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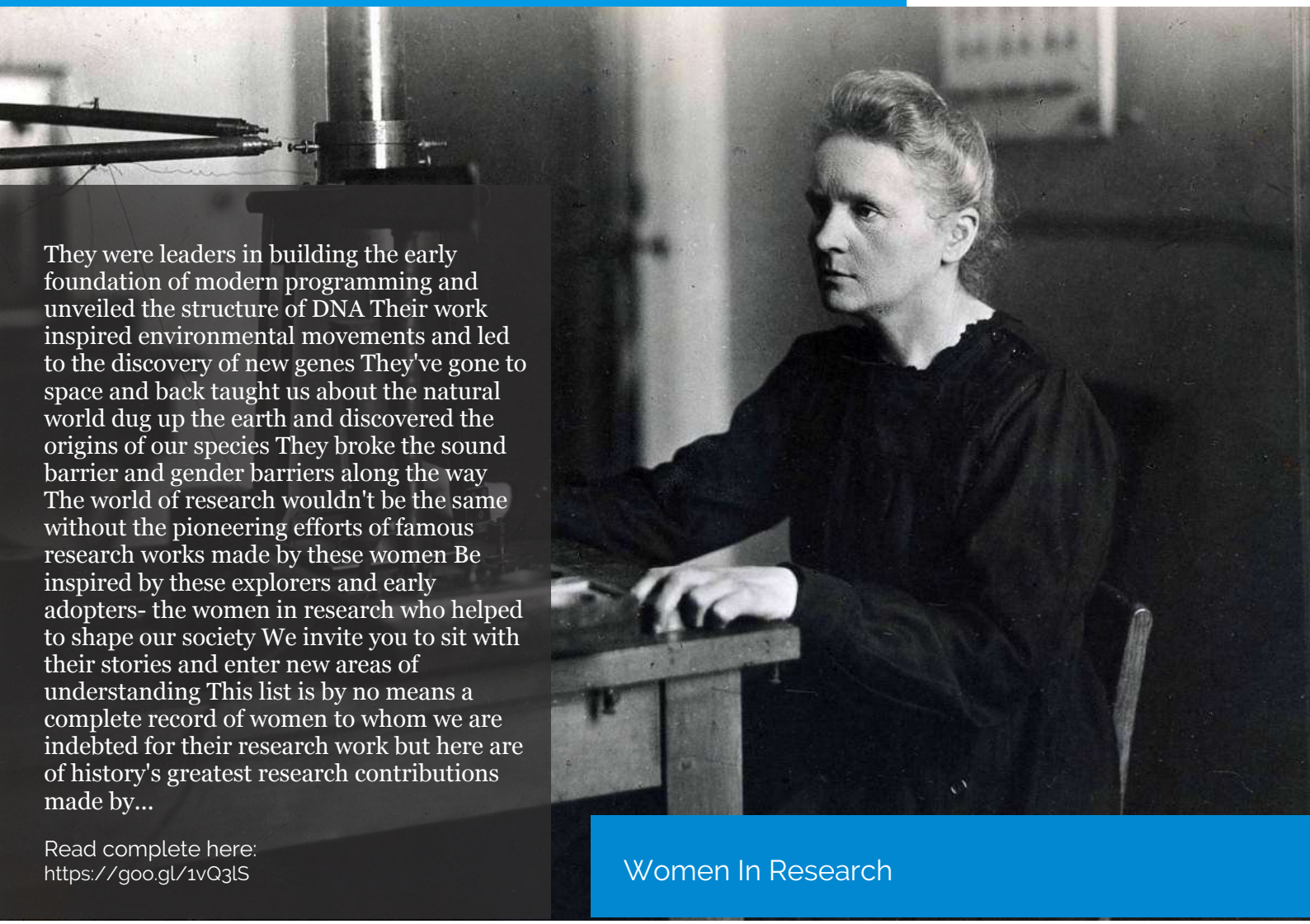

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Primary Tokophobia Rooted in Developmental Trauma: A Case Study of Reproductive Fear in Adulthood

Kodjo Anahlui, Afiwa Agbobli & Bassantéa Lodegaèna, Kpassagou

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ABSTRACT

This case study explores a rare presentation of tokophobia-an intense, pathological fear of childbirth in a 38-year-old Togolese woman named Afi, situated within a highly pro-natalist cultural context. Unlike the normative expectations in Togo, where motherhood is celebrated and socially reinforced from an early age, Afi developed a persistent fear of pregnancy and childbirth following early developmental trauma and using an integrative therapeutic approach-including cognitive-behavioral therapy, imagery desensitization, psychoeducation, and observational exposure the intervention aimed to reframe traumatic associations and restore autonomy in reproductive decision-making. The treatment was framed within a solution-focused and brief therapy model and culturally adapted to fit collective values. The findings underscore the importance of trauma-informed, culturally sensitive interventions in underrecognized mental health conditions such as tokophobia, particularly in African contexts where such cases are seldom documented. While the results are not generalizable, this case contributes to the emerging literature on reproductive trauma in non-Western settings.

Keywords: tokophobia, developmental trauma, cognitive-behavioral therapy, reproductive mental health, cultural psychiatry, togo.

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I. INTRODUCTION

Tokophobia-derived from the Greek *tokos* (childbirth) and *phobos* (fear)-is defined as a persistent, irrational, and often debilitating fear of pregnancy and childbirth. It is classified as a Specific Phobia, Other Type (F40.298), in the *Diagnostic and Statistical Manual of Mental Disorders*, 5th Edition, Text Revision (*DSM-5-TR*; American Psychiatric Association, 2022). Two subtypes are generally distinguished: *primary tokophobia,* which develops in individuals without prior pregnancy experience-often rooted in childhood trauma or negative cultural conditioning-and *secondary tokophobia*, which arises following traumatic obstetric experiences such as complicated delivery, miscarriage, or perinatal loss (Hofberg & Brockington, 2000; O'Connell, Leahy-Warren, & Khashan, 2017).

In high-income Western countries, tokophobia has gained increasing clinical and academic attention due to its impact on reproductive decision-making, maternal mental health, and obstetric outcomes. Research has associated it with increased rates of elective cesarean sections, fertility avoidance, and comorbid anxiety or depressive disorders (Saisto & Halmesmäki, 2003; Zar et al., 2001). However, in many African settings, including Togo, the phenomenon remains underrecognized and underdocumented. The sociocultural context in Togo is heavily pronatalist: womanhood is commonly defined by the ability to conceive and bear children, and childbirth is celebrated as a collective triumph rather than a personal struggle (Attané & Tabutin, 2004). In such environments, expressing fear or ambivalence toward pregnancy may be stigmatized, silenced, or dismissed as weakness or selfishness.

This article presents the rare and clinically significant case of Afi, a 38-year-old financially independent Togolese woman who has primary tokophobia. Despite having access to modern obstetric care, Afi was consumed by fear of pregnancy, frequently seeking reassurance through pregnancy tests and avoiding intimate relationships. Her psychological distress stemmed from an early traumatic incident involving menstruation and the firsthand witnessing of a painful childbirth, which became symbolically and emotionally fused in her psyche.

The therapeutic intervention described in this article drew upon multiple theoretical frame-worksincluding cognitive-behavioral therapy (Beck, 1976), exposure and imagery rescripting (Arntz, 2012), developmental trauma theory (van der Kolk, 2005), attachment theory (Bowlby, 1988), emotion schema theory (Izard, 2009), and feminist and sociocultural psychology (Kirmayer, 2001). A culturally sensitive, solution-focused, and time-limited therapy model was implemented over 24 sessions, with attention to trauma-informed care and reproductive autonomy.

This case aims not to establish generalizable treatment standards but to contribute to a growing literature on culturally nuanced psychotherapeutic practices. By documenting a rare presentation of tokophobia in the Togolese context, this study underscores the need to acknowledge silent suffering in societies where childbirth is idealized. It fills a critical gap in maternal mental health research in West Africa. It invites further exploration of how gendered cultural scripts, early trauma, and psychological defenses intersect in shaping reproductive fears.

II. LITERATURE REVIEW AND PREVIOUS RESEARCH RELATED TO THE CASE STUDY

2.1 Introduction to the Topic

Tokophobia, defined as a pathological fear of childbirth, can manifest as primary (prior to any childbirth experience) or secondary (after a traumatic birth). Although largely documented in Western contexts (Hofberg & Brockington, 2000), the condition remains under-researched in African countries, including Togo, where cultural, spiritual, and gender-based factors may strongly influence women's reproductive experiences.

2.2 Global and African Prevalence of Childbirth-Related Fear

International research estimates that 6–14% of women experience clinical levels of fear of childbirth (O'Connell et al., 2017). In Sub-Saharan Africa, however, the *fear of childbirth* is often framed not in psychological terms, but through *spiritual, communal, and gendered narratives*, making it difficult to isolate as a clinical diagnosis. In Togo, anecdotal and qualitative evidence suggests women may attribute childbirth risks to *ancestral displeasure, curses, or failure to meet ritual or familial obligations,* rather than psychological conditions (Attipou & Dovi, 2012).

2.3 Cultural Beliefs in Togo: Motherhood, Fear, and Stigma

In Togolese society, *motherhood is a cultural expectation,* and a woman's value is often deeply linked to her reproductive capacity. Women who fear childbirth may internalize *shame or guilt,* especially in contexts where pregnancy is seen as a rite of passage and a source of social status. Traditional narratives often discourage the open expression of fear, and women may turn to *spiritual or religious interventions* rather than mental health services (Dosseh, 2017).

