ORIGINAL REPORT

Cell-free DNA Levels in Herpes Zoster: A Cross-sectional Longitudinal Study

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This study investigates serum cell-free DNA fluctuations in patients with herpes zoster or post-herpetic neuralgia, offering insight into the tissue damage and inflammatory dynamics associated with these conditions. A single-centre combined cross-sectional and longitudinal study was conducted with 59 patients to assess cell-free DNA levels in herpes zoster and post-herpetic neuralgia. Cell-free DNA was extracted from blood samples of patients with herpes zoster or post-herpetic neuralgia and compared with healthy controls. The findings demonstrated elevated cell-free DNA levels in patients with herpes zoster, which remained elevated for 3 months or longer following treatment. These results suggest the presence of a subacute inflammatory state after herpes zoster infection. Furthermore, patients who developed post-herpetic neuralgia did not show elevated cell-free DNA levels, while those who did not develop post-herpetic neuralgia exhibited increased levels. This indicates that post-herpetic neuralgia is likely a localized response to prior nerve damage rather than a systemic inflammatory process with acute tissue damage.

Key words: herpes zoster; post-herpetic neuralgia; human herpes virus; cell-free DNA; inflammation marker.

Submitted Sep 30, 2024. Accepted after revision Dec 2, 2024

Published Jan 3, 2025. DOI. 10.2340/actadv.v105.42137

Acta Derm Venereol 2025; 105: adv42137.

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SIGNIFICANCE

Our research on cell-free DNA (cfDNA) levels in herpes zoster and postherpetic neuralgia reveals how herpes zoster may have long-term effects on the body, even after treatment. We found elevated cfDNA levels in patients with herpes zoster months after recovery, indicating ongoing tissue damage and inflammation. Interestingly, postherpetic neuralgia patients did not show these increases, suggesting that postherpetic neuralgia pain might not be linked to inflammation as is herpes zoster. These findings provide new insights into the development of postherpetic neuralgia.

Herpes zoster (HZ) is a relevant public health issue due to its significant global morbidity and burden on the healthcare system (1). The disease is a result of reactivation of latent varicella zoster (VZ) infection in the sensory and dorsal root ganglia and presents as a painful vesicular rash distributed in a dermatomal distribution. The most common complication of HZ is post-herpetic neuralgia (PHN), a chronic condition that is characterized by persistent burning pain in a dermatomal distribution that can be lasting or intermittent (2). Patients with HZ or PHN can experience debilitating pain that may negatively impact their quality of life, cause increased economic burden, and put them at a higher risk of developing depression and anxiety (3–5). Understanding disease severity and relevant risk factors for complication development is vital to providing adequate care. To better understand the progression of HZ, studies have highlighted biomarkers present in patients with HZ and PHN (6). However, circulating cell-free DNA (cfDNA), a promising biomarker being studied in other inflammatory tissue-destructive conditions such as COVID, sepsis, and other viral infections, has minimal data on HZ and PHN (7–10). These studies have identified it as a promising biomarker for infection, and it has been associated with increased mortality and severity of illness. These DNA fragments are released from cells undergoing apoptosis, lysis, or necrosis of affected tissue, and in inflamed tissue a major source of cfDNA is actively released from neutrophils in the form of neutrophil extracellular traps (NETs) (7), a matrix primarily composed of DNA. There has been work done using cfDNA in diagnosing HZ, but we lack research on how cfDNA can indicate the development of complications or successful recovery (11, 12). Our study aims to analyse cfDNA levels in HZ patients throughout their recovery and in patients who develop PHN to see if cfDNA levels correlate. We hypothesize that patients with HZ will have increased cfDNA levels that drop after treatment. In addition, we predict that patients who develop PHN will also show changes in their cfDNA levels.

MATERIALS AND METHODS

We conducted a single-centre, combined cross-sectional and longitudinal study examining the levels of serum cfDNA in patients with HZ compared with healthy controls. Data were gathered from patients seen by dermatologists at the dermatology department of

the Soroka University Medical Center (SUMC) from April 2017 to November 2018. SUMC, a 1,200-bed university-affiliated referral centre, serves as a tertiary hospital for up to 1 million residents in Southern Israel. The study was conducted in accordance with the declaration of Helsinki and all appropriate amendments. The study was reviewed and approved by the local Ethics Committee of SUMC, Israel (approval number 0338-16-SOR). Written informed consent was obtained from each patient before entering the study.

