as well as the town of Halabja, which is now its own governorate in northern Iraq's Kurdistan region near the Iraq–Iran border, were attacked with multiple chemical warfare agents, including mustard gas, sarin, and tabun. This attack has been characterized as the largest chemical strike against civilians in modern history, causing thousands of casualties among women, children, and elderly individuals. Although many exposed individuals died immediately or shortly after

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Corresponding author's e-mail: Hawbash M. Rahim, Department of Biology, College of Science, University of Sulaimani, Kurdistan Region, Iraq. E-mail: hawbash.amin@uhd.edu.iq

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the attack, hundreds of exposed survivors remain in the Kurdistan region today [2], [3].

Long-term follow-up studies of chemical attack survivors reveal that chemical weapon exposure has been associated with various health complications, including respiratory, ocular, dermatological, and immunological disorders [4], [5]. Sulfur mustard is an immunotoxic compound; exposure has been reported to lead to impaired cell-mediated and humoral immunity decades after exposure. In animal and human studies, mustard exposure reduced total T lymphocytes by ~50%, decreased helper-inducer $CD4^+$ T cells, increased suppressor T cells, and lowered phagocytic activity to 20% of normal levels. Such immune dysregulation could increase susceptibility to chronic or latent viral infections [6]–[8].

Prior research on Halabja and other exposed populations reveals a significant prevalence of chronic morbidities [1], yet investigations focusing on viral infection patterns in this unique exposed cohort remain scarce [2]. Studying the prevalence of these viruses in this unique cohort will enhance understanding of long-term health consequences of chemical warfare, inform surveillance and vaccination strategies, and support targeted clinical management.

This study, therefore, aims to determine the epidemiological pattern of viral infections caused by hepatotropic viruses and herpesviruses in survivors of the Anfal chemical attacks across various areas of Iraqi Kurdistan in comparison with non-exposed residents of those same areas.

2. MATERIALS AND METHODS

2.1. Study Setting and Study Population

This was an observational study conducted over a period of 6 months, from November 07, 2023 to May 11, 2024, that assessed the virological profile of 221 chemical warfare survivors who were exposed during the Anfal champaign attacks on Iraqi Kurdistan (Fig. 1). Participants were enrolled from Halabja City, Goptapa, Sewsenan, Khurmal, Serwan, and Balisan, and they were compared to 100 people currently living in the same areas who were not exposed during the bombardments. The participants were assessed for Hepatitis B virus (HBV), Hepatitis C virus (HCV), Hepatitis D virus (HDV), Cytomegalovirus (CMV), Epstein–Barr virus (EBV), and Varicella–Zoster virus (VZV) positivity.

2.2. Ethical Approval

The study was approved by the ethics committee of the College of Health Sciences at the University of Human Development, Sulaimani, Kurdistan, Iraq. Written and verbal informed consent were acquired from the participants.

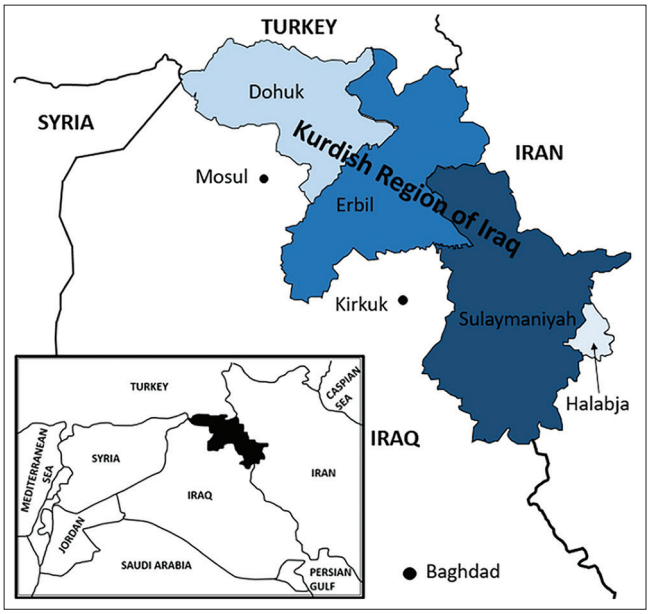

Fig. 1. Map of Kurdistan Region [9].