Additionally, *complications during childbirth* are frequently interpreted within the framework of *supernatural causes* (e.g., witchcraft, divine punishment), rather than medical or psychological explanations. This can create barriers to mental health intervention and delay psychological diagnosis or care.

2.4 Theoretical Perspectives: Trauma, Anxiety, and Culture

Western models such as *Cognitive Behavioral Theory (Beck, 1976)* or *Trauma-Informed Therapy (Van der Kolk, 2014)* may not fully capture the experiences of Togolese women. For instance, *intergenerational trauma* related to

childbirth deaths or forced marriages may shape women's fears, yet this may be culturally unspoken.

Furthermore, *Ubuntu-based frameworks,* which emphasize the individual as embedded in community, may help reframe the condition from an isolating psychological disorder to a *relational and collective concern* (Mkhize, 2004). Applying such culturally congruent models is crucial in making therapy relevant and effective.

2.5 Treatment Approaches in African Contexts

While evidence-based practices like *exposure therapy, imagery rescripting,* and *CBT* have been successful globally, their adaptation in African contexts remains limited. Studies from Ghana and Nigeria suggest that integrating *psychoeducation with cultural rituals and community support groups* enhances treatment outcomes (Ayonrinde, 2020).

In Togo, the few available mental health services are centralized and often under-resourced. Traditional healers and spiritual leaders play a prominent role in mental healthcare, and collaboration with these actors may be essential in culturally appropriate interventions.

2.6 Gaps in the Literature and Contribution of the Case Study

This case study addresses an essential gap: the *intersection of tokophobia, trauma, and cultural expectations in Togo*. It provides insight into how fear of childbirth can be both a *psychological and sociocultural issue,* requiring sensitive clinical approaches that are trauma-informed, community -aware, and spiritually literate.

III. CONCEPTUAL FRAMEWORKS AND THEORETICAL ANALYSIS

3.1 Cognitive-Behavioral Model of Specific Phobia

The cognitive-behavioral model (Beck, 1976) posits that irrational fears emerge from maladaptive beliefs that are reinforced by avoidance and safety behaviors. In Afi's case, the core belief- "pregnancy equals destruction"- developed from early exposure to traumatic childbirth and negative cultural messaging. Her persistent avoidance of intimacy, along with compulsive reassurance-seeking behavior (such as repeated pregnancy checks), maintained her phobia by preventing disconfirmation of her fears. These behaviors represent a classic anxiety-maintaining cycle, wherein short-term relief strengthens long-term distress.

3.2 Developmental Trauma Theory

The developmental trauma framework (van der Kolk, 2005; Herman, 1992) helps contextualize Afi's early emotional experiences and their impact on her adult functioning. Afi's exposure to maternal suffering and distress during childbirth-coinciding with her menarche at age 10-created an overwhelming emotional imprint. This moment became a nexus where her gender identity, reproductive capacity, and fear of suffering were tightly linked. These early, unprocessed sensory and emotional memories persisted as somatic flashbacks, influencing her nervous system's sensitivity to reproductive themes. Her current phobia is not just cognitive but embodied-fueled by stored sensory-emotional trauma.

3.3 Attachment Theory

Afi's pattern of initiating romantic relationships but later withdrawing may reflect a fearful-avoidant attachment style, consistent with Bowlby's and Main's work on insecure adult attachment. Her conflicting needs-desiring closeness yet fearing entrapment-suggest unresolved early attachment ruptures. Such patterns are commonly seen in individuals who grew up in unpredictable or emotionally invalidating environments. Afi's anxiety around intimacy may be a projection of her fear of eventual suffering or abandonment tied to reproductive roles.

3.4 Feminist and Sociocultural Perspectives

From a feminist and sociocultural standpoint, Afi's tokophobia is not solely intrapsychic but also embedded in collective narratives about woman hood in her cultural context. In Togo, as in many African societies, women are socially conditioned from a young age to value motherhood above all

else. However, the lived reality often includes exposure to maternal mortality, limited medical care, and the suppression of reproductive autonomy. Afi internalized symbolic messages from her environment: witnessing public shaming of pregnant women and hearing her mother express despair. These experiences conveyed that childbirth equals suffering, loss of agency, and socioeconomic hardship. Such cultural scripts, when absorbed unconsciously, can manifest as enduring emotional conflicts and somatic fears (Kirmayer, 2001).

3.5 Integrated Theoretical Discussion

Afi's case provides a compelling example of how multiple theoretical models intersect to explain the development and maintenance of tokophobia. Developmental trauma theory underscores the profound and disorganizing effects of early, unintegrated traumatic events. When trauma occurs during critical developmental windows-especially related to gender identity and the body-it can fragment the self and lead to chronic fear responses (Van der Kolk, 2014; Van der Hart et al., 2006). Afi's first menstruation became symbolically fused with the horror of witnessing unmedicated childbirth, generating a maladaptive schema equating femininity with inevitable suffering.

Emotion schema theory (Izard, 2009) further explains how these early affective experiences crystallized into habitual emotional responses. Each menstrual cycle served as a monthly trigger, reviving her original fear. Through classical conditioning (Pavlov, 1927), menstruation-initially a neutral physiological event-became paired with auditory and visual trauma cues, forming an enduring phobic response.

Social learning theory (Bandura, 1977) also plays a role in understanding how Afi internalized fear from observing and hearing about childbirth-related suffering in her community. Cultural pronatalist norms in Togo, while idealizing motherhood, may inadvertently silence fear and trauma, leading to under-recognized forms of reproductive anxiety. In such environments, tokophobia may be particularly underreported, making Afi's case both rare and clinically significant.

Finally, attachment theory and feminist frameworks shed light on the relational and societal components of her phobia. Afi's fear of intimacy and withdrawal from relationships mirror insecure attachment patterns, while the cultural messages about womanhood reinforce internal conflict. These overlapping systems of influence-personal, interpersonal, and cultural-converge to maintain a complex, multidimensional disorder that cannot be understood through any single lens.

IV. CASE DESCRIPTION

Afi, a 38-year-old woman with a master's degree in accounting, was referred by her obstetrician to psychological services after visiting the obstetrics department four times within three months, each time seeking confirmation that she was not pregnant. Despite reassurances and negative test results, Afi experienced persistent anxiety, which she described as "a consuming fear that something has started growing inside me."

4.1 Study Framework and Ethical Considerations

The study was conducted at the General Hospital of Adjido (CHP-Adjido), located in Aného. It was a descriptive, analytical, and prospective case study carried out from September 2011 to September 2012. A convenience sampling method was applied, as the participant was selected based on accessibility and referral.

At the time of the study, there was no institutional review board (IRB) in place at hospitals in Togo to formally approve research protocols. However, the study was conducted with respect for basic ethical principles. In line with local cultural practices, *verbal informed consent* was obtained from the client after explaining the purpose and procedures of the study. The participant's confidentiality was respected throughout the process.

In Togo, verbal consent is a culturally accepted norm, especially in clinical and community settings, and was considered appropriate in this

context. The study adhered to ethical principles such as respect for persons, beneficence, and confidentiality, adapted to the realities and norms of the local environment.

4.2 Relationship History

Afi reported four marriages, each ending in divorce initiated by her. "None of them were bad men," she clarified. "I just could not handle intimacy. Every time things got serious, I ran." She attributed these breakups primarily to her inability to engage in sexual activity without an overwhelming fear of pregnancy.

Afi avoided intimacy except during menstruation and expressed discomfort with contraceptives due to a latex allergy. She admitted to sometimes sabotaging relationships to escape the potential of sexual contact. She reported no history of sexual abuse, substance use, or mental health diagnoses.

4.3 Developmental and Cultural Context

Afi described her childhood as marked by economic hardship and emotional instability. Raised by a single mother in poverty, she witnessed her mother's suffering during childbirth, which occurred without medical care in a rural village. Afi recalled, "She screamed. I thought she would die. We had no money, no help."

Her stepfather was emotionally abusive, and Afi began working early to support her family. She reported that her mother often equated motherhood with poverty and shame.

In middle school, a classmate named Carol became pregnant and was expelled. The incident became a moral lesson for teachers and students: "Carol was used as an example of how smart girls ruin their lives," Afi called. "Even today, when I think about being intimate, I see her."

4.4 Current Presentation

Afi reported being asexual for over a decade, with no sexual relationships or desire for intimacy. She occasionally experienced fleeting attraction to women, which she viewed as safer: "At least with women, I knew I could not get pregnant." However, she later withdrew from relationships altogether, describing a profound disinterest and fear.

After protected sex, Afi would frequently rush to the hospital seeking confirmation that she was not pregnant. She acknowledged that this was irrational but stated, "Hearing it from a doctor is the only thing that calms me down."