Study population

Our study included adult patients (>18 years old), including those clinically diagnosed with HZ, and healthy controls, age- and sex-matched. Patients diagnosed with other conditions that affect the levels of cfDNA in the serum were excluded from the study, including psoriasis, pregnancy, rheumatoid arthritis, lupus, and active malignant disease.

The distribution of the disease, intensity of the pain, and development of post-herpetic neuralgia were recorded. Intensity of the pain was measured using the Numeric Pain Rating Scale (13). Epidemiological information such as age and gender were also collected. Complete physical examinations, including a skin examination, were conducted, with ophthalmologic and neurologic exams performed in relevant cases. Venous blood samples of 1–2 mL were collected at diagnosis and again 3 months later. All patients with HZ were treated with acyclovir for 7 days, along with pain medication such as non-steroidal anti-inflammatory drugs (NSAIDs), acetaminophen, dipyrone, or narcotics when indicated.

Lab analyses

cfDNA measurement. Blood samples were collected in commercial gel tubes using BD Vacutainer® SST II plastic serum tubes with silica (clot activator) gel (Becton-Dickinson, Plymouth, UK). Sera were separated by centrifugation (4°C, 2,000 g, 10 min) and kept at –20°C until assayed. cfDNA was quantified on coded serum samples by a rapid SYBR® Gold fluorometric assay, which does not require prior processing of samples, i.e., DNA extraction and amplification (14). Briefly, 20 µL of patient serum was applied in duplicate to black 96-well plates (Greiner Bio-One; Frickenhausen, Germany); 80 µL of diluted SYBR® Gold (Invitrogen, Paisley, UK) was added to each well (final dilution 1:10,000), and fluorescence was measured with a 96-well fluorimeter (SpectraMax Paradigm plate reader, Molecular Devices, San Jose, CA,USA) at an emission wavelength of 535 nm and an excitation wavelength of 485 nm. Concentrations of unknown samples were calculated by extrapolation in a linear regression model from a standard curve (39–5,000 ng/mL) of sonicated Salmon sperm DNA (Sigma-Aldrich, St Louis, MO, USA).

Statistical analyses

Statistical analysis was performed using Prism 9.02 software (https://www.graphpad.com/features); an unpaired *t*-test and a Mann–Whitney *U* test were used to compare serum cfDNA levels between the following groups, as the results did not follow a normal distribution: patients with HZ vs healthy controls, and cfDNA levels in patients with HZ at the time of initial diagnosis vs during the recovery period following treatment. ANOVA was performed to compare cfDNA values in patients who developed PHN, patients who did not, and healthy controls. A significance level of $p<0.05$ and 80% statistical power was selected. A *t*-test was performed to ensure that the study and control groups did not differ significantly in age.

Table I. Demographics of study group and control group

Factor	Patients with HZ	Healthy controls
Total number	36	23
Female (male)	20 (16)	16 (7)
Median age	71	59
Mean age	64	59
Range	24–87	31–73

HZ: herpes zoster; PHN: postherpetic neuralgia; cfDNA: circulating cell-free DNA.

RESULTS

Group demographics

Our study consisted of 59 patients overall, including 36 with HZ, 55% of which were women ($n=20$). The mean age of the patients was 64 years, with a median age of 71 years (range: 24–87 years). The control group consisted of 23 healthy individuals, of which 69% were women ($n=16$). The mean age of the control group was 59 years, with a median age of 59 years (range: 31–73 years). The age difference between the 2 groups was insignificant ($p=0.29$), as shown in **Table I**.

cfDNA levels in herpes zoster patients vs controls

The median cfDNA level in the patient group at the time of hospitalization was 848 ng/mL (95% CI: 841–1,445), significantly higher than the control group's median level of 534 ng/mL (95% CI: 410–587, $p = 0.0002$), as shown in **Table II** and **Fig. 1**.

