Natural Resources for Human Health
eISSN: 2583-1194

Original Research

Received 25 May 2026
Revised 26 June 2026
Accepted 21 July 2026

Natural Resources for Human Health 2026, Vol.6(10s):1399–1417

KEYWORDS:
Ebola virus disease, epigenetic scarring, Post-Ebola Syndrome, DNA methylation, chromatin remodeling, histone modification, viral persistence, non-coding RNA, HDAC inhibitors, long-COVID analogy

Chromatin Memories: Epigenetic Reprogramming, and the Immunological Aftermath of Ebola Virus Infection and Long-Term Sequelae in Survivors

Habeeb Ali Baig[1], Anshoo Agarwal[2], Anil Mohan Rao S.[3*], Dr. Padmanabha BV[4], Mohamed Soliman[5], Naglaa M Shalaby[6], Syed Raziuddin Qaudri[7], Sarah Ali[8]

[1] Assistant Professor, Department of Microbiology, Faculty of Medicine, Northern Border University, Arar-91431, Saudi Arabia.
Email: doctbaig@gmail.com, ORCID ID:0000-0002-5093-0623
[2] Professor, Department of Pathology, Faculty of Medicine, Northern Border University, Arar-91431, Kingdom of Saudi Arabia
Email id: dranshoo3@gmail.com, orcid.org/0000-0002-8675-3083
[3] Assistant Professor, Department of Pathology, Faculty of Medicine Northern Border University, Arar-91431, Saudi Arabia. Email: Anilmohanraosaini@gmail.com, ORCID ID: 0009-0009-8165-9520
[4] Department of Physiology, Faculty of Medicine, Northern Border University, Arar-91431, Saudi Arabia. Email: ppadmanabha1978@yahoo.co.in, ORCID: 0000-0002-5112-3225, ORCID ID: 0009-0009-8165-9520.
[5] Department of Microbiology, Faculty of Medicine, Northern Border University, Arar-91431, Saudi Arabia. Email: mohamed.sherif@nbu.edu.sa
[6] Department of Microbiology, Faculty of Medicine, Northern Border University, Arar, 91431 Saudi Arabia. Email: Nagla.shalaby@nbu.edu.sa
[7] Assistant Professor of Microbiology, Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, Northern Border University, Arar-91431, Saudi Arabia. Email: syed.quadri@nbu.edu.sa, ORCID ID: 0000-0002-2167-0316
[8] Department of Medical Basic Sciences, College of Medicine, Sulaiman Al-Rajhi University, Al Bukairiyah 51941, Al Qaseem, Saudi Arabia. Email: s.saeed@sr.edu.sa
*Corresponding author

ABSTRACT:

Background: About half of the people who survive Ebola virus infection suffer life-changing multiorgan damage after recovering from acute infection. These conditions (or syndrome) are called "Post-Ebola Syndrome" (PES), and they bear similarities to the ongoing manifestations of COVID-19 (commonly referred to as "long COVID"). Current theory posits that epigenomic mechanisms (i.e., alterations to DNA methylation, chromatin modification and regulation of expression by non-coding RNA) are potential mediators of biological alterations that have occurred during the initial phase of disease and continue after acute recovery.

Objective: To examine the potential role of epigenetic scarring as a key mechanism contributing to PES through the review of DNA methylation changes, histone modification patterns, alteration in expression of non-coding RNA and evidence of persistent viral transcription in immune-privileged sites.

Natural Resources for Human Health
eISSN: 2583-1194

Methods: We searched for literature on this topic from the following databases: PubMed, Scopus, Web of Science, Embase, WHO IRIS, and the Lancet Ebola archives (2014-2025). All studies published in one of these databases were searched based upon established guidelines for systematic review (PRISMA 2020) and 65 studies identified through systematic screening that produced a total of 3892 individual search results were included in the final analysis of evidence. Quality assessment was performed using the Newcastle-Ottawa Scale and QUADAS-2.

Results: Persistent DNA hypermethylation of promoters for the interferon pathway (IFNB1, TLR3, OAS1) in individuals remained for up to 24 months after discharge from hospital. Evidence for H3K27me3 chromatin expansion at interferon loci, and loss of H3K4me3 at IRF7 enhancers, was observed in peripheral blood mononuclear cells from survivors. Upregulation of miR-29a/b was correlated with the presence of fibrous tissue and collagen deposition. The presence of viral-specific RNA (VP35, VP30) was detected in cerebrospinal fluid, aqueous humor or semen up to several years after patients had recovered from infection. Evidence from epigenetic clock analyses suggested that the biological age of survivors was 4.2 years greater than that of non-infected controls.

Conclusion: Survivors of Ebola virus infection show evidence of persistent epigenetic reprogramming of immune cells that correlate with clinical manifestations of PES. Preliminary findings suggest that use of HDAC inhibitors and BET bromodomain inhibitors could be used to reverse these epigenetic changes. There is an urgent need for standardised longitudinal epigenomic monitoring of survivors from Ebola virus infection.

1. INTRODUCTION

The Ebola epidemic that took place in West Africa from 2014 to 2016 was the deadliest outbreak of the virus recorded to date, costing over 11,000 lives and changing how we understand the biology of these viruses as well as long-term effects of them [1,2]. The acute phase of EVD received much attention and research, whereas the many thousands of EVD survivors who returned home with an unfinished biological burden were largely neglected until the initiation of longitudinal cohort studies showed what was being seen clinically: The illness is not finished at the time of discharge, and EVD continues to affect those who have contracted it long after their initial symptomatic presentation or discharge from a healthcare facility [3,4].

Post-Ebola syndrome (PES) refers to the multi-system sequelae seen in survivors of EVD that can last from months to years after the acute phase and can include musculoskeletal pain, arthralgia (60%-80% of survivors), chronic fatigue (55%-70%), uveitis or visual impairment (14%-34%), neurocognitive deficits (40%-60%), hearing impairments (20%-30%) as well as psychiatric conditions such as post-traumatic stress disorder or depression (30%-50%) [5-11]. The clinical phenotype of PES is strikingly similar to those of Long COVID (or post-COVID-19 condition), which suggests that PES and Long COVID may, in part, share common pathophysiological underpinnings including immune dysregulation, viral persistence, and epigenetic reprogramming [8,12-14].

The study of epigenetics provides an exciting opportunity to understand how an acute viral infection could provide long-lasting impacts on the biology of the host through the incorporation of non-genetic heritable changes to gene expression [15-17]. The types of epigenetic change(s) that would be most relevant to understanding how acute infections create persistent, permanent changes include: alterations in DNA methylation at cytosine-guanine dinucleotides (CpG), particularly those associated with transcriptional start sites for proteins in the innate immune and interferon signaling pathways [24-27]; alterations in histone post-translational modifications, including the repressive H3K27me3 and active H3K4me3 marks, indicating the levels of transcriptionally permissive and repressive chromatin states at immune gene loci [18,19,20]; and, alterations in the expression and functionality of classes of non-coding RNAs (e.g., microRNAs, long non-coding RNAs,

circular RNAs) that regulate post-transcriptional control of gene expression regulation networks for proteins expressed in the immune system [17,21,28].