4.5 Diagnosis

Using the DSM-5-TR:

- *Primary Diagnosis:*
  - *F40.298 – Specific Phobia, Other Type (Childbirth-related)*
- *Rule-Out Considerations:*
  - *Post-Traumatic Stress Disorder (PTSD)*: trauma from witnessing childbirth
  - *Obsessive-Compulsive Disorder (OCD)*: due to reassurance-seeking
  - *Avoidant Personality Disorder Traits*

V. THERAPEUTIC INTERVENTION

Treatment for Afi's tokophobia was conducted in three progressive phases across therapy sessions, combining theoretical exploration, cognitive restructuring, exposure-based methods, and real-world observation. These methods were chosen in alignment with prior research, which has shown that *cognitive-behavioral therapy (CBT), psychoeducation, and exposure therapy* are among the most effective strategies for treating tokophobia (Saisto & Halmesmäki, 2003; O'Connell et al., 2017).

Phase 1: Anamnesis and Cognitive Exploration

The first phase focused on *anamnesis,* building rapport, and exploring Afi's *personal history and core traumatic experiences*. Her earliest significant trauma occurred at the age of 10, during the onset of menstruation, when she witnessed a woman in her village undergoing a *painful and complicated childbirth*. Soon after, Afi was told: *"You are a woman now; you can get pregnant and go through the same pain."* This moment formed two lasting cognitive associations:

1. Menstruation = inevitable painful childbirth.
2. Childbirth = trauma and suffering.

Another powerful secondary association emerged when Afi recalled a teenage boy, born from that traumatic labor, telling his mother, *"I did not ask you to give birth to me."* This statement echoed in Afi's mind for years, reinforcing fears of maternal regret and offspring rejection.

These internalized beliefs led to emotional conflicts on a monthly basis. Despite no sexual activity, Afi felt simultaneous relief and dread during menstruation-relief at not being pregnant and dread from being reminded of the "inevitability" of childbirth. This ambivalence reflects *cognitive dissonance,* where Afi's values (motherhood is honored) clash with her fears (childbirth equals suffering and rejection).

During this phase, therapy emphasized:

- *Validating her emotional pain*
- *Identifying and challenging cognitive distortions*
- *Providing psychoeducation on childbirth options* (e.g., cesarean section, epidural anesthesia, modern delivery techniques)
- *Normalizing her fears within a psychological framework*

Afi was also informed that her traumatic memory occurred in a rural setting with minimal medical resources, unlike her current situation, where she lives in an urban area with access to quality hospitals and maternal care.

Phase 2: Exposure and Observational Desensitization

Building on theoretical insights, the second phase introduced *imaginary therapy and controlled exposure*. These sessions were held in a nearby childbirth room at the hospital. Some sessions occurred without live births, serving as preparatory desensitization; others coincided with deliveries.

When a birth occurred, Afi was guided to *listen to the natural sounds of labor-screams, breathing, and joy-and to observe the whole emotional arc.* After the birth, she and the therapist visited the postpartum room to meet the new mothers. These encounters offered powerful *counter-narratives* to Afi's trauma. She witnessed women who, despite the pain, expressed overwhelming joy and fulfillment at meeting their child for the first time.

Critically, Afi a met mothers who had given birth to their third or fourth child, helping her *reframe childbirth as survivable and even desirable.*

This phase aligns with research suggesting that *graduated exposure*-either through virtual, imagined, or live experiences-can reduce phobic responses by *retraining the emotional memory system* (Arntz, 2012; Hofberg & Ward, 2003).

Phase 3: Integration and Meaning Reconstruction

In the final phase, Afi processed her mixed emotions and *integrated her new experiences.* One central issue remained: her fixation on the boy who told his mother he never asked to be born. This statement had become symbolic of the perceived futility or ungratefulness associated with motherhood.

Through cognitive reframing and guided conversation, Afi was helped to:

- Recognize the *immaturity and impulsivity of adolescence,* including her past behaviors.
- Understand *frontal lobe development* and its role in emotional regulation and empathy (Blakemore, 2012).
- Accept that she *held only one part of the story* and had used it, consciously or not, to justify her fears.

When asked whether she had ever seen that same mother and son interact positively, Afi acknowledged witnessing many joyful and harmonious moments between them. This admission marked a *cognitive breakthrough,* showing that her trauma had selectively filtered information to support her fears.

By the end of the therapeutic plan, Afi no longer experienced terror at the thought of childbirth. Her sessions were gradually spaced from once a week during the active treatment phase to once every two weeks, and eventually to monthly follow-ups. Although some residual apprehension

remained-common even among individuals without phobias-heravoidance behaviors, anxiety levels, and intrusive thoughts had markedly diminished. This successful outcome is consistent with findings in the broader literature and contributes to the limited research on tokophobia within African populations, where the condition often remains underrecognized (Bayrampour et al., 2019; Saisto & Halmesmäki, 2003).

VI. ANALYSIS AND CRITIQUE OF THE THERAPEUTIC INTERVENTION

6.1 Strengths of the Treatment Plan

6.1.1 Holistic Approach

The treatment plan thoughtfully combined cognitive-behavioral therapy (CBT), imagery rescripting, psychoeducation, and gradual real-world exposure. This aligns well with best practices for treating phobias and trauma-related conditions (Hofmann et al., 2012).

6.1.2 Cultural Sensitivity

The therapist recognized the cultural context of Togo, where motherhood is a social expectation and tokophobia is taboo. By framing the therapy in a culturally appropriate manner and validating Afi's fear rather than pathologizing it too early, the plan respected her background and belief system-an essential component of effective mental health care in non-Western settings (Kirmayer, 2001).

6.1.3 Use of Exposure in a Controlled Setting

A unique and commendable element of this treatment was the observational exposure near actual childbirth rooms. This provided direct emotional experiences to recondition Afi's fear responses and introduce new emotional associations with childbirth-joy, support, and a sense of survival. Exposure therapy is widely recognized as a core treatment for specific phobias (Craske et al., 2014).

6.1.4 Trauma-Informed Care

The therapist explored and processed Afi's core trauma, linking early menstruation, reproductive responsibility, and the observation of a traumatic childbirth. The use of cognitive reframing and psychoeducation to break irrational associations was appropriately aligned with trauma-focused CBT (Foa et al., 2009).

6.2 Lack of Standardized Outcome Measures

A notable limitation of the intervention was the absence of validated psychometric tools at intake, midpoint, and discharge to assess Afi's fear levels objectively. The lack of standardized measures, such as the Tokophobia Questionnaire (Hofberg & Brockington, 2000) or the Fear of Birth Scale (Slade et al., 2019), weakens the empirical rigor and generalizability of the case findings. Without quantitative metrics, it is challenging to track treatment efficacy, symptom trajectory, or to compare results across studies.

6.3 Limited use of Emotional Regulation Techniques

While the therapeutic plan incorporated key cognitive-behavioral strategies such as cognitive restructuring and graded exposure, there was insufficient integration of emotional regulation methods to support distress tolerance during triggering moments. Evidence-based trauma treatments emphasize the importance of grounding techniques, diaphragmatic breathing, and mindfulness to support safety and emotional regulation, particularly during exposure work (Linehan, 1993; Najmi et al., 2014). The omission of such tools may have limited Afi's ability to self-soothe and potentially increased the risk of retraumatization during live-birth observations.

6.4 Ethical Considerations in Live-Birth Exposure

The inclusion of live childbirth observation as an exposure technique raises important ethical considerations. Introducing a client into hospital recovery areas-especially in contexts involving postpartum women-necessitates careful attention to informed consent, confidentiality, and the risk of vicarious trauma (Zur, 2007). While cultural context matters-indeed, in many African collectivist societies, childbirth is a public and celebrated event-the lack of explicit discussion around ethical safeguards for the postpartum

mothers is concerning. In this specific case, the hospital's recovery room was described as communal and accessible, and the psychologist was a member of the hospital staff. Even so, adherence to ethical standards-including respect for dignity, privacy, and voluntary participation -remains essential in research and therapeutic interventions (American Psychological Association, 2017).

6.5 Insufficient Emphasis on Reproductive Autonomy

The therapeutic intervention firmly focused on reducing Afi's fear of childbirth and reframing her beliefs toward embracing motherhood. However, there was limited exploration of reproductive autonomy as a valid therapeutic goal. While challenging maladaptive cognitions is a key component of therapy, care must be taken not to impose culturally influenced expectations around motherhood. Feminist clinical psychology highlights the importance of affirming a woman's right to make informed decisions about her reproductive future, including the right not to become a mother (Ussher, 2006). Although Afi was eventually educated about her right to choose motherhood or not, the therapeutic process may have benefited from deeper work around this autonomy, disentangling cultural scripts from authentic desire.

Future interventions should include structured exploration of the client's reproductive values and goals, alongside fear reduction, to ensure alignment with the client's valid preferences rather than cultural expectations.

6.6 Absence of Long-Term Follow-Up or Relapse Prevention

The treatment plan appeared to conclude following symptom reduction at the end of 24 sessions, without a detailed relapse prevention or maintenance strategy. Research on specific phobias and trauma recovery highlights the importance of preparing clients for potential future triggers and providing booster sessions to consolidate gains (Foa et al., 2007). Afi may still encounter anxiety in contexts such as initiating a romantic relationship, facing a medical issue, or contemplating pregnancy. The omission of a structured follow-up plan may leave her vulnerable to future relapses.

Incorporating a relapse prevention module, including booster sessions, identification of early warning signs, and coping strategies for anticipated life transitions involving reproduction or intimacy.