cfDNA levels in herpes zoster patients at initial diagnosis vs recovery period (3 months later)

During the recovery period, 3 months following HZ infection, the median cfDNA level was 804 ng/mL (95% CI: 717–1,135). When compared with the cfDNA values gathered at initial diagnosis, 848 ng/mL (95% CI: 841–1,445), the decrease in cfDNA was not found

Table II. Circulating cell-free DNA (cfDNA) levels in patients with herpes zoster (HZ)±post-herpetic neuralgia (PHN) and controls

Factor	Healthy controls	HZ all patients	HZ PHN negative	HZ PHN positive	HZ 3+ months after treatment
Number of patients	23	36	20	16	21
25% percentile	339	554	554	565.3	584
Median cfDNA (ng/mL)	534	848	884	841.5	804
75% percentile	600	1,455	2,142	1,068	1126
Mean cfDNA (ng/mL)	499.2	1,143	1,373	856.6	925
Lower 95% CI of mean	410	841	855	651	717
Upper 95% CI of mean	587	1,445	1,890	1062	1135
p-value		0.0002	<0.001	>0.05	>0.05

CI: confidence interval.

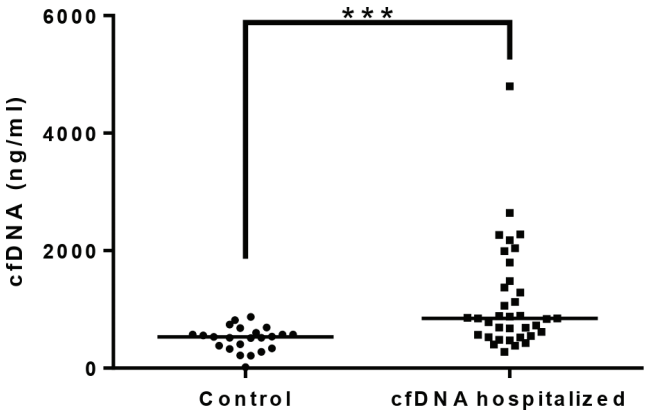

Fig. 1. Circulating cell-free DNA (cfDNA) levels in patients with herpes zoster (HZ) vs controls. A significant increase in cfDNA levels (ng/mL) can be seen in patients with HZ compared with the control group ($p = 0.00020$).

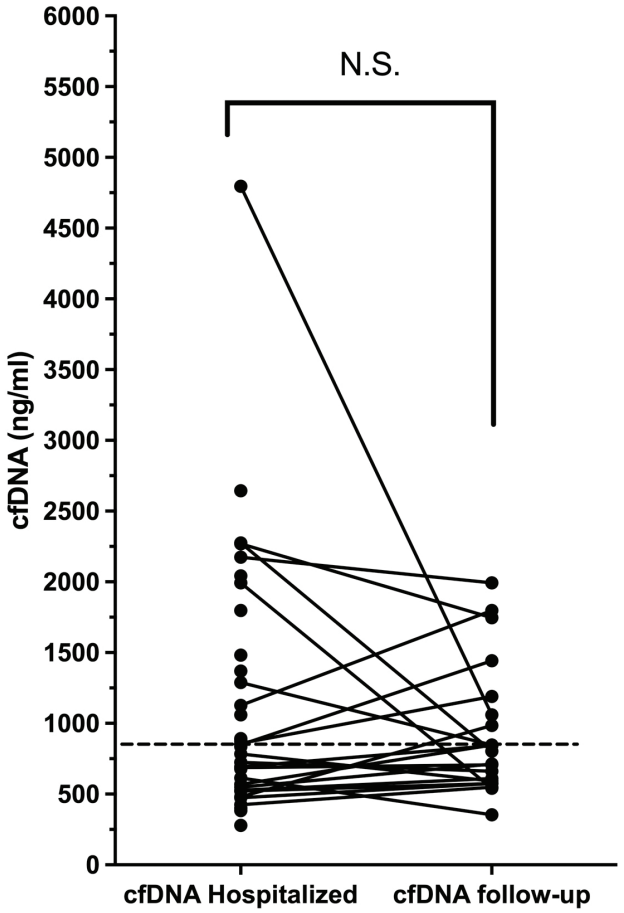

Fig. 2. Circulating cell-free DNA (cfDNA) levels in patients at the time of diagnosis vs at follow-up (3 months later). No significant difference in cfDNA levels. NS = not significant.