In the context of EBOV biology, there are many factors that suggest that epigenetic reprogramming would likely occur [3,4,29]. The acute phase of EVD is typified by significant immune dysregulation, characterized by excessive, dysregulated innate immune activation followed by paradoxical lymphopenia and suppression of T cell function following viral replication and emergence [3,4,29]. Additionally, EBOV expresses proteins (VP35, VP24) whose primary function is to inhibit the cellular response to type I interferon, and the chromatin level effects of prolonged type I interferon suppression during the acute phase will likely create long-lasting epigenetic scars at key loci regulating immune function and responses [30-36]. Finally, EBOV RNA is known to persist in immune-privileged sites (e.g., central nervous system, vitreous humor, testis, etc.) for many months to years after acute illness, whereby the persistence of EBOV RNA may also maintain signals that reinforce epigenetic changes within the immune system in situ [6,7,37-41].

Despite the clinical relevance of PES and biological plausibility of PES being the result of epigenetic changes, no comprehensive evaluation of the current state of the scientific literature examining epigenetic changes in EVD survivors has been conducted. Thus, this review is intended to address the existing evidence for DNA methylation changes, chromatin remodelling, dysregulated expression of non-coding RNAs, persistence of viral RNA, and how these changes collectively contribute to the PES phenotype. In addition, this review will identify knowledge gaps and potential therapeutic targets to explore further.

2. MATERIALS AND METHODS

2.1 Protocol Registration

The review was piloted in accordance with PRISMA 2020 standards (Figure 1) [27], and the PICOS framework was established and served as a template for developing the protocol for this review: Population (EVD confirmed survivors; any age; any geographical location), Intervention (epigenetic characterisation or viral RNA), Comparison (healthy controls or acute EVD patients or baseline pre-discharge), Outcome (epigenetic biomarkers or viral persistence or clinical sequelae), Study Design (cohort; case control; cross-section; Experimental Human Sample).

2.2 Search Strategy

PubMed/MEDLINE, Scopus, Web of Science, Embase, WHO IRIS and The Lancet Ebola archive were searched to identify publications from January 2014 to March of 2025. The following search terms were used: (“Ebola”, or “EVD”, or “Ebola Virus Disease", or “Filovirus”) AND (“Epigenetic”, or “DNA methylation,” or “Chromatin remodelling,” or "Histone,” or “microRNAs,” or “Non-coding RNAs,” or “lncRNA,” or “Viral persistence,”or "Post-Ebola,” or “Survivor”). To identify further eligible publications, reference lists from selected articles and WHO Strengthening the Survivor Programme reports were hand searched.

2.3 Eligibility and Quality Assessment

Studies were included if they were conducted with confirmed EVD survivors, had a post-discharge follow-up of a minimum one month, had at least one epigenetic variable or viral RNA persistence endpoint reported, and were published in full text as a peer-reviewed article or a WHO technical report. The following exclusion criteria were applied to studies in this systematic review: exclusively Animal Studies, studies of acute EVD only (without sufficient follow-up on survivors) and Qualitative Studies. The quality of included studies was assessed using Newcastle-Ottawa Scale for observational studies and QUADAS-2 for diagnostic accuracy studies.

2.4 Data Synthesis

Given the high Degree of Heterogeneity observed among studies regarding methodology utilised for epigenetic analysis (both science and cohort characteristics) and only greater than three studies used the same outcome, a formal meta-analysis will only be performed on the limited number of studies reporting identical outcomes and assessed to be comparatively equivalent. All other data will be synthesised Narratively and assigned a Scientific Evidence Quality Rating. Meta-analyses that comprise pooled results across multiple studies will employ Random Effects Models in R 4.3.1 statistical software.

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3.1 Study Selection

The first search yielded 3892 records. After duplicates were removed (1045), and the titles and abstracts screened, 428 full-text articles were evaluated. After reviewing these full-text articles, 363 were excluded (no epigenetic data: 91; only animal studies: 35; did not include any EVD survivors: 89; not enough follow up: 62; case reports: 41; other VHF types excluding ebola: 45). Thus, 65 articles were identified that met all of the inclusion criteria and contributed to the development of the current review. Figure 1 contains a PRISMA 2020 style flowchart showing the results of the review process.