6.7 Generalizability, Cultural Context, and Therapeutic Framework

This study represents a rare and context-specific case of tokophobia in Togo, where childbirth is typically normalized and celebrated within a strong pro-natalist cultural framework. Given the unique cultural, psychological, and sociomedical factors surrounding Afi's presentation, the findings and therapeutic approach may not be broadly generalizable to all populations. The primary aim of this study was not to establish universal treatment standards but to document and explore the psychotherapeutic management of a culturally rare and clinically significant phobia within a Togolese context.

Additionally, one of the guiding principles of the treatment approach was the application of *solution-focused and brief therapy (de Shazer et al., 1986)*. This orientation emphasizes identifying goals, building on existing strengths, and facilitating practical, time-limited interventions rather than prolonged, insight-oriented therapy. The goal was to rapidly reduce Afi's fear response and support her in constructing new cognitive and emotional associations around childbirth, without necessitating long-term dependence on the therapeutic process. The 74 -session framework was thus designed to deliver targeted therapeutic impact in a culturally appropriate, efficient, and ethically mindful way.

As such, this case contributes to the growing literature on *culturally sensitive, trauma-informed, and solution-oriented mental health care,* and it highlights the importance of recognizing diverse presentations of fear and resistance surrounding childbirth-especially in underrepresented populations and health systems

where tokophobia remains under-recognized and under-treated.

VII. CONCLUSION

This case study sheds light on a rare but clinically meaningful presentation of primary tokophobia in a Togolese woman. In a culture where motherhood is deeply valorized and childbirth is often framed as a joyful communal event, Afi 's fear highlights the complex interplay between early trauma, sociocultural conditioning, and individual psychological vulnerability. The integration of cognitive-behavioral, developmental, and culturally grounded frameworks proved effective in reducing her fear and restoring a sense of agency in reproductive decision-making.

Notably, the study illustrates how early traumatic experiences, especially those linked to identity-forming milestones such as menarche, can profoundly shape beliefs, emotions, and behavioral patterns into adulthood. Through culturally sensitive, trauma-informed care that included both theoretical insight and experiential exposure, the therapy helped Afi challenge maladaptive associations and reconstruct new meanings around womanhood and childbirth.

While the findings are not broadly generalizable, they offer valuable insight into how tokophobia may manifest and be treated in African contexts, where the topic remains largely unexplored. Future research should aim to validate assessment tools, consider ethical frameworks for exposure-based interventions, and support more inclusive reproductive mental health services across diverse cultural landscapes.

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Long-Term Anamnesis of Chronic Thromboembolic Pulmonary Disease. Does it Predict the Results of Pulmonary Thromboendarterectomy?

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ABSTRACT

Introduction: Pulmonary thromboendarterectomy (PEA) is the main method of treatment for patients with chronic thromboembolic pulmonary hypertension (CTEPH). The residual pulmonary hypertension (PH) after CTEPH surgical treatment is a risk factor with increasing hospital mortality.

Objective: To analyze and evaluate the results of PEA in patients with different persistent time of medical history as a possible prognostic factor of residual PH and outcome.

Methods: Retrospective and prospective analysis of the PEA results in 87 patients operated on from April 2012 to February 2022 was conducted. The patients were divided into 3 groups. The 1st group - 45 patients with a medical history from 3 months to 1 year, the 2nd- 20 patients from 1 year to 3 years and the 3rd - 22 patients with long-term history more than 3 years.

Keywords: pulmonary hypertension, chronic thromboembolic pulmonary hypertension, thromboendarterectomy, pulmonary vascular resistance, long-term medical history.

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Long-term Anamnesis of Chronic Thromboembolic Pulmonary Disease. Does it Predict the Results of Pulmonary Thromboendarterectomy?

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Introduction: Pulmonary thromboendarterectomy (PEA) is the main method of treatment for patients with chronic thromboembolic pulmonary hypertension (CTEPH). The residual pulmonary hypertension (PH) after CTEPH surgical treatment is a risk factor with increasing hospital mortality.

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Results: The average age of the patients was 48.7± 13.5 years, including 56.7 % males and 43.3 % females. Due to New York Heart Association (NYHA), 7 (8.0%) patients preoperatively belonged to class II, 60 (69.0%) to class III and 20 (23.0%) to class IV. Postoperatively the mean pulmonary artery pressure (mPAP mmHg) and pulmonary vascular resistance (PVRdyn·s·cm^{-5}) significantly decreased in all groups(mPAP :1st group from 45±13 to 23±6, 2nd – from 49±14 to 25±6 and 3rd from 58±12 to 31±7; PVR: 1st group from 797±262 to 290±135, 2nd – from 925±383 to 376±159 and 3rd – from 1248±332 to 505±189). Hospital mortality after PEA was 0 in patients with medical history less than 1 year, 5% from 1 to 3 years and 31.8% in patients with long-term anamnesis.

Conclusions: PEA is an effective surgery with mPAP and PVR decreasing in the early postoperative period. However, long-term medical history and PVR>1000 dyn·s·cm^{-5} are very important risk factors with increasing of hospital mortality.

Keywords: pulmonary hypertension, chronic thromboembolic pulmonary hypertension, thromboendarterectomy, pulmonary vascular resistance, long-term medical history.

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I. INTRODUCTION

Chronic thromboembolic pulmonary hypertension is a severe cardiovascular disease with a highly unfavorable prognosis if not treated in time. CTEPH is a complication of pulmonary embolism (PE) and a treatable cause of PH. The pathology is a combination of mechanical obstruction due to failure of clot resolution, and a variable degree of microvascular disease. The first decision remains assessment of operability, and the best improvement in symptoms and survival is achieved by the mechanical therapies, pulmonary endarterectomy and balloon pulmonary angioplasty. With the advances in multimodal

therapies, excellent outcomes can be achieved with 3-year survival of >90%.This is a rare form of PH develops due to obstruction of the elastic pulmonary arteries by thrombi or emboli and usually occurs in 1–4% of cases as a late complication of acute PE [1, 2]. Its incidence, which had been estimated at 5–6 cases per million inhabitants per year, is reaching 13 cases per million inhabitants per year when a systematic PE follow-up is organised [3].

The haemodynamic thresholds defining PH in CTEPH are adopted from the revised and now accepted thresholds based on definition of normal versus abnormal pulmonary haemodynamic parameters [4, 5]. That is why a complete haemodynamic evaluation by right heart catheterisation (RHC) including cardiac output is recommended, because the calculated PVR is important to assess the prognosis and the risks associated with PEA [6].

Factors contributing to the development of chronic thromboembolism of the pulmonary artery (PA) include ineffective use of anticoagulants, recurrent PE episodes, thrombi formation in the PA in patients with thrombophilia, or due to changes in the vessel wall after a prior PE. The disease progresses with development of microvasculopathy and gradual remodeling of the right heart chambers with elevation of mPAP and PVR and decreasing cardiac output (CO). PH is considered severe when mPAP is more than 45–50 mmHg and PVR is greater than 1000–1200 dyn·s·cm^{-5}. [7, 8]

Nowadays PEA is recognized as the best treatment for CTEPH. Long-term surgical outcomes have demonstrated significantly higher survival rates among patients who underwent surgery compared to those who did not. The outcome and early postoperative results depend on several factors: the degree and nature of PA lesions, the level of cardiovascular insufficiency, and the experience of the surgical team [2, 5]. One of the major risk factors is the baseline value of thePVR. Studies have shown that mortality among patients with PVR >1000 dyn·s·cm^{-5} was 3–4 times higher than in patients with lower PVR. Extremely high PVR that does not correlate with imaging and angiographic findings may indicate irreversible microvascular changes, which can ultimately lead to residual PH after surgery, postoperative complications, or even death [7, 8].

In order to assess potential perioperative risks, a multidisciplinary team (MDT) must identify correlation of the type and severity of PA lesions and the level of PH. Unfortunately, there is no currently unified algorithm capable of predicting the scope of surgical intervention and its possible complications. We hypothesize that evaluation of the CTEPH medical history may be a useful method for predicting and assessing the risks of surgical intervention.

II. METHODS

2.1 Study Design

A retrospective and prospective study was conducted in 87 patients after a CTEPH surgical treatment undercardiopulmonary bypass (CPB), moderate hypothermia, cardioplegia and without deep hypothermic circulatory arrest from April 2012 to February 2022 at the Heart Disease with Progressive Pulmonary Hypertension Surgical Treatment Department in the Bakulev National Medical Research Center for Cardiovascular Surgery under the Russian Federation Health Ministry (Moscow, Russian Federation) were collected and thoroughly analyzed.Written and informed consents from the patients were received. The study protocol was approved by the local ethics committee at the institution where the research was conducted. All the patients were examined before the surgery, immediately after operations and at the hospital discharge. After operation the patients were evaluated with clinical assessment, transthoracic echocardiogram, computed tomography angiogram (CTA), invasive PA pressure (PAP) measurement; the data received were collected. The inclusion criteria suggested by the American College of Chest Physicians were the following: 1) NYHA class symptomatology; 2) preoperative PVR > 300 dyn·s·cm^{-5}; 3) surgical accessibility of thrombi in main, lobar, segmental PA as seen in CTA; 4) no debilitating comorbidities. All the patients with acute PE did not fit CPB undergoing and only

after adequate anticoagulant therapy for at least 3 months after confirmed PE. Demographic variables, PAP (systolic (PAPs), mPAP, diastolic (PAPd)), right ventricle (RV) dysfunction measured by tricuspid annular peak systolic excursion (TAPSE) on 2D echo, central venous pressure, pulmonary arterial wedge pressure (PAWP), oxygen saturation (SatO2), CPB time, location of thrombi as described by Jamieson's classification as well as the University of California, San Diego (UCSD), surgical classification and any postoperative complications were studied. PAPs, mPAP and PAPd, as well as CO, were invasively measured before and after the operation with the subsequent PVR calculation.