to be statistically significant ($p > 0.05$), as indicated in Table II (**Fig. 2**).

cfDNA levels in patients who developed post-herpetic neuralgia

Of the 36 patients included in the study, 16 developed PHN. The median cfDNA level in patients who developed PHN was 841 ng/mL (95% CI: 651–1,062) (Table II), with no significant difference compared with controls ($p > 0.05$) (Table II). Patients who did not develop PHN had a cfDNA median of 884 ng/mL (95% CI: 855–1,890), which was a significant increase compared with controls (884 vs 534 ng/mL, $p < 0.001$), as shown in Table II (and see **Fig. 3**).

DISCUSSION

In our study, we investigated cfDNA levels in patients with HZ and PHN and compared them with healthy controls. cfDNA levels were significantly higher in patients with HZ. Additionally, we found that patients who developed PHN did not exhibit increased cfDNA levels, while those who did not develop PHN showed elevated cfDNA levels. Levels of cfDNA did not decrease as expected 3 months or more post-treatment. Elevated levels of cfDNA present in HZ patients align with the understanding of cfDNA as an established biomarker of inflammation and tissue damage in inflammatory conditions such as myelofibrosis, COVID-19, bacteraemia, and sepsis (15–20). Dermatological conditions such as hidradenitis suppurativa and dermatomyositis have been shown to display increased levels

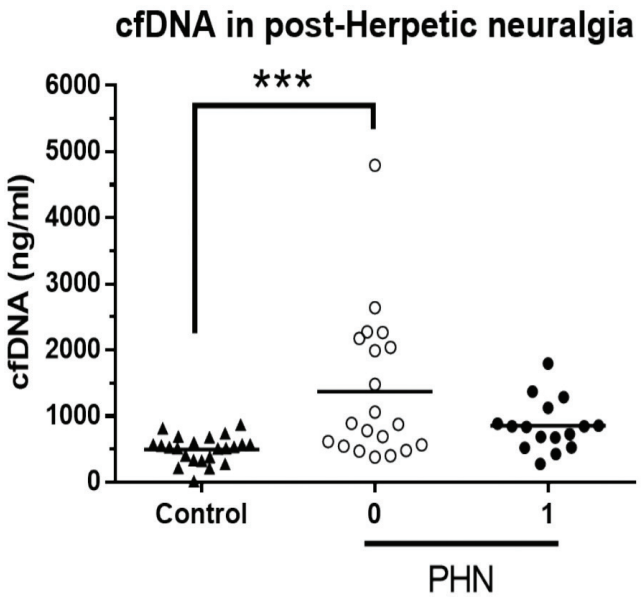

Fig. 3. Circulating cell-free DNA (cfDNA) levels in patients with post-herpetic neuralgia (PHN). Group "0" represents herpes zoster (HZ) patients without PHN; group "1" represents patients with PHN. No significant increase in cfDNA levels between patients with PHN and controls. cfDNA levels are significantly higher in HZ patients without PHN ($p < 0.001$).

of cfDNA and a correlation between elevated CRP and cfDNA (21, 22). Given that HZ infection induces neural and perineural damage, it is likely that this damage, along with the associated inflammation, leads to the release of cfDNA into the bloodstream, resulting in elevated levels.

Our findings in patients with PHN were unexpected, as they did not exhibit elevated cfDNA levels compared with controls. At the same time, those who did not develop PHN showed significant increases in circulating cfDNA levels. This result is counterintuitive, as we had anticipated elevated cfDNA in patients with PHN due to the severity of their condition. However, according to the ectopic pacemaker hypothesis, pain in PHN results from ectopic firing that is only moderately exacerbated by an inflammatory mediator, while HZ causes pain through inflammation (23). Given that HZ is associated with increased inflammation and tissue damage, it is reasonable to expect elevated levels of cfDNA in patients with HZ, as the damage occurs to the nerve and surrounding tissues. However, in PHN, hyperexcitable ectopic pacemaker sites located at various points in primary sensory neurons may cause pain but do not cause tissue destruction to the same extent as the inflammation seen in HZ.