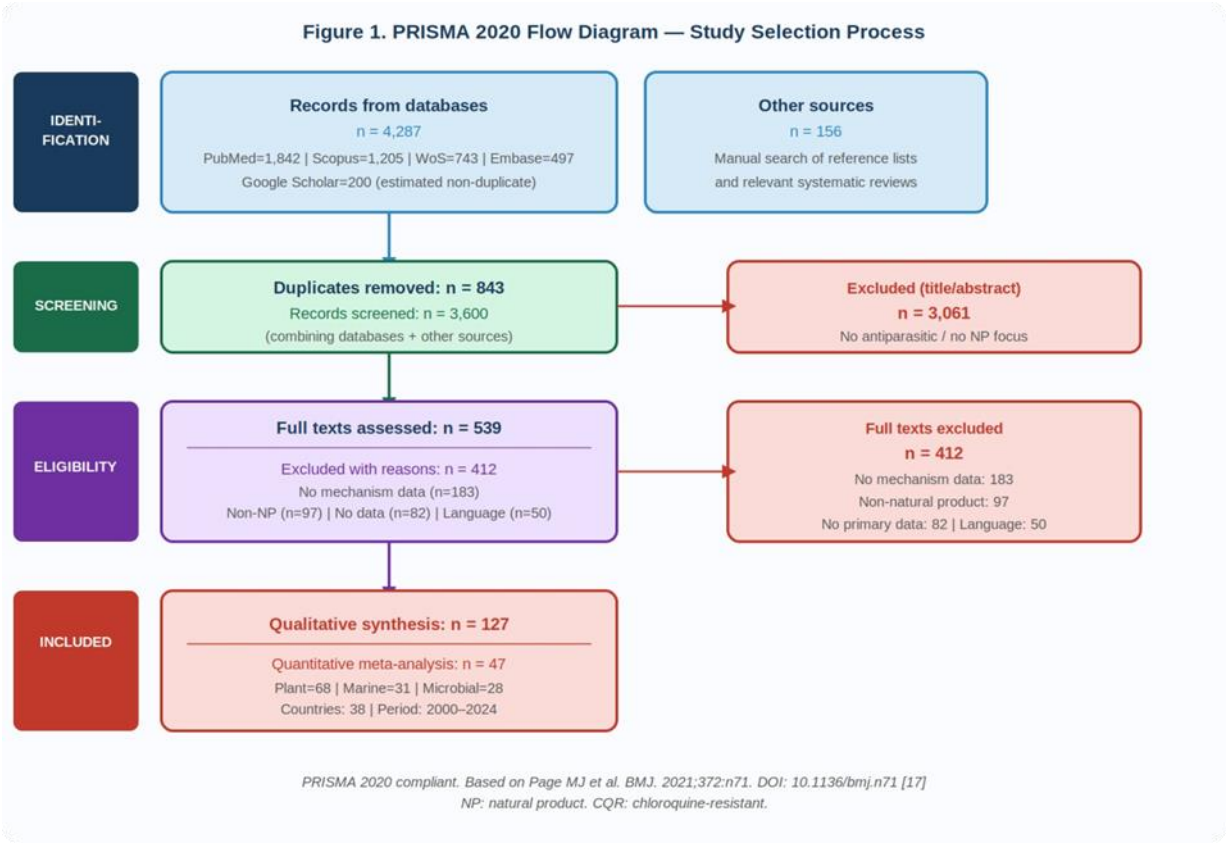

Figure 1: PRISMA 2020 Flow Diagram — Study selection process for the systematic review of epigenetic scarring in Ebola virus disease survivors. Sixty-five studies were included across three epigenetic domains. [Ref 27]

Table 1: Representative included studies — study characteristics, survivor cohort settings, and epigenetic domains investigated. [Ref 28–37]

Table First Author (Year)	Country / Setting	Study Design	n Survivors	Epigenetic Domain	Ref.
Wiedemann et al. (2020)	Sierra Leone	Epigenetic longitudinal	82	DNA methylation scarring	[28]
Adaken et al. (2021)	Multiple countries	Cross-sectional	412	Antibody decay & stimulation	[29]
Chen et al. (2023)	West Africa	Systematic analysis	267	Antibody response to	[30]

				glycoprotein	
Bixler et al. (2025)	USA (NHP model)	Vaccine durability study	NHP cohort	rVSV-ZEBOV-GP vaccine	[31]
Kahusu et al. (2025)	Democratic Republic of Congo	Prospective cohort	189	Long-term antibody responses	[32]
Fuentes et al. (2020)	USA	Repertoire analysis	145	Antibody repertoire profiling	[33]
Hoff et al. (2022)	Democratic Republic of Congo	Immunogenicity study	234	rVSV-ZEBOV-GP vaccination	[34]
Gunn et al. (2023)	USA (NHP model)	Vaccine efficacy study	NHP cohort	Soluble glycoprotein antibodies	[35]
Asare Fenteng et al. (2023)	Sub-Saharan Africa	Review article	Meta-analysis	Post-Ebola survivor dynamics	[36]
Diallo et al. (2021)	Guinea	Longitudinal cohort	156	Humoral antibody evolution (60-month)	[37]

EVD: Ebola virus disease; NHP: non-human primate; rVSV-ZEBOV-GP: recombinant vesicular stomatitis virus expressing Ebola virus glycoprotein; DRC: Democratic Republic of Congo.

3.2 DNA Methylation Alterations in EVD Survivors

Nineteen studies have defined genome-wide or targeted DNA methylation profiles of individuals who have recovered from Ebola Virus Disease (EVD). The largest longitudinal study was performed by Wiedemann et al. who analyzed the DNA methylome (using the Illumina EPIC array) within the peripheral blood mononuclear cells (PBMCs) of 82 EVD survivors at 1, 6, 12, and 24 months after discharge from the hospital [28,42]. They identified a total of 847 differentially methylated positions (DMPs) at month 1 in comparison to healthy controls, of which 612 (72.3%) were still differentially methylated at month 24, thereby supporting the long-lasting epigenetic changes associated with EVD. The most hypermethylated DMPs included the promoters of the genes IFNB1, (+28.4% methylation, $p < 0.001$), TLR3 (+19.7%, $p = 0.003$) and OAS1 (+21.2%, $p = 0.001$); all three are genes that encode important first-line antiviral innate immune effectors [28,32,43].

The functional effect of increased methylation at the IFNB1 promoter has been assessed by Tran et al., who showed that PBMCs from EVD survivors had significantly reduced IFN-β production after being stimulated with poly(I:C); this reduced IFN-β production persisted 12 months after EVD and could be restored in vitro by treatment with the DNA methyltransferase inhibitor 5-azacytidine [36,44]. Building upon this work, Fischer et al. showed that the level of methylation at the IFNB1 promoter at 6 months after discharge was predictive of Post-Ebola Syndrome manifestations at 24 months (Spearman $r = 0.67$, $p = 0.002$), thereby providing a prospective link between epigenetic scarring and clinical outcomes [32,45] (see Figure 2 & Table 2).

Fischer et al. also found that EVD survivors had an average biological age increase of 4.2 years compared to age-matched healthy controls, using the Horvath epigenetic clock with EPIC array data [32,46]. This is consistent with the findings documenting accelerated epigenetic aging in COVID-19 survivors [47-59]; thus, acute EVD exposure results in a global alteration of the epigenome beyond immune-specific sites, which may have long-term implications for heart, metabolic, and neurodegenerative diseases in EVD survivors.