Patients were divided into three groups depending on the time of medical history. The first group consisted of 45 patients with a medical history from 3 months to 1 year; the second group - 20 patients with a history from 1 to 3 years; and the third group - 22 patients with a long-time history more than 3 years. We also analyzed the results of PEA in patients with PVR less or more than 1000 dyn·s·cm^{-5} in all three groups.

2.2 Surgical Technic

Methods and peculiarities of CPB, anesthetic management and surgical technic were described previously [9, 10].The surgical technique remained unchanged throughout the study period. CPB was established in a standard fashion with moderate hypothermia, cardioplegia and without deep hypothermic circulatory arrest. An arteriotomy of the right pulmonary artery (RPA) was performed between the aorta and superior vena cava, followed by thromboendarterectomy from the lobar and segmental branches of the RPA. After thrombi removal was completed, the arteriotomy was closed. Access to the left pulmonary artery was achieved by opening the main pulmonary artery trunk and extending the incision into the left branch, followed by thrombus removal. Restoration of cardiac activity occurred spontaneously or after defibrillation. CPB was discontinued under monitoring of PAP and cardiac filling. During surgery, repeated measurements of pulmonary and systemic hemodynamics were performed using a Swan–Ganz catheter. Cardiac output was measured using the thermodilution method. The type of pulmonary artery lesion was clarified according to Jamieson's classification. After surgery, patients were transferred to the intensive care unit (ICU) for stabilization.

2.3 Intensive Care Unit Management

The outcomes of the surgical intervention were analyzed, including CPB duration and aortic cross -clamp time. In the early postoperative period, hemodynamic parameters obtained via Swan–Ganz catheter, duration of mechanical ventilation (MV), length of stay in the ICU and in hospital, echocardiographic results, functional tests, laboratory parameters at discharge, complication rate, and mortality were evaluated.All patients received correction of infusion and sedative therapy and other medical procedures during the first 24 hours after surgery. Patients with elevated PAP received prophylactic therapy with iloprost at a dose of 20 μg, administered via nebulizer during MV, 4–8 times per day. After the restoration of normal external respiration and absence of signs of heart failure, tracheal extubation was performed. If severe PH persisted (mean PAP > 35 mmHg, PVR > 500 dyn·s·cm^{-5}), extubation was delayed for several days. Postoperatively, all patients were prescribed anticoagulant therapy in therapeutic doses.

2.4 Statistical Analysis

For statistical analysis of the results, IBM SPSS Statistics version 26 was used. Quantitative variables following a normal distribution were presented as the mean ± standard deviation; quantitative variables with a non-normal distribution were presented as the median and interquartile range (25th–75th percentiles). To assess the normality of the distribution, the Kolmogorov–Smirnov and Shapiro–Wilk tests were used. Qualitative variables were presented as absolute values and percentages. To compare groups of quantitative variables with a normal distribution, Student's t-test was used. Groups of quantitative variables with a non-normal distribution or with different types of distribution were compared using the Wilcoxon and Mann–

Whitney tests. Qualitative variables were analyzed using Pearson's Chi-square test and Fisher's exact test. Differences were considered statistically significant at $p < 0.05$. To compare more than two groups of quantitative variables with a normal distribution and equal variances, one-way ANOVA for independent groups was used. When more than two groups of quantitative variables with a non-normal distribution and/or unequal variances had to be compared, the Kruskal–Wallis test was applied. Levene's test was used to check for homogeneity of variances. If differences between groups were found ($p < 0.05$), pairwise comparisons between these groups were conducted. For variables with a normal distribution, Tukey's test was used for pairwise comparison, while for variables with a non-normal distribution, the Mann–Whitney test was applied. In the case of pairwise comparisons, the Bonferroni correction was used to determine the new significance threshold.

III. RESULTS

The present data analysis included 87 patients who underwent surgery in the period from April 2012 to February 2022. The average age of the patients was 48.7 ± 13.5 years, including 56.7 % males and 43.3 % females. Due to NYHA, 7 (8.0%) patients preoperatively belonged to class II, 60 (69.0%) to class III and 20 (23.0%) to class IV. The patients had some classical symptoms, i.e. dyspnea on exertion (DOE) (in all 87 patients), palpitations (in 62 patients, 71%), pedal oedema (in 56 patients, 64%), and a cough (in 16 patients, 18%). The main CTEPH cause was deep venous thrombosis in 69 patients (79.3%), thrombophilia in 9 patients (10.3%) and was unidentified in 10 patients (11.5%). Location and extent of thrombi were evaluated by CTA. According to Jamieson's classification 49 (56.3%) patients had type I *(Figure 1)* and 38 (43.7%) patients had type II thrombi. None of the patients had type III orIV thrombi. The baseline demographic and preoperative clinical characteristics of the study population are presented in *Table 1 and 2*.

Table 1: The Baseline Demographic and Preoperative Clinical Characteristics

Comparative Characteristics of Patients				
Parameters	Group 1 (from 3 months to 1 year; n = 45)	Group 2 (from 1 to 3 years; n = 20)	Group 3 (> 3 years; n = 22)	*p*
Male, n (%)	26 (58%)	13 (65%)	9 (41%)	
Female, n (%)	19 (42%)	7 (35%)	13 (59%)	
Age, years	46 ± 14	47 ± 12	50 ± 13	>0.05
BSA, m^2	1.98±0.63	1.96±0.5	1.95±0.4	>0.05
NYHA Functional Class, n (%) II III IV	 7 (15.5%) 35 (77.78%) 3 (6.67%)	 0 (0%) 16 (80%) 4 (20%)	 0 (0%) 9 (40.9%) 13 (59.1%)	

Note: BSA - Body Surface Area

Table 2: Preoperative Clinical and Hemodynamic Parameters

Comparative Characteristics of Patients				
Parameters	Group 1 (n = 45)	Group 2 (n = 20)	Group 3 (n = 22)	
Functional Tests				
6MWT, m	259 ± 64	230 ± 65	216 ± 82	
DOE Borg Dyspnea Scale, score	4.1 ± 1,0	4.4 ± 1,1	6.7 ± 0,9	
Echocardiography				
RV EDD, cm	5.0 ± 0,7	5.2 ± 0,6	5.5 ± 0,7	
RV EDV, mL	127 ± 24	135 ± 35	171 ± 42	
RA width, mm	56 ± 11	62 ± 11	62 ± 13	
RA length, mm	58 ± 11	61 ± 11	65 ± 11	
RV SV, mL	26 ± 6	29 ± 5	33 ± 3	
RV DV, mL	38 ± 7	42 ± 6	46 ± 4	
RA SV, mL	25 ± 6	28 ± 5	32 ± 3	
RA DV, mL	34 ± 7	39 ± 5	42 ± 3	
TAPSE, mm	15.2 ± 3,3	14.1 ± 1,7	12.0 ± 1,7	
RV EF, %	41 ± 8	41 ± 7	37 ± 6	
sPAP, mmHg	75 ± 23	92 ± 23	97 ± 17	
Right Heart Catheterization				
PVR, dyn·s·cm^{-5}	797 ± 262	925 ± 383	1248 ± 332	
mPAP, mmHg	45 ± 13	49 ± 14	58 ± 12	
PAWP, mmHg	15.7 ± 2,5	17.0 ± 3,8	18.0 ± 2,8	
CO, L/min, median (IQR)	4.0(3.4-5.2)	5.1 (3.6-8.1)	3.5 (3.1-4.4)	
CI, L/min/m^2	2.3 ± 0.5	2.6 ± 0.9	2.0 ± 0.3	
Laboratory Diagnostics				
NT-pro BNP, pg/mL, median (IQR)	138 (81-223)	243 (151-345)	503 (324-705)	
Pulmonary Vascular Resistance				
PVR < 1000 dyn·s·cm^{-5}, n (%	37 (82)	12 (60)	7 (32)	
PVR ≥ 1000 dyn·s·cm^{-5}, n (%)	8 (18)	8 (40)	15 (68)	

Note: NT-proBNP – brain natriuretic peptide; EDD – end-diastolic dimension; EDV – end-diastolic volume; EF – ejection fraction; CI – cardiac index; 6MWT – 6-minute walk test; DV - diastolic volume; SV - stroke volume; RA – right atrium.

There was no statistical difference in CPB time, aortic cross-clamp duration, or body temperature between the groups. *Figure 2* shows the thrombotic masses removed at the time of surgery. The most patients with PVR > 1000 dyn·s·cm^{-5} were in group 3 (68%) while in group 1 only 18%.