2.3. Inclusion and Exclusion Criteria

Inclusion criteria were based on the documents offered by the Chemical Victims' Society (CVS), which is a local non-governmental organization that maintains the official medical document of all victims of the chemical attack. The subjects who resided in these regions were chosen as survivors. In selecting the participants who did not get exposed, matching was done for an equal proportion of both groups. Participants were excluded if they had incomplete demographic or contact data, were receiving immunosuppressive therapy, or were unwilling or unable to provide informed consent.

2.4. Data Collection

The potential subjects were determined through the patients' records sent by CVS, and contact with the victims from a local healthcare practitioner assisted in verifying these records and the current living status of the survivors. Complete demographic and clinical information was gathered from the survivors and an equal number of Halabja residents who were not exposed to the chemical weapons. Data were mostly obtained through personal interview sessions and questionnaires; however, some information was extracted from the CVS records, including the degree of exposure, which was determined by professionals at the time of first registering them in their records. The personal interviews

took place in the homes of the respondents and the local clinics based on the preference of the subjects.

2.5. Serological Laboratory Assays

When conducting the blood draws from each participant, an amount of 5 mL of blood was collected aseptically using vacuum tubes with no anticoagulant for serum production. The blood was promptly taken to the lab in a temperature-controlled refrigerated box, not more than 4 h after sampling. The serum was then obtained from the samples by centrifuging the samples at 3000 rotations/min for 10 min, not more than 6 h after collection.

To determine the seropositivity of HBV, HCV, and HDV among the study participants, an indirect Enzyme-linked immunosorbent assay (ELISA) was performed using several kits. The Bioelisa Hepatitis B surface antigen (HBsAg) kit (Biokit, Spain) was used for the qualitative detection of HBsAg. Bioelisa HCV kit (Biokit, Spain) was used to detect anti-HCV antibodies, and the Bioelisa HDV kit (Biokit, Spain) for anti-HDV antibodies. The CMV IgG ELISA kit (MyBioSource, USA) and CMV IgM ELISA kit (MyBioSource, USA) detected anti-CMV Immunoglobulin G (IgG) and anti-CMV IgM antibodies. For EBV, the EBV IgG ELISA kit (MyBioSource, USA) and the EBV IgM ELISA kit (MyBioSource, USA) were used to detect anti-EBV IgG and anti-EBV IgM antibodies. The VZV IgG ELISA kit (MyBioSource, USA) and VZV IgM ELISA kit (MyBioSource, USA) were used for anti-VZV IgG and anti-VZV IgM antibodies. The ELISA tests took place in a controlled laboratory environment, with trained staff strictly following the manufacturer's protocols. Serum samples were thawed and brought to room temperature before testing. Each sample was placed in assay wells that were pre-coated with specific antigens or antibodies. After incubation and washing steps, enzyme-labeled conjugates and substrates were applied to create a colorimetric reaction. Viral markers were evaluated using optical density values measured at 450 nm with an ELISA reader.

2.6. Statistical Analysis

The data were entered and organized into Microsoft Excel 2019 sheets. Version 25.0 of the Statistical Package for the Social Sciences software was used to evaluate the data. A descriptive analysis was performed, showing categorical data as frequencies and percentages. The Chi-square (χ^2) test analyzed the qualitative data, and Fisher's exact test was used when one of the cells had fewer than five. A $P \leq 0.05$ was considered statistically significant.