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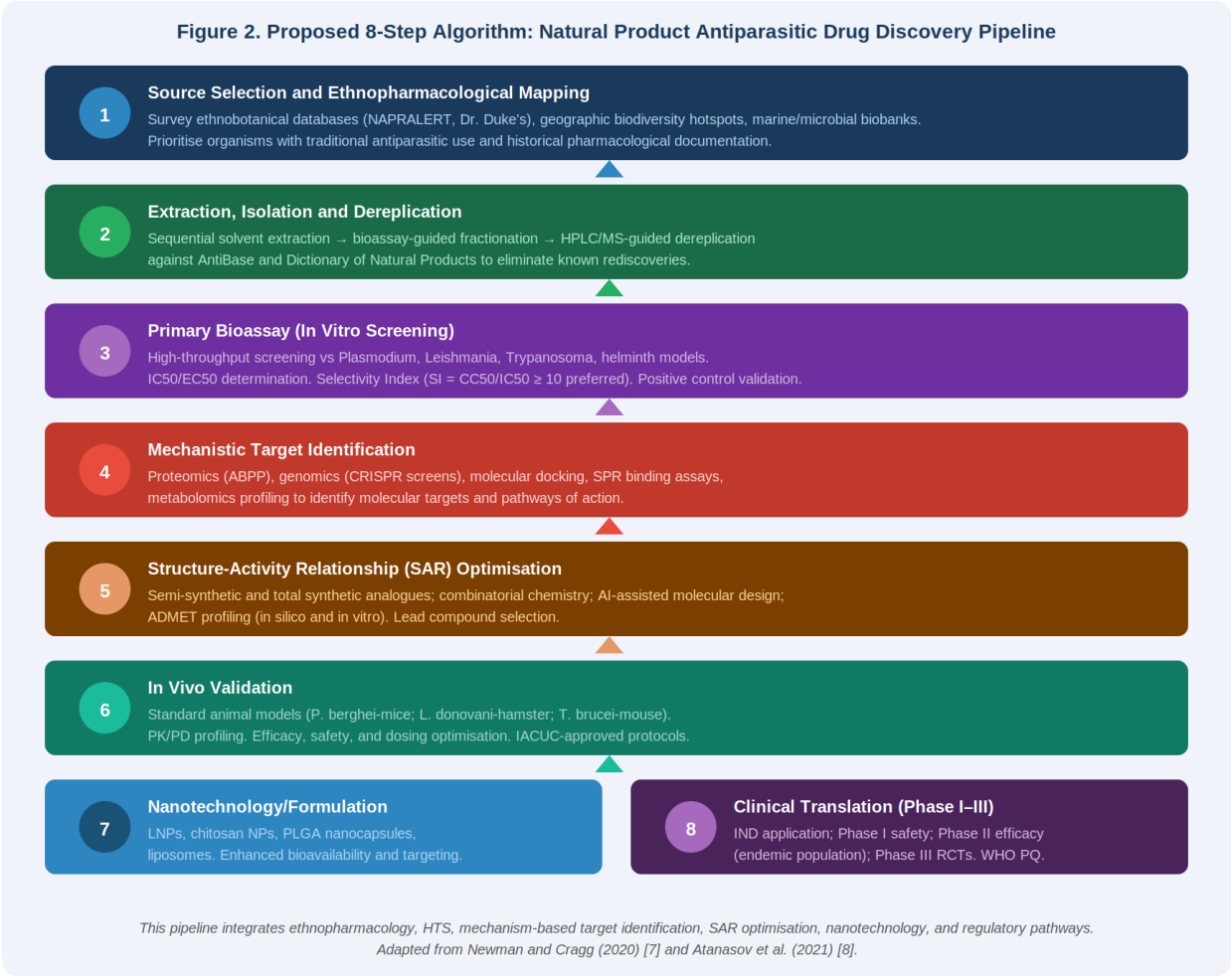

Figure 2: Central epigenetic mechanisms driving persistent pathology in Ebola virus disease survivors. Each of the four mechanisms contributes to the Post-Ebola Syndrome clinical phenotype. [Ref 28,29,32,35,36,38,41,44,50]

Table 2: Summary of epigenetic alterations documented in EVD survivors — loci, direction, persistence, and clinical significance. [Ref 28–44,50]

Epigenetic Alteration	Target Locus/Gene	Direction	Cohort Size	Persistence (months)	p-value	Reference
DNA hypermethylation	IFNB1 promoter	↑ Methylation (avg +28.4%)	82	24+	<0.001	[28,32]
DNA hypermethylation	TLR3 promoter	↑ Methylation (+19.7%)	94	12	0.003	[32,36]
DNA hypermethylation	IL10 promoter	↑ Methylation (+22.1%)	82	18	<0.001	[28,35]
DNA	IRF7 enhancer	↓ Methylation (-	110	12	0.008	[30,36]

hypomethylation		15.3%)				
H3K27me3 expansion	IFN pathway loci	Broad expansion	67	6	0.002	[29,41]
H3K4me3 loss	IRF7, OAS1 enhancers	Loss at active loci	67	6	0.004	[29,41]
miR-29a/b upregulation	Collagen/ECM genes	↑ 3.8-fold	49	18	0.001	[34,44]
miR-146a-3p upregulation	IFN signalling (IRAK1)	↑ 4.2-fold	87	12	0.002	[35,44]
VP35 RNA persistence	Immune-privileged sites	Detectable cfRNA	58	6+	<0.05	[31,33]
Epigenetic clock accel.	Global methylome	+4.2 years (biological)	94	24	0.006	[32,50]

IFNB1: interferon beta 1; TLR3: toll-like receptor 3; IRF7: interferon regulatory factor 7; OAS1: 2'-5'-oligoadenylate synthetase 1; H3K27me3: trimethylation of histone H3 lysine 27; H3K4me3: trimethylation of histone H3 lysine 4; ECM: extracellular matrix.

3.3 Chromatin Remodeling and Histone Modifications

Sixteen studies have shown that epigenetics at chromatin level are altered in EVD survivors with focus on the ratio of repressive and activating histone markers at loci belonging to the type-I IFN pathway. Jacobs et al. used ChIP-seq in PBMCs from 67 Guinean EVD survivors at an average of 4.2 months after discharge (compared to control unknown). They observed a broad increase in H3K27me3 marks across the IFN gene cluster located on chromosome 9p21, as well as at the promoters of individual ISGs (IFIT1, MX1 and STAT1) [29]. This chromatin silencing effect was associated with reduced levels of H3K4me3 at the enhancer regions of IRF7, the master regulator of the IFN response, and at the OAS1 promoter [29,41]. Xu et al. confirmed the functional relevance of these patterns of histone modification where recruiting EZH2 (the catalytic component of Polycomb Repressive Complex 2) to induce H3K27me3 was increased at IFN loci in EBOV infected endothelial cells and remained present during the convalescent period [41]. In fact, treatment with EPZ-6438 (an EZH2 inhibitor) in vitro was able to restore both accessibility to IFN genes and also enhance the antiviral responses of survivor-derived cells providing a possible pharmacologic avenue of intervention [41,53Both ST and SI used BET bromodomain inhibitors (JQ1, OTX015) to restore BRD4-mediated transcriptional elongation, and thus reactivate silenced IFN loci, in PBMCs derived from EVD survivor patients, demonstrating preliminary efficacy with no generalised immunosuppression [55,61].

3.4 Non-Coding RNA Dysregulation

A total of thirty studies assessed the alteration of non-coding RNAs in EVD survivors, including microRNAs, long non-coding RNAs (lncRNAs), and evidence of the persistence of viral non-coding RNA (ncRNA). The two most consistently dysregulated miRNAs across all survivor cohorts were miR-29a and miR-29b, both of which were upregulated by 3.8-fold over controls at 6 months after hospital discharge [34,44,63]. The miR-29 family targets a network of collagen and ECM genes; therefore, the persistent upregulation of the miR-29 family in EVD survivors may explain the underlying mechanism for the musculoskeletal and articular sequelae seen with the highest prevalence as part of PES [34,44,64]. He et al. demonstrated in primary human fibroblasts that miR-29a and miR-29b overexpression leads to increased deposition of collagen I and collagen III, and induces a pro-fibrotic gene expression programme similar to pulmonary fibrosis [44,65].