Table 3: Postoperative Clinical and Hemodynamic Parameters

Comparative Results				
Parameters	Group 1 (n=45)	Group 2 (n=20)	Group 3 (n=22)	*p*
Functional Tests				
6MWT, m	434 ± 55	415 ± 36	373 ± 47	p_{1-2} =0.291 p_{1-3} =0.005 p_{2-3} =0.013
DOE Borg Dyspnea Scale, score	1.8 ± 0.5	2.2 ± 0.4	2.8 ± 0.4	p_{1-2} =0.004 p_{1-3} <0.0001 p_{2-3} =0.001
Echocardiography				
RVEDD, cm, median (IQR)	4.5 (4.0-5.1)	4.3 (4.1-5.1)	5.0 (4.4-5.3)	>0.05
RVEDV, ml	93 ± 11	97 ± 15	102 ± 22	>0.05
RA width, mm	48 ± 8	51 ± 11	55 ± 10	>0.05
RA length, mm	46 ± 8	49 ± 7	51 ± 7	>0.05
RV SV, ml	16 ± 4	17 ± 4	20 ± 2	p_{1-2} =0.048 p_{1-3}<0.0001 p_{2-3} =0.007
RV DV, ml	24 ± 5	26 ± 3	28 ± 3	p_{1-2} =0.045 p_{1-3} =0.003 p_{2-3} =0.099
RA SV, ml	14 ± 4	16 ± 2	18 ± 2	p_{1-2} =0.009 p_{1-3}<0.0001 p_{2-3} =0.059
RA DV, ml	21 ± 4	23 ± 3	25 ± 2	p_{1-2} =0.008 p_{1-3}<0.0001 p_{2-3} =0.117
TAPSE, mm	18.5 ± 2,2	18.4 ± 2,1	17.2 ± 1,7	>0.05
RV EF, %	51 ± 6	49 ± 5	47 ± 4	>0.05
sPAP, mmHg	37 ± 16	45 ± 18	53 ± 9	p_{1-2} =0.047 p_{1-3} =0.001 p_{2-3} =0.11
Right Heart Catheterization				
PVR, dyn·s·cm^{-5}	290 ± 135	376 ± 159	505 ± 189	p_{1-2} =0.037 p_{1-3}<0.0001 p_{2-3} =0.071
mPAP, mmHg	23 ± 6	25 ± 6	31 ± 7	p_{1-2} =0.179 p_{1-3}<0.0001 p_{2-3} =0.01
dPAP, mmHg	10.7 ± 4,0	10.5 ± 3,3	9.6 ± 4,2	>0.05
CO, L/min, median (IQR)	5.4(4.6-7.6)	6.2 (4.7-25.0)	4.0 (3.8-7.0)	>0.05
CI, L/min/m^2, median (IQR)	2.7 (2.5-3.4)	3.5 (2.7-4.1)	2.5 (2.3-3.5)	>0.05
Laboratory Diagnostics				

NT-pro BNP, pg/ml, median (IQR)	39 (13-63)	67 (46-75)	94 (87-105)	p_{1-2} =0.01 p_{1-3}<0.0001 p_{2-3} =0.001
Stay in ICU				
MV, hours, median (IQR)	13 (8; 19)	18 (8; 32)	24 (11; 264)	p_{1-2} =0.086 p_{1-3}=0.002 p_{2-3} =0.271
ICU, hours, median (IQR)	21 (18; 23)	22 (21; 67)	43 (20; 288)	p_{1-2} =0.012 p_{1-3} =0.002 p_{2-3} =0.205
Stay in Hospital				
Length, days, median (IQR)	10 (8-12)	14 (9-18)	20 (17-36)	p_{1-2} =0.004 p_{1-3} <0.0001 p_{2-3} =0.013

Note: IQR – Interquartile Range

Decreasing of PVR after surgical intervention was statistically significant in all groups. In group 1, PVR decreased to 290 ± 135 ($p < 0.001$), in group 2 to 376 ± 159 ($p < 0.001$), and in group 3 to 505 ± 189 dyn·s·cm^{-5} ($p < 0.001$). Decreasing of NT-pro BNT was also observed in all three groups. In group 1 – to 39 pg/ml, in group 2 – to 69 pg/ml and in group 3 – to 94 pg/ml. Simultaneously, the 6MWT and TAPSE showed a significant increase . In group 1 to 434 m and 18.5 mm, in group 2 – to 415 m and 18.4 mm and in group 3 – to 373 m and 17.2 mm respectively. There were no significant differences between groups 1 and 2 in the time of mechanical ventilation or length of stay in the ICU. However, these indicators showed statistically significant differences between groups 1 and 3 *(Table 3)*.

Postoperative incidence of complications is presented in *Table 4*. We also analyzed an affecting of baseline PVR >1000 dyn·s·cm^{-5} on the developing a composite endpoint reflecting all identified postoperative complications within the groups (*Tables 4, 5)*. Non-lethal complications, such as moderate heart failure, pneumonia, and arrhythmias (paroxysmal form of atrial fibrillation) were revealed in group 1.

Table 4: Complications in Early Postoperative Period

Comparative Results			
Complications, n (%)	Group 1 (n = 45)	Group 2 (n = 20)	Group 3 (n = 22)
Reperfusion pulmonary oedema	0 (0)	3 (15)	9 (41)
Pneumonia	1 (2.2)	1 (5)	2 (9.1)
Heart failure	7 (16)	10 (50)	11 (50)
Cardiac arrhythmia	1 (2.2)	2 (10)	1 (4.5)
Residual pulmonary hypertension	2 (4.4)	4 (20)	8 (36)
Mortality	0 (0)	1 (5)	7 (32)
Composite endpoint	10 (22)	12 (60)	20 (91)

In group 3 the complication rate was significantly higher, and the early postoperative period was notably more severe. No cases of reperfusion pulmonary oedema were observed in group 1, whereas its incidence was 15% and 41% in groups 2 and 3, respectively ($p < 0.0001$). Additionally, residual PH was observed in 2 patients in group 1, 4 in group 2, and 8 in group 3. We hypothesized that this may be due to the long medical history with development of microvasculopathy with distal changes of pulmonary arteries. In-hospital mortality was not observed in group 1, while it was 5% in group 2 and 31,8% in group 3 ($p < 0.0001$).

Table 5: Development of Postoperative Complications based on the Composite end Point in Patients with PVR More and Less than 1000 dyn·s· cm^{-5} and with Different Time of Medical History

Groups (n)	PVR ($dyn \cdot s \cdot cm^{-5}$)	Complication rate (composite endpoint), n (%)
1 (37)	PVR<1000	7 (19.0)
1 (8)	PVR≥1000	3 (37.5)
2 (12)	PVR<1000	5 (41.6)
2 (8)	PVR≥1000	7 (87.5)
3 (7)	PVR<1000	6 (85.7)
3 (15)	PVR≥1000	14 (93.3)

Surgical outcomes of all patients were thoroughly analyzed and compared with data of PVR changes before and after surgery in all three groups *(Figures 3, 4)*. Residual PH and outcome depend on the preoperative level of PVR.

IV. DISCUSSION

PEA performed in specialized centers contributes to the normalization of the main parameters of the pulmonary circulation, improves long-term prognosis in patients with CTEPH, and is the preferred treatment method for patients with proximal pulmonary artery lesions. However, there is no universal algorithm for patient selection for surgery that could predict outcomes, especially with different times of medical history. Due to the nonspecific clinical presentation of CTEPH, misdiagnosis is common in clinical practice. As a result, there is often a significant delay in referring patients to expert centers for diagnosis confirmation and assessment for surgical treatment [5, 11]. In our center, as well as in other clinics worldwide, candidates for pulmonary PEA are selected by a multidisciplinary team, including a cardiovascular surgeon, anesthesiologist-intensivist, and cardiologist. As mentioned earlier, candidate selection is based on the overall patient condition, the characteristics and extent of pulmonary vascular lesions, and the presence of comorbidities [9]

Anatomically, two different vascular lesions participate in the increasing of PVR in patients with CTEPH: an obstruction of pulmonary arteries by unresolved thrombi and a microvasculopathy observed in both obstructed and nonobstructed lung areas [12]. A microvasculopathy is also observed in completely obstructed lung areas and is attributed to the development of systemic bronchial arteries anastomosing with pulmonary arterioles and venules [13]. Simonneau G. and colleagues presented similar data regarding the possibility of collateral blood flow development bypassing pulmonary vascular obstructions in patients with long-term medical history, which increases the likelihood of residual PH postoperatively and an unfavorable surgical outcome [14]. The presence of microvasculopathy is suspected when mechanical obstruction does not correlate with the haemodynamic severity. The microvasculopathy may explain the persistence of PH after PEA [15]. Microvasculopathy is suspected when mechanical obstruction does not correlate with the haemodynamic severity and may explain the persistence of residual PH after PEA [15].

Historically, high PVR was considered as a potential contraindication to surgery [16], however, even in patients with the most severe forms of disease, characterised by a preoperative PVR of more than 1000 $dyn \cdot s \cdot cm^{-5}$, the operative mortality has decreased to <5% [7, 8]. Madani M.and colleagues in their study reported that a baseline PVR value over 1000 $dyn \cdot s \cdot cm^{-5}$ leads to a fourfold increase in preoperative mortality [17]. According to data from an international registry, the mortality rate was 10.6% in patients with a baseline PVR above 1200 $dyn \cdot s \cdot cm^{-5}$. Elevated PVR values exceeding 1600 $dyn \cdot s \cdot cm^{-5}$, regardless of the lesion type, indicate a severe form of CTEPH and may be a contraindication for

pulmonary PEA. However, a clearly expressed lesion in the proximal pulmonary artery, even with a high PVR, indicates potential effectiveness and safety of surgical intervention [18].