3. RESULTS

In total, 321 participants were enrolled in this study, of whom 221 were exposed survivors, and 100 were non-exposed control participants. The overall mean age was 55.3 ± 11.2, with the survivors being 58.6 ± 9.0 and controls being 48.0 ± 12.0 years old. The majority of the participants were aged between 50 and 59 years (32.7%), followed by 60–69 years (29.9%), then 40–49 years (15.9%), and 30–39 years constituted 12.1%, with the lowest percentage being those aged between 70 and 79 years (9.3%). Males constituted 56.4% of the overall study population, with similar proportions within the survivors and controls; 58.4% and 52.0%, respectively. The majority of the participants were from Halabja (39.3%), followed by Serwan (15.9%), Goptapa (13.7%), Balisan (13.7%), Sewsenan (13.1%), and Khurmal (4.4%); the proportion of participants from each location is intentionally equalized between the survivors and controls. Of the survivors, 95% were either moderately or severely exposed to chemical warfare agents, with only 5% being mildly exposed. Further details are provided in Table 1.

Table 2 summarizes the seroprevalence of the investigated viral infections. Overall, HBsAg positivity was detected in 14 (4.4%) participants, with no significant difference between survivors and controls (5.4% vs. 2.0%, $P = 0.240$). HCV seropositivity was observed in 10 (3.1%) participants and was significantly higher among survivors than controls (4.5% vs. 0.0%, $P = 0.034$), while HDV was detected only among survivors (1.4% vs. 0.0%) without a significant group difference ($P = 0.555$). In the case of herpesviruses, CMV IgG was not significantly different between the two groups (56.6% in survivors and 61.0% in controls; $P = 0.467$), but CMV IgM was only found in survivors (7.7% vs. 0.0% $P = 0.002$). The prevalence of EBV IgG between the two groups was also similar (91.4% vs. 92.0%, $P = 1.000$), and EBV IgM was not common and only observed in survivors (1.8% vs. 0.0%, $P = 0.314$). VZV IgG seropositivity was also much greater in controls than in survivors (91.0% vs. 81.4% $P = 0.031$), whereas VZV IgM was only detected in survivors (2.7% vs. 0.0% $P = 0.182$).

Sex-specific distribution of serological markers is shown in Fig. 2. HBsAg seropositivity and HCV seropositivity were greater in males than females (11/129, 8.5% and 8/92, 8.7% respectively), and greater in females than males (2/129, 1.6%). HDV was rare among survivors, detected in 2/129 (1.6%) males and 1/92 (1.1%) females. CMV IgG, EBV IgG, and VZV IgG were also highly prevalent in both genders, with the highest seropositivity of EBV IgG among survivors (93.0%

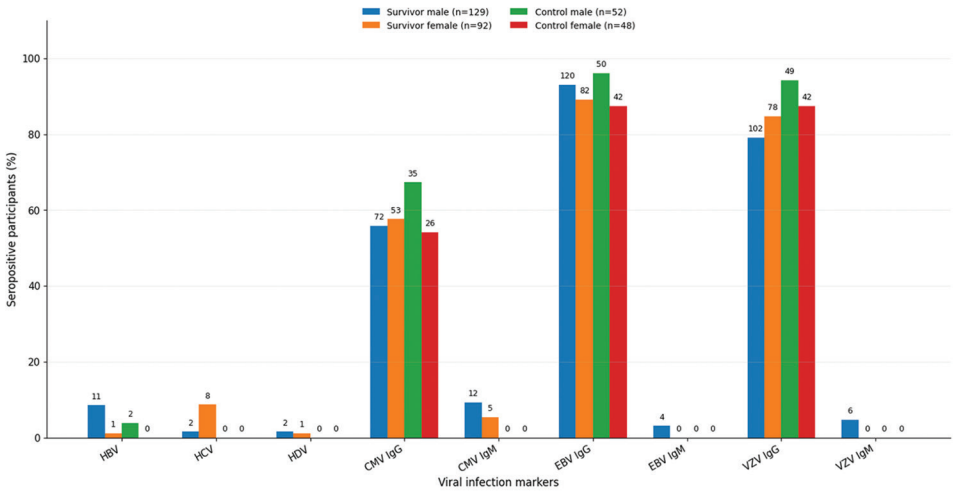

Fig. 2. Sex-specific distribution of positive serological markers for viral infections among chemical-attack survivors and control participants.

in men and 89.1% in women). HBsAg was only detected in males (2/52, 3.8%) in the controls, and neither HCV nor HDV was detected. Both CMV IgG, EBV IgG, and VZV IgG were also prevalent among both male and female controls. Remarkably, only survivors had CMV IgM, EBV IgM, and VZV IgM, and none of the controls had an IgM positive.