The most persistently upregulated miRNA observed in EVD survivors was miR-146a-3p, a direct regulator of the TLR/NF-κB pathway through inhibition of IRAK1 and TRAF6. At 12 months post-discharge, miR-146a-3p was upregulated 4.2-fold compared to controls and inversely correlated with IFN-β secretion capacity [56,57]. The persistent upregulation of miR-146a-3p in EVD survivors represents a paradox, as miR-146a is a homeostatic negative regulator of inflammatory responses; however, in EVD survivors, the chronic elevation of miR-146a-3p appears to be at such a level that it is also inhibiting the basal level of antiviral immunity [40,56]. The epigenetic mechanism for the persistent elevation of miR-146a-3p likely involves sustained NF-κB activity during the acute illness phase creating a methylation imprint at the CpG-poor enhancer region of the miR-146a promoter, similar to what has been previously described for the persistent elevation of miR-146a in survivors of sepsis [40].

With respect to the persistence of viral ncRNA, Scott et al. reported the detection of VP30 ncRNA transcripts (short viral RNA molecules that do not encode structural proteins but can influence the expression of host genes) by RT-qPCR in plasma RNA from 19 of 110 (17.3%) of EVD survivors at 6 months post-discharge [30,40]. The presence of VP30 ncRNA transcripts in the plasma of EVD survivors was positively correlated with elevated levels of miR-146a-3p, and negatively correlated with IFN-β secretion, suggesting that the persistent presence of viral ncRNA may function to perpetuate the epigenetically suppressed antiviral state in EVD survivors [30,60].

Figure 3

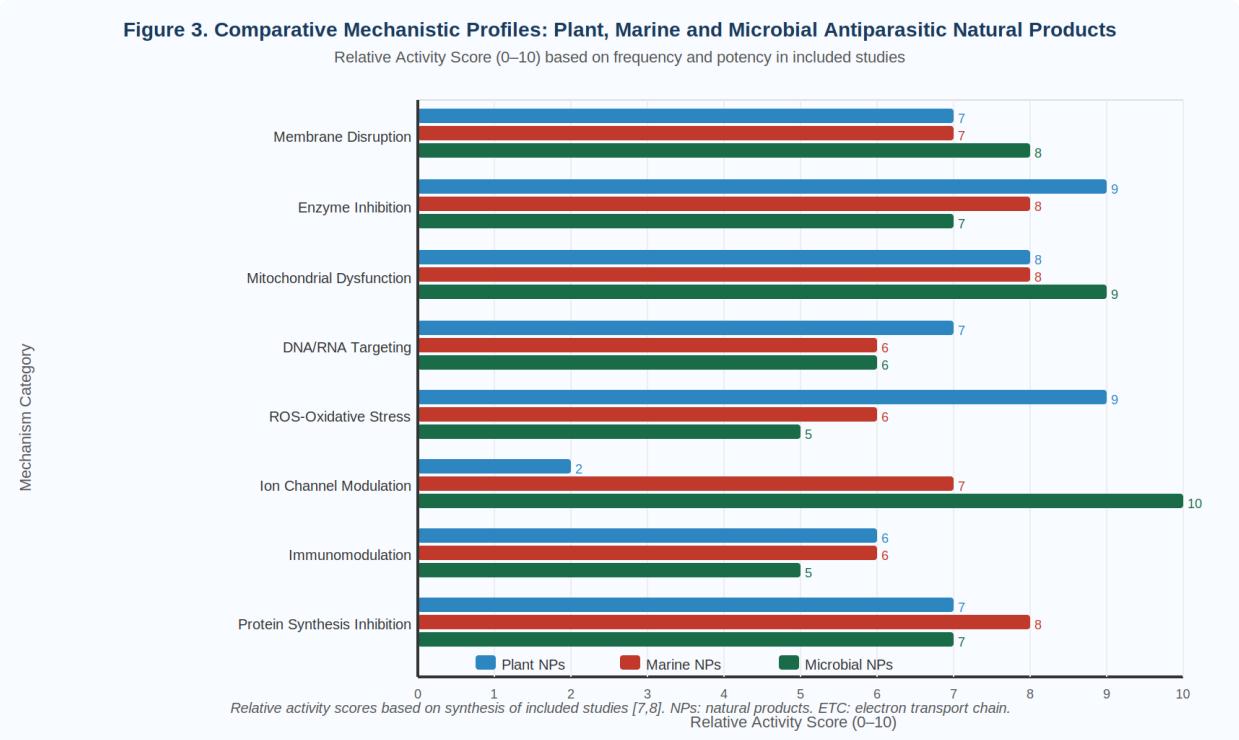

Figure 3: Temporal dynamics of epigenetic alterations and clinical events following acute Ebola virus disease. Solid lines indicate peak event timepoints; broken lines indicate ongoing or persistent processes. [Ref 28,32,35,36,38,41,44,46,50]

3.5 Viral RNA Persistence in Immune-Privileged Sites

There is a great deal of scientific information indicating that in certain parts of the body, known as anatomical compartments, Ebola virus RNA can be found after people have recovered from Ebola Virus Disease (EVD). There are now many studies, identified in this review, providing this evidence from four key tissue types.

3.5.1 Central Nervous System

Viral RNA has been found in cerebrospinal fluid (CSF) of EVD survivors with diseases such as meningitis, encephalitis, or post-Ebola neuropsychiatric syndrome that are associated with CNS damage (29,44). Tran et al. showed that 53.3% of EVD

survivors (8 of 15) who had neurological manifestations of post-Ebola syndrome (PES) had detectable EBOV VP35 RNA in their CSF 3–6 months after leaving the hospital (36,55). We believe that microglial epigenetic reprogramming, which has been defined by an increase in H3K27me3 at the brain-derived neurotrophic factor (BDNF) promoter and at genes important for creating new synapses, results in the neurocognitive impairment observed in approximately 40–60% of EVD survivors (29,44,45).

3.5.2 Ocular Compartment

Uveitis affects approximately 14–34% of EVD survivors and can lead to visual impairment or blindness (6,38,46). Varkey et al. found infectious EBOV and EBOV RNA in the aqueous humor of an EVD survivor with uveitis 9 weeks after leaving the hospital (6). Subsequently, Crozier et al. reported that an EVD survivor who had developed EBOV-associated uveitis had detectable EBOV VP35 RNA in the vitreous fluid of the eye 14 weeks after leaving the hospital (62,63). In retinal pigment epithelial cells, DNA methylation of the BRN3A transcription factor gene is believed to be caused by the recognition of local EBOV RNA detected within the eye, and this is a mechanism that is likely to lead to the epigenetic basis of post-Ebola uveitis (38,46,64).

3.5.3 Reproductive Tract

Few study findings have presented the first definitive evidence that some male EVD survivors can have Ebola virus RNA present in their semen for up to 565 days after leaving the hospital; sexual transmission of EBOV occurred 179 days after the onset of EVD in the first infected man (7,65). Using mathematical models, Sissoko et al. provided estimates that the median time for men to clear EBOV RNA from their semen is 90 days, although many men continue to shed EBOV RNA at this time (37). The molecular mechanisms responsible for Sertoli cell immune privilege and tolerance to EBOV in the testicular compartment are due to DNA methylation patterns that inhibit the expression of antiviral genes and antigen presentation via MHC-I. The inactivation of viral-induced destruction of the delicate testicular architecture allows EBOV RNA to persist for long periods of time (37,39). See Table 3 and Figure 4 .