Bergin C. and colleagues confirmed that in patients with similar characteristics, unfavorable postoperative outcomes were associated not only with PVR >1000 dyn·s·cm^{-5} but also with the presence of secondary microvasculopathy [19]. Residual PH rates ranged from 8.2% [20] to 41.9% [21]. Some previous studies provided solid evidence that patients with persistent or residual PH immediately after PEA have an increased risk of in-hospital death [18, 22]. These studies showed that mPAP > 30 mmHg after PEA was relevant for the prognosis. On the other hand, the United Kingdom National Cohort showed that mPAP > 38 mmHg measured 3 - 6 months postoperatively correlated with a poor long-term survival and a higher risk of CTEPH related death [23]. In our previous study we described , that it is rational to use 35 mmHg as a cut-off value, which is a mean data between 30 mmHg and 38 mmHg [10].

A long time before correct diagnosis may play a crucial role in the development of microvasculopathy and right heart failure. This leads to a worse prognosis with increasing complications ' rate and operative mortality [24]. Hsieh W. and colleagues conducted a meta-analysis dedicated to residual PH after surgical treatment of CTEPH. The study showed that after PEA PAP and PVR usually decrease even in patients with distal-type lesions [25]. Our results confirm this conclusion, demonstrating significant improvement in the hemodynamic parameters of the pulmonary circulation in all groups. All patients in the third group were included in study retrospectively, and at the time of surgery they were identified as potential candidates considering the proximal type of the pulmonary vascular lesions, despite the high PVR. This means that there was a relative correspondence between the severity of the arterial lumen obstruction and the degree of PH.In our study, we also referred to the work from the Meshalkin E. N. Institute of Circulation Pathology [26]. Authors concluded that the time of medical history does not affect the possibility of PH reduction in the postoperative period. We confirmed that PEA significantly reduces PVR and improves hemodynamic parameters in all patients including those with long-term medical history. Nevertheless, complications such as residual PH, reperfusion pulmonary oedema and severe heart failure in the early postoperative period were significantly higher in patients with long-term medical history (anamnesis more than 3 years), with high hospital mortality.

The extent of microvasculopathy significantly affects the development of disease severity and postoperative outcomes. Therefore, analysis of small vessel damage in the lungs may be key to achieve the best surgical results [27, 28, 29]. Further research in this field will help identify patients who achieve the best treatment outcomes using all available strategies and develop new therapies to prevent disease progression to irreversible right ventricular dysfunction.

V. CONCLUSION

PEA is an effective and safe treatment of CTEPH in patients with PVR less than 1000 dyn·s·cm^{-5}. In patients with PVR more than 1000 dyn·s·cm^{-5} and a clearly established time of medical history more than 3 years, PEAmay be effective in reducing of right heart failure and PH,however, the early postoperative period is associated with a higher frequency of complications and a high surgical mortality.

Limitations

Our research cannot definitively determine whether the above-mentioned patients with long-term medical history of CTEPH (more than 3 years) and with PVR more than 1000 dyn·s·cm^{-5} were unsuitable for surgical intervention, leaving the question of operability open for further study.

What is Already Known?

After PEA PAP and PVR usually decreases even in patients with distal-type lesions.

What Does This Study Adds?

The long-term medical history of CTEPH (more th an 3 years) is a risk factor for severe residual PH after PEA with high hospital mortality.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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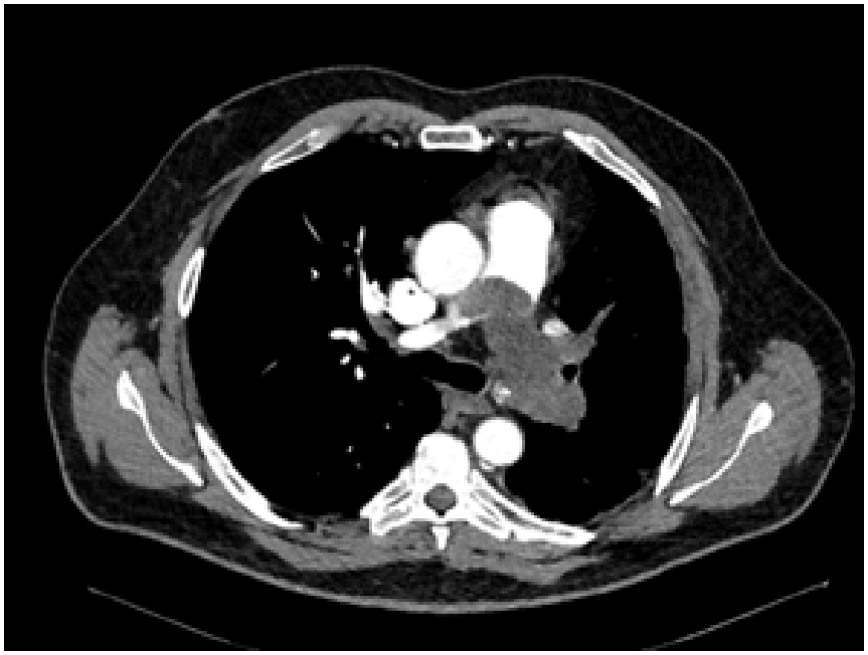