4. DISCUSSION

The present study constitutes an important contribution to the understanding of long-term susceptibility to viral infections in individuals exposed to chemical weapons, particularly in the context of the Kurdish survivors of the Anfal Campaign. The research offers one of the rare comparative sero-epidemiologic descriptions of the selected hepatotropic viruses (HBV, HCV, HDV) and herpesviruses (CMV, EBV, VZV) in Kurdish survivors of chemical attacks in multiple areas versus the controls in the non-exposed community.

Even though HBsAg positivity was numerically greater in survivors than in the controls (5.4% vs. 2.0%), the difference was not statistically significant in this sample. Notably, HBsAg is a biomarker of present infection (acute or chronic) and not previous exposure [10]. The prevalence of HBsAg in the survivors is observed to be greater than some Iraqi population data (e.g., a large household survey has been cited as having an HBsAg prevalence of approximately 1.6 in Iraq), though the estimates differ by region and risk profile of the population [11]. Since the survivors of chemical attacks often have chronic health issues and increased healthcare use over time, it might be the case that there are differences in opportunities of exposure to HBV, but the present study was not intended to cause, nor was it intended to confound cohort effects with exposures related to healthcare.

The finding of a much greater HCV antibody seropositivity in survivors (4.5% vs. 0) is a clinically significant finding since HCV is mainly a blood-borne infection, and the presence of antibody reactivity would suggest previous or current infection; active infection would be confirmed by HCV RNA. Another possible explanation is that survivors, owing to chronic respiratory/ocular/dermatologic sequelae and long-term follow-up, might have undergone more invasive medical procedures, injections, dental procedures, transfusions, or other healthcare-associated exposures over decades, all of which are well-established pathways of HCV transmission in most settings [12]. The lack of HCV seropositivity of controls in this study can also be explained by the rather small size of the control group ($n = 100$) and the overall low prevalence in several of the reported Iraqi blood-donor and community datasets [13]. These data justify the incorporation of reflex HCV RNA testing (when all anti-HCV reactive samples are tested) in future studies and, clinically, enhancing the linkage-to-care pathways among survivors receiving access to the curative direct-acting antiviral therapy.

Seropositivity of HDV was only significant in survivors (1.4% vs. 0%), which was not statistically significant, probably because of small numbers. However, HDV is significant, as it depends on HBV to replicate, and HDV-HBV co-infection is regarded as the most dramatic type of chronic viral hepatitis with accelerated cirrhosis and mortality due to liver diseases compared to HBV-only infection [14]. The

clinically significant implication is operational: All HBsAg-positive patients should be assessed for HDV as per current recommendations, since the absence of HDV may cause the underestimation of future liver disease and various monitoring/treatment choices [15].

The seroprevalence of EBV IgG was extremely high (>91) and almost the same between the survivors and controls. This is consistent with long-standing epidemiology over 90% of adults display signs of previous EBV infection and antibody profiles often indicate distant infection and not recent illness [16].

IgG seroprevalence of CMV was moderately high in both groups (~5861), although it was lower than that of many countries worldwide; a large systematic review of seroprevalence estimates high CMV IgG seroprevalence in most of the world, with the highest levels found in the WHO Eastern Mediterranean region [17]. This variation may be due to assay properties/cutoffs, sampling variations, or other uncontrolled variables, and may be confirmed in future studies with assay-specific performance data and/or on a different platform.

The result that CMV IgM positivity was only observed among the survivors (7.7% vs. 0%; $P = 0.002$) must be considered with caution. CMV IgM is an imperfect marker: it can be a sign of primary infection, but it can also be a result of reactivation/reinfection, or a false positive, and dual/overlapping IgM patterns between CMV and other herpesviruses have been reported. CMC IgM may be a plausible indication of intermittent reactivation as opposed to primary infection in a population with chronic inflammatory disease, such as chemical-attack survivors, who might also have altered immune regulation. But these possibilities cannot be differentiated by the existing data set. Future research can include CMV IgG avidity or CMV polymerase chain reaction (PCR) when IgM is positive, which can better distinguish between a recent primary infection and non-primary reactivation/reinfection [18].