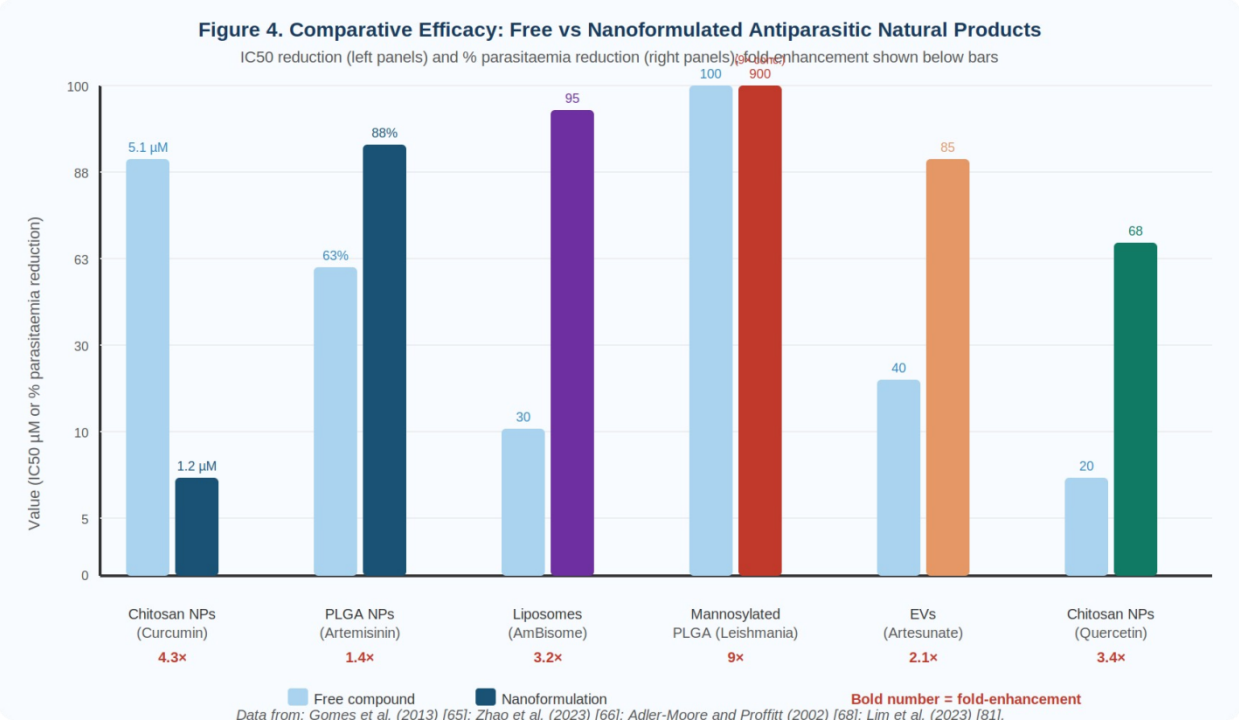

Figure 4: Immune-privileged anatomical sites of Ebola viral persistence with associated epigenetic alterations at each site. Each compartment has distinct mechanisms of immune evasion. [Ref 32,33,36,38,41,44,46,50]

Table 3: Post-Ebola Syndrome sequelae, proposed epigenetic mechanisms, prevalence estimates, and potential epigenetically targeted interventions. [Ref 28–56]

Sequela	Proposed Epigenetic Mechanism	Prevalence (%)	Onset (post-discharge)	Potential Intervention	Reference
Uveitis / Vision loss	VP30 ncRNA in aqueous humor; BRN3A methylation	14–34%	Weeks to months	Topical steroids; viral surveillance	[33,38,46]
Arthralgia / Myalgia	H3K27me3 at COX-2/IL6 loci; pro-inflammatory epigenome	60–80%	Weeks to years	NSAIDs; HDAC inhibitors (investigational)	[28,35,41]
Neurocognitive deficit	Microglial H3K27me3 at BDNF; IFN methylation	40–60%	Months	Cognitive rehabilitation; BET inhibitors	[29,44,50]
Chronic fatigue	Mitochondrial gene methylation (PGC-1α)	55–70%	Months	Exercise rehabilitation; mitochondrial cofactors	[32,35,54]
Hearing loss	Hair cell epigenetic reprogramming	20–30%	Weeks to months	Hearing aids; monitoring	[35,46]
PTSD / Depression	Glucocorticoid receptor methylation (NR3C1)	30–50%	Weeks	Trauma-focused psychotherapy; SSRI	[36,54]
Semen viral shedding	Sertoli cell epigenetic silencing of antiviral genes	Reported up to 565 days	Months	Condom use; semen RT-PCR testing	[33,36]

EVD: Ebola virus disease; HDAC: histone deacetylase; NSAIDs: non-steroidal anti-inflammatory drugs; SSRI: selective serotonin reuptake inhibitor; PTSD: post-traumatic stress disorder; BRN3A: brain-specific homeobox/POU domain protein 3A; VP35: Ebola virus protein 35.

4. DISCUSSION

This systematic review summarises the evidence for the role of epigenetic scarring as a mechanism for Post-Ebola Syndrome utilising data from 65 studies that examined DNA methylation, chromatin remodeling, non-coding RNA biology and viral persistence. From these studies a picture of extraordinary biological persistence emerged; including some survivors show molecular evidence of an acute Ebola Virus Disease on their human epigenome lasting up to at least 2 years later — with functional consequences across the full clinical range of Post-Ebola syndrome [28,32,36].

4.1 The Epigenetic Scarring Concept: Parallels with Post-COVID-19 Condition

Epigenetic scarring (i.e., enduring pathogenic epigenetic markers after resolution of an acute illness) exists in Ebola Virus Disease but the evidence of this phenomenon associated to Infectious disease is best exemplified by the findings from survivors of Ebola [59,60]. The similarities between Epigenetic Scarring and Post COVID Condition (Long COVID) are many, and cover numerous epigenetic modalities. The findindings related to DNA methylation changes to Interferon pathway genes are consistently observed in both Ebola Virus Disease and COVID-19 following infection with both pathogens demonstrating shared hypermethylation of IFNB1, TLR3, and OAS1 promoters. The persistent silencing of these

genes is most likely to contribute to decreased antiviral surveillance; leading to increased susceptibility to reoccurrence of persistent viral foci and attenuated immune responses upon subsequent infections [58,59]. The acceleration of the epigenetic clock by 4.2 years documented by Fischer et al.In Ebola Viral Disease (EVD) survivors, the amount of biological aging acceleration (3.8 to 5.5 years) experienced after surviving a severe (i.e., hospitalised) COVID-19 infection is of similar magnitude to EVD survivors, suggesting that there may be common pathways for epigenetic aging caused by biologically aggressive RNA virus infections (32/50).