Three months post-treatment, we examined the levels of cfDNA and observed no significant decrease. This finding was unexpected, as the patients had received treatment and were asymptomatic. We anticipated a substantial decline in cfDNA levels compared with those observed at initial diagnosis; however, this was not the case. Interestingly, the cfDNA levels remained elevated, contrary to the pattern in patients recovering from COVID-19, whose cfDNA levels returned to normal within 4 months post-treatment (24). Furthermore, microbial cell-free DNA (mcfDNA) has been shown to significantly decrease following treatment for endocarditis, a trend that contrasts with our findings (25). Similarly, mcfDNA levels tend to decline after the diagnosis of fungal infections, such as aspergillosis, with most patients exhibiting undetectable *Aspergillus* mcfDNA 1 month after diagnosis (26). Patients who had undergone surgical resection of extramammary Paget disease also showed decreased cfDNA levels (27). Our findings deviate from these established patterns; therefore, we propose that patients with HZ continue to maintain a subacute inflammatory state regardless of symptom resolution. This theory is supported by work that labels the first year after HZ infection to be a time period of increased risk for Major Adverse Cardiac and Cerebrovascular Events (MACCE) (28, 29). Given that patients may experience prolonged inflammation following infection, as indicated by the presence of cfDNA, it could serve as a valuable tool in investigating inflammatory conditions such as MACCE or post-infection autoimmunity, including acquired protein S deficiency (30).

Our study possesses several strengths that contribute to its scientific relevance. A key strength is the novel focus on cfDNA levels following treatment for HZ infection. Additionally, we are the first to investigate cfDNA within the context of PHN, for which limited data exist. The integration of cross-sectional and longitudinal analyses further enhances the robustness of our findings by providing insight into the cfDNA fluctuations that take place over time. However, there are some limitations to consider. Data were gathered 3 months post-treatment, and the study could have benefited from additional data collected later after treatment to understand the timeline of cfDNA level changes better. Additionally, while patients with conditions known to affect cfDNA levels, such as psoriasis, pregnancy, rheumatoid arthritis, lupus, and active malignant disease, were excluded from the exposed group, there may have been other unknown conditions that were not accounted for.

Further research is warranted to investigate the potential presence of a subacute inflammatory state following HZ infection. Additionally, we recommend that physicians assess the risk of inflammatory complications, as patients may remain in a subacute inflammatory state after the apparent resolution of HZ. Future studies could also trace the tissue origin of cfDNA to better understand which specific tissues are being damaged in the context of HZ infection.

To conclude, cfDNA emerges as a promising biomarker for detecting the presence of HZ and as an indicator of systemic inflammation. We propose that cfDNA could be a valuable minimal invasive complement to other clinical diagnostic tools and a useful resource for researchers investigating systemic inflammation across various disease states, especially HZ. Moreover, our findings support the ectopic pacemaker hypothesis by showing the absence of tissue damage and inflammation in PHN, which are commonly observed in HZ. These results underscore the need for further investigation into the prolonged inflammatory state observed in patients following infection.

ACKNOWLEDGEMENTS

The authors would like to thank Mrs Valeria Frishman for her technical assistance.

The authors acknowledge the use of OpenAI's ChatGPT for assistance in refining the phrasing, grammar, and spelling throughout the manuscript. All final content decisions were made by the authors.

All authors accept and agree with the UN's Declaration of Human Rights.

IRB approval status: The study was conducted in accordance with the Declaration of Helsinki and all appropriate amendments. The study was reviewed and approved by the local Ethics Committee of SUMC, Israel (approval number 0338-16-SOR). Written informed consent was obtained from each patient before entering the study.

The authors have no conflicts of interest to declare.

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