In VZV, IgG seropositivity was much less in survivors than in controls (81.4% vs. 91.0%). This comes as a surprise to some extent, considering that survivors were older on average and cumulative exposure to varicella usually increases with age. Varicella epidemiology and vaccination policies in the Middle East differ widely between countries and across time, resulting in mixed patterns of seroprevalence [19]. The observed group difference can thus be due to cohort effects, history of dissimilar childhood exposure, unmeasured socioeconomic factors, assay sensitivity, or chance variation. Speaking of VZV IgM (detectable in only survivors), it is important to mention that IgM testing is less sensitive than PCR in lesions, and cannot, reliably, tell primary infection and reinfection/reactivation apart; IgM tests also have specificity problems [20]. Therefore, in case the aim is to assess clinically meaningful reactivation (zoster), the combination of serology with clinical history and PCR (in the presence of lesions) would enhance inference.

One of the major hypotheses in this work is that exposure to chemical warfare and specifically sulfur mustard can have long-term systemic effects that can change the predisposition to infection or viral reactivation. Chronic multi-organ morbidity and enduring hematologic/immunologic dissimilarities years following exposure have been recorded in long-term research in mustard-exposed groups, and biologic plausibility of altered host-virus interactions in survivors has been observed [21]. A report by Hama *et al*., stipulated that the chemical warfare agents have long-term effects on the survivors of the Halabja attack, especially on the immune system, both the antibody-mediated and cell-mediated immunity are affected [6]. Although causality cannot be determined, the trend that is observed in the current study (i.e., the pattern of IgM signals in survivors only) (CMV IgM, EBV IgM, VZV IgM) is congruent with (but not demonstrative of) either the risk of recent exposure or the changes in the immune control that result in a higher frequency of reactivation/boosting. To proceed to inference rather than plausibility, confirmatory virologic testing and longitudinal follow-up would be required.

In terms of practical applications, the available evidence suggests that it is necessary to enhance interventions related to viral hepatitis following chemical attacks. First, HCV seropositivity should lead to further investigation with HCV RNA testing [12]. Second, appropriate follow-up of HBV tests regarding phase and status of infection, as well as HBV vaccination, is crucial for effective preventive interventions [22]. Finally, patients who test positive for HBsAg require further assessment for HDV infections due to the significant danger associated with HBV-HDV co-infections [14]. Regarding herpesviruses, the clinical importance will be determined by the link between serology findings and patient history (such as CMV IgG avidity/PCR for acute CMV or lesion PCR for zoster).

A key strength of our study is the focus on a unique and vulnerable population, sampling survivors from multiple historically exposed Kurdish areas and including controls

residing in the same communities. However, several limitations should be acknowledged. (1) The design is observational, so temporality and causal attribution are not fully possible. (2) Serology alone—particularly IgM—has known diagnostic limitations; HCV antibody requires RNA confirmation, and CMV/VZV IgM can represent reactivation or false positivity. (3) The small number of positives for some markers (e.g., HDV, EBV IgM, VZV IgM) limits statistical power and prevents us from conducting further statistical analysis to detect true associations between variables. (4) Clinical data, such as transfusion history, were not available. (5) Due to budget limitations, only 100 controls were enrolled in the study with somewhat unequal age grouping.

5. CONCLUSION

Increased seropositivity to HCV and exclusive CMV IgM positivity in Kurdish individuals exposed to chemical attacks; meanwhile, VZV IgG seropositivity was higher among the control participants. This indicated either enhanced possibilities of infection or different ways of regulating immunity, or both. Further research in the form of appropriate virological testing, namely detection of HCV RNA and IgM positivity by PCR and avidity testing, is needed. It would also be reasonable to include tests of viral hepatitis among other examinations to provide comprehensive care for exposed patients.

6. ACKNOWLEDGMENT

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