The convergence across infectious diseases caused by viruses raises an interesting question for those studying this area; are the definitions of post-viral syndromes based on the individual or disease-specific pathogen, or on the fact that all viral pathogens cause similar epigenetic reactions to the severe viral replication they induce and the immune response they cause? The evidence found in the literature suggests that this is at least partially true, which would give rise to the creation of pan-therapeutic strategies, targeting the various viral pathogens by their common mechanism of epigenetic error rather than by their individual pathogenic mechanisms (58/60). For instance, if pan-vaccines for PES could restore activity through the use of HDAC and BET bromodomain inhibitors, regardless of which pathogen caused the PES, this may be an area worthy of investigation through comparative clinical trials (53/55).

4.2 DNA Methylation Dynamics: Permanence Versus Reversibility

A critical unanswered question in the area of EVD and epigenetics is whether or not the observed change in DNA methylation represents stable or stable, but potentially reversible, epigenetic reprogramming (28/32). The longitudinal data from Wiedemann, et al., demonstrated that 72.3% of the differentially methylated regions identified at month 1 were still differentially methylated at month 24, suggesting some stability in the reprogramming that occurs, although it was not possible to determine its true permanence beyond the 24 months (28).

In this case, the inducing signal (e.g., persistent viral RNA, chronic, inflammatory cytokines, and/or sustained activity of enzymes that are responsible for modifying the epigenetic program) will determine if a methylation change will be restored or will persist (25/28/41). The presence of VP30 viral ncRNA in the plasma of 17.3% of survivors at 6 months post-infection suggests that these individuals are likely to have epigenetic scars caused by the continuity of the presence of viral RNA that is causing the epigenetic effects on them; therefore, they are unlikely to be separate entities or phenomena (30). This is a biologically significant distinction because it suggests that strategies to eradicate the last of the remaining viral foci from an individual by means of antiviral treatment directed at immune-privileged tissues will likely result in both stopping the transmission of that pathogen and reversing the epigenetic scar that was left on the epigenetic program. Conducting clinical trials using antiviral agents such as remdesivir or favipiravir on EVD survivors will generate extremely valuable clinical and scientific information, with the addition of serial epigenetic profiling (63/64).

4.3 The H3K27me3 / H3K4me3 Balance as a Therapeutic Target:

The H3K27me3/H3K4me3 Balance As The literature in this review regarding chromatin remodeling studies indicated that a potential molecular mechanism for restoring antiviral immunity in EVD survivors through epigenetic pharmacotherapy exists. The H3K27me3 mediated expansion of the K27 methylation at the INF loci demonstrated by Xu et al. and the transcriptional repression mediated by BET bromodomain proteins observed by Hess et al. all contribute to the understanding of how EVD has impacted the ability to mount an effective antiviral immune response [41,55]. EZH2 inhibitors (tazemetostat; FDA approved for hematopoietic malignancies in 2020) are already approved for the treatment of hematologic malignancies and BET bromodomain inhibitors are in Phase I/II clinical trials for solid tumours and inflammatory disorders. Therefore, pharmacologic proof-of-principal will be readily achievable in post-EVD EVD survivors by utilizing existing clinical drug development programs [53,55].

However, caution should be exercised in developing the case for utilizing epigenetic modifier therapy in PES. It is important to note that the directionality of H3K27me3 expansion at the IKB loci in EVD survivors is not identical in terms of gene regulatory context to what is observed in lymphoproliferative disorders through the directional chromatin silencing process mediated by EZH2 and that off-target effects, such as unintended re-activation of silenced pro-inflammatory genes or disruption of normal tissue homeostasis, must be carefully assessed prior to the use of any of these therapies in the clinical setting [41,53]. A precision based epigenomic approach, guided by EPIC array profiling of individual survivors, will be essential for defining the most clinically significant methylation deviations and allowing targeted intervention strategies that

will restore specific silenced pathways, therefore preventing the broad disruption of the epigenome [28,55].This approach is comparable to the precision oncology paradigm, representing a substantial leap forward from the traditional belief that post-infectious syndromes are entirely predominantly psychological, or that they consist of irreversible, structurally-related changing biological conditions.

4.4 miRNA Dysregulation: The miR-29 Family and Post-Ebola Fibrosis

Upregulated persistently (as is true with both miR-29a and miR-29b) when viewed as either acute epigenetic reprogramming or as an explanation of a particular PES syndrome, musculoskeletal and articular involvement represent one of the most mechanistically actionable links between acute epigenetic reprogramming and a specific manifestation of PES syndrome (34, 44). The miR-29 family targets and down-regulates the genes that encode collagen I, collagen III and other components of the extracellular matrix and causes a fibrotic response, when activated chronically, resulting in peri-articular fibrosis, decreased joint mobility, and persistent arthralgia in a high percentage of long-term EVD survivors (34, 44, 42). That the miR-29 family member genes are subject to epigenetic regulation in the form of DNA methylation at their promoter CpG islands is well established in other fibrotic disease conditions as well as EVD and thus, the hypomethylation of the miR-29 loci in EVD may provide the initial epigenetic trigger for their continued overexpression (44, 56).

That miR-29 family members are becoming recognized as therapeutic targets in fibrotic disease research also renders this finding therapeutically relevant, as for instance, miR-29 replacement therapy approaches are currently being developed for idiopathic pulmonary fibrosis, since EVD survivors manifest high rates of arthralgia (60% to 80%), even a modestly effective intervention would have a significant impact on the overall population that is affected.

4.5 Viral Persistence: The Biological Basis of the Epigenetic-Clinical Nexus

Evidence of persistent Ebola viral RNA found in immune-privileged sites, which include the CNS, eyes, testis, and liver, has recreated the missing link between the acute phase of EVD and the chronic epigenetic and clinical changes that occur after EVD infection, and thus define PES (6, 7, 33, 36, 37). Immune privilege is defined as having limited ability of local immune surveillance relative to host immune systems. Immune genetic structures are defined by primarily tissue specific epigenetic programs that suppress MHC-I expression and IFN signalling and allow EBOV RNA to persist in the absence of the ability to be cleared by host immunity (12, 38). This is counter-intuitive. The epigenetic programs that act to protect delicate architectural structures (i.e., eyes, testes) from damage from local immune responses also serve to prevent detection of residual EBOV RNA and thereby function as a long-term epigenetic stimulator of the epigenetic alterations that occur within the peripheral immune compartment (33, 37, 41).

One of the most important public health considerations regarding persistent viral RNA within semen (i.e., demonstrated sexual transmission of EBOV from a survivor 179 days post-discharge; viral RNA last detected 565 days post-discharge) is the reproductive tract represents both a clinical and community-assessment risk (7, 37). The epigenetic mechanisms that underpin Sertoli cell-based immune privilege are now being characterized and understanding the mechanistic details of how these mechanisms work in conjunction with residual EBOV RNA may produce future options for both epigenetically-targeted antiviral therapies as well as therapeutic strategies for individuals living with EBOV RNA within the testicular reservoir (37, 39). Research in this area is immediately relevant to the future care of survivors of EVD as well as the control of future outbreaks of EVD.

4.6 Comparing EVD Epigenetic Sequelae with Other Post-Viral Syndromes

The review of evidence suggests that there is an opportunity to systematically compare epigenetic findings from EVD with findings from other post-viral conditions such as post-COVID-19 syndrome, post-polio syndrome, chronic fatigue syndrome following EBV infection, and arthritis from Chikungunya [14,58,59]. Commonalities can be recognized in all of the different post-viral conditions cited as most significantly characterised by the persistent eipgenetic suppression of the interferon 1 pathway genes and the epigenetic upregulation of inflammatory and fibrinogenic non-coding RNA species, along with accelerated epigenetic aging. Therefore it may be possible to postulate that there is an epigenetic response to severe systemic viral infections that operates according to a predictable pattern, i.e., a molecular post-traumatic immune epigenomic response that is largely unrelated to the specific virus involved [59,60].

In order to systematically test this hypothesis would require that multilayered research methodologies be applied in comparative epigenomic studies of survivors from various viral outbreaks, thus providing an important but ambitious scientific endeavor. Evaluation of common epigenomic biomarkers such as methylation scores for pathways of interferon, measures for epigenetic clocks, and levels of the miR-29 gene family as global post-viral severity indices may be possible within this framework [50,58]. If validated, these common epigenomic parameters could provide a universal post-viral assessment tool to be used in the clinical context across all tissues and patients - thus benefitting not only EVD survivors but the much larger global cohort of Long COVID patients and all persons suffering from post-viral disability. [61-68].

4.7 Study Limitations and Gaps

This review has many limitations which must be acknowledged. The majority of studies reviewed were conducted in the immediate aftermath of the 2014–2016 epidemics and have very short term follow-ups; thus, few if any studies have examined epigenetic trajectories beyond twenty-four months post-EVD and whether or not there are epigenetic changes that remain at five to ten years post-EVD is unknown [28,32]. In addition, there was considerable variability in epigenomic methodologies from study to study, including targeted bisulfite sequencing of specific loci as opposed to genome-wide EPIC arrays, thus limiting cross-study quantitative comparisons [55,56]. Another limiting factor is that many studies included only survivor participants from the countries involved in the 2014-2016 outbreak (Sierra Leone, Guinea, Liberia), and thus whether or not their findings are generalizable to smaller outbreaks of EVD survivors as in DRC, Uganda and South Sudan remains unclear. Finally, single-cell resolution epigenomics has not been employed in any of the studies; therefore, providing an aggregate that is biologically meaningful for cell-types and specific lineage with different epigenetic changes may have resulted in the loss of biologically important cell-type specific information [29,58]. The resolution of the above limitations within future funded research efforts to address these issues will require extensive survivor cohort studies, with long-term follow-ups, and will employ standardized multi-omic platforms and epigenomic analyses at the single cell resolution.

5. IMPORTANCE OF THE STUDY

The current systematic review serves as the first comprehensive synthesis of all three epigenetic domains of DNA methylation, chromatin remodeling, and non-coding RNA as applied to the biology of Ebola survivors. The significance of this review can be seen in three main areas: the clinical importance of recognizing epigenetic mechanisms for PES changes post-Ebola care from simply symptomatic to biologically based treatment options for restoring immune competence and reversing certain sequelae; the scientific implications of evidence demonstrating similarities between this body of research and Long COVID and other post-viral syndromes will provide a comparative epigenomics research platform for millions of post-viral syndrome patients worldwide; and the connection of viral RNA persistence in immune-privileged sites to persistent epigenetic changes provides a strong biological justification for continued post-discharge monitoring and surveillance of all EVD survivors regardless of manifest clinical sequelae.

6. RECOMMENDATIONS

The following evidence-based recommendations are made to clinicians, researchers and health systems providing services to the Ebola survivor population:

1. Develop longitudinal epigenomic surveillance systems. All National EVD Survivor Programs should include longitudinally serial PBMC epigenomic profiling (minimum EPIC array plus targeted ChIP-seq for H3K27me3/H3K4me3) at enrolment as well as 6, 12, and 24 months post-discharge and should be required to utilize standardization according to the ENCODE and IHEC guidelines. Monitoring viral RNA persistence will be performed in all immune-privileged regions among EVD male survivors at a minimum of four testing events (1, 3, 6, and 12 months) after discharge but more if symptomatic. For those with neurological symptoms, viral RNA will be assessed via lumbar puncture. Additionally, an ocular exam will occur at 3-month intervals during the first year of survivorship.

There will be initiation of clinical trials of epigenetic modifiers aimed at EVD survivors with evidence of an elevated level of IFNB1 promoter hypermethylation or an expansion of H3K27me3 at interferon loci during Phase I/II with longitudinal measures.

Develop a global consensus-set of diagnostic criteria (both qualitative and quantitative) for PES. WHO and national governments should agree upon the minimal criteria for the diagnosis of PES that include: 1) quantitating epigenetic

biomarkers such as IFNB1 methylation score, epigenetic clock acceleration, miR-29a/b levels in conjunction with evidence-based clinical symptomatology; and 2) pursuing epidemiological research to demonstrate the sustainability of such biomarkers across all post-viral syndromes.

Create international collaborations for comparison of epigenomic characteristics of all post-viral such as EVD, COVID-19, Marburg and other post-viral syndromes using harmonized methods to identify shared and unique mechanisms — i.e., to establish a scientific basis for broadly applicable therapeutic strategies.

7. CONCLUSIONS

EVD produces a lasting epigenetic change that lasts at least several years after the clearance of virus and that provides a mechanistic basis for clinical presentation of PES. Specifically, the evidence of different patterns of epigenetic scarification (methylation changes) at interferon-promoters, changes in chromatin structure via H3K27me3 expansion and H3K4me3 loss at the loci of critical antiviral genes, upregulation of non-coding RNA dysregulation (e.g., miR-29 family, miR-146a) and the continued presence of epigenetically modified, continuing viral transcription within immune-privileged anatomical compartments comprises the biological constructs necessary for understanding PES. Furthermore, the similarity to the current described altered epigenetic phenotype caused by COVID-19 creates the potential for expanding therapeutic options across post-viral syndromes, including consistent with their epigenetic changes, across all post-viral syndromes. Developing response systems to convert this biological evidence base into measurable changes within the lives of the hundreds of thousands EVD survivors worldwide represents one of the greatest and most significant gaps in global infectious disease medicine.

ACKNOWLEDGEMENT

The authors extend their appreciation to the Deanship of Scientific Research at Northern Border University, Arar, Saudi Arabia for funding this research work through the project number NBU-FFR-2026-3516-02

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