Figure 1: Type I Thrombi According to Jamieson's classification

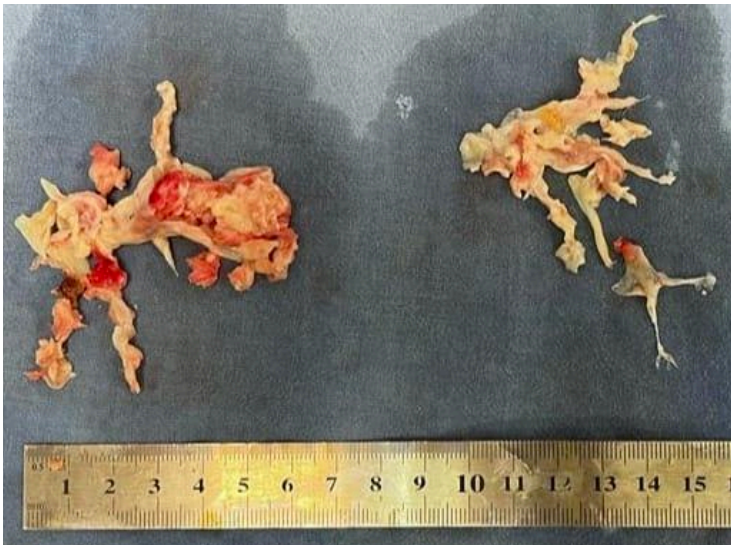

Figure 2: Thrombotic Material Removed During Surgery

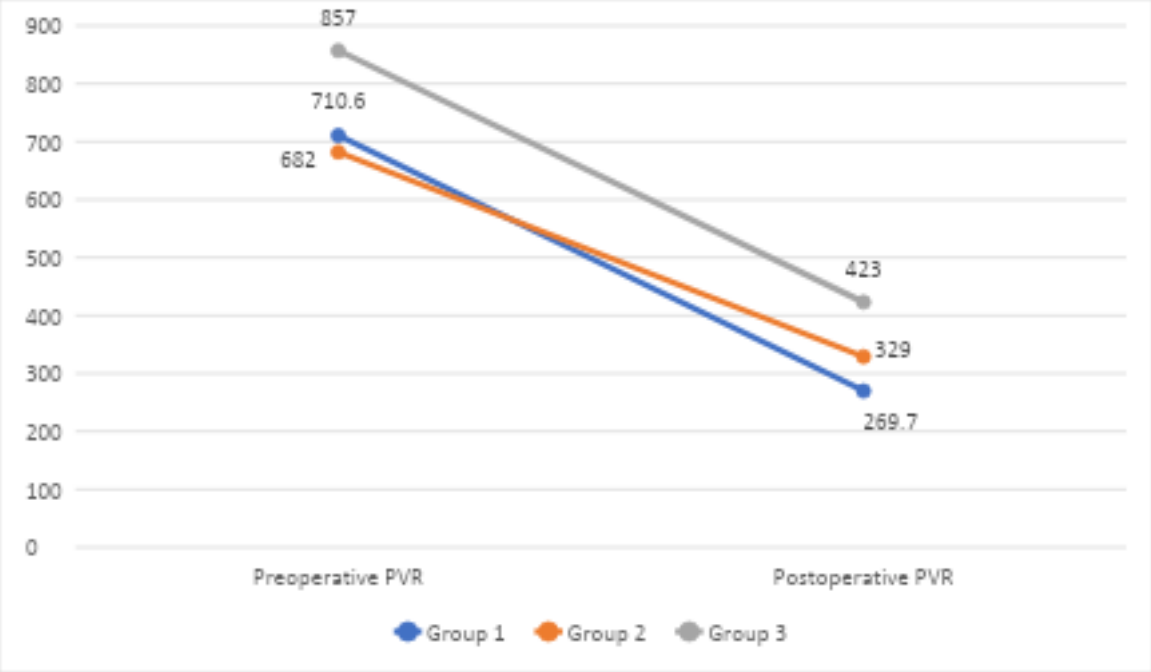

Figure 3: Dynamics of PVR in Patients with Initial PVR <1000 dyn*sm*sec-5

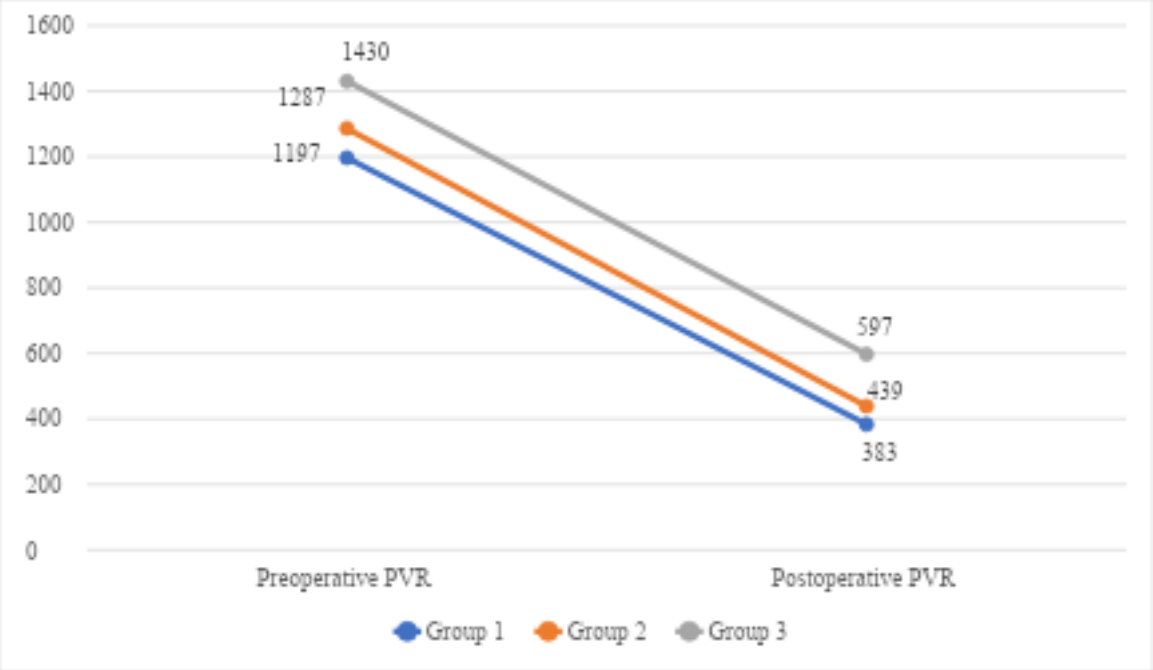

Figure 4: Dynamics of PVR in Patients with an Initial PVR > 1000 dyn*sm*sec-5

Developing RNase P Ribozymes for Therapy of Herpes Simplex Virus 1

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ABSTRACT

RNase P ribozymes, derived from the M1 RNA of *Escherichia coli*, have shown great promise as a novel nucleic acid-based gene interference approach to modulate gene expression. When the M1 RNA component of RNase P is covalently linked with a guide sequence (GS), it can be engineered into a sequence-specific endonuclease M1GS ribozyme. As the GS base-pairs with target mRNAs, it forms a structure that mimics a pre-tRNA-like substrate, allowing for M1 RNA's structure-based recognition mechanism. These M1GS constructs function catalytically and irreversibly, capable of cleaving target mRNA substrates without relying on host proteins. M1GS activity enhancement has been achieved through an in vitro selection process that introduced mutations in the catalytic core of M1 RNA. This process generated ribozyme variants with greatly improved cleavage efficiency and substrate affinity. M1GS ribozymes have been successful in inhibiting herpes simplex virus 1 (HSV-1) by targeting genes critical for viral infection.

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ABSTRACT

RNase P ribozymes, derived from the M1 RNA of Escherichia coli, have shown great promise as a novel nucleic acid-based gene interference approach to modulate gene expression. When the M1 RNA component of RNase P is covalently linked with a guide sequence (GS), it can be engineered into a sequence-specific endonuclease M1GS ribozyme. As the GS base-pairs with target mRNAs, it forms a structure that mimics a pre-tRNA-like substrate, allowing for M1 RNA's structure-based recognition mechanism. These M1GS constructs function catalytically and irreversibly, capable of cleaving target mRNA substrates without relying on host proteins. M1GS activity enhancement has been achieved through an in vitro selection process that introduced mutations in the catalytic core of M1 RNA. This process generated ribozyme variants with greatly improved cleavage efficiency and substrate affinity. M1GS ribozymes have been successful in inhibiting herpes simplex virus 1 (HSV-1) by targeting genes critical for viral infection. HSV-1 is the causative agent of cold sores and may lead to severe morbidity and mortality in neonates and immuno-compromised individuals. HSV-1 establishes lifelong latent infection, and novel anti-HSV-1 strategies are needed to block and eliminate viral latency and reactivation. Using HSV-1 infection as an example, this review will summarize the function of RNase P and its catalytic RNA, the enhancement and engineering of M1GS ribozymes, and their potential as a gene-targeting agent for therapeutic applications against HSV-1.

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I. INTRODUCTION

Nucleic acid-based gene targeting strategies have been at the forefront of major advancements in the field of molecular biology and therapeutic development. Among these strategies, antisense oligonucleotides, ribozymes, RNA interference (RNAi), and the genome editing technology based on the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR- associated protein (Cas) RNA-guided nuclease systems have shown significant promise as tools for targeted gene regulation [1-3]. Although the mechanisms vary greatly, ranging from passively blocking translation through complementary base pairing to introducing double-stranded breaks in DNA with site-specific endonuclease activity, each strategy provides a powerful and distinct approach to gene regulation. For example, hairpin and hammerhead ribozymes have demonstrated their potential in therapeutic applications such as the treatment of AIDS [1, 2]. These ribozymes contain a substrate-binding domain that, through Watson-Crick interactions, guides them to the target mRNA sequence, where the catalytic RNA domain can then hydrolyze a specific sequence [4-6].

RNA interference (RNAi) is a nucleic acid-based gene interference strategy that offers multi-turnover potential [1, 2]. Through its recruitment of cellular machinery, small interfering RNA (siRNA) directs the degradation of target mRNA substrates [7]. However, at high siRNA concentrations, host protein complexes can be overwhelmed, potentially leading to off-target effects [8, 9]. CRISPR-Cas9 systems can be programmed to target DNA or RNA, but they may

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carry the risk of off-target cleavage and permanent genomic alterations [3, 10].

Among the various nucleic acid-based gene interference strategies that interact with RNAs, the M1GS ribozyme offers a unique advantage of its endogenous catalytic activity guided by structural recognition [4, 11]. The M1GS ribozyme is derived from the catalytic RNA subunit (M1 RNA) found in *Escherichia coli* RNase P, and is covalently linked to a guide sequence (GS) (Figures 1 and 2) [12]. This guide sequence (GS) binds to target mRNAs in a sequence-specific manner and reshapes them into a pre-tRNA-like structure that M1 RNA can recognize and cleave [13]. M1GS acts catalytically and irreversibly, allowing one M1GS to cleave multiple copies of the target mRNA. M1GS functions exclusively at the mRNA level, avoiding direct genomic alterations [4, 11]. Recent progress on the structures and functions of RNase P and M1 RNA has been summarized in recently published reviews and is outside the purview of this article [12, 14-16]. In this review, we will discuss the potential of RNase P-derived gene-targeting ribozymes for treating herpes simplex virus 1 (HSV-1), highlighting their design, optimization, and application in targeting HSV-1-encoded mRNAs.

II. HERPES SIMPLEX VIRUS 1

HSV-1 is a large, double-stranded DNA virus that establishes lifelong latency within sensory neurons [17, 18]. HSV-1 enters through mucosal epithelial tissue, where it replicates productively before traveling through the nerve termini to reach the sensory neurons, where latent infection is established and provides a viral reservoir for periodic reactivation [17, 18]. HSV-1 can cause symptoms such as cold sores and genital lesions, and in severe cases, can lead to severe morbidity or mortality [19]. The viral life cycle for HSV-1 depends on the expression of conserved genes that encode proteins such as ICP4, a major transcription activator required for the expression of early and late genes during lytic infections, and thymidine kinase (TK), an enzyme important for nucleotide metabolism during replication in vivo [18, 20] .

Acyclovir is a widely used antiviral for HSV-1 [19]. This compound is structurally similar to guanosine but lacks the 3' hydroxyl group required for DNA elongation and is used to disrupt the viral replication process. Acyclovir is phosphorylated by thymidine kinase (TK), converting it into acyclovir monophosphate [19]. Host kinases convert it to its active triphosphate form. This active form competes with deoxyguanosine triphosphate and is preferentially incorporated by HSV-1 DNA polymerase, stalling elongation and terminating replication [19]. In immunocompromised individuals, resistance to acyclovir has emerged, as some HSV-1 strains carry TK-deficient mutations, and other strains express TK with structural mutations that prevent acyclovir from binding and getting phosphorylated [21, 22]. While acyclovir is considered safe due to its dependence on TK, high systemic concentrations of acyclovir in patients have been associated with renal impairments and symptoms such as confusion, agitation, or seizures [19]. These risk factors are elevated in elderly patients with underlying renal disease. These limitations highlight the need for novel strategies that can act through alternative mechanisms. The use of ribozymes, catalytic RNA molecules, may show promise and can be designed to degrade viral mRNA, inhibiting replication by targeting the mRNA level.

HSV-1 encodes immediate early (IE) genes that initiate reactivation, early (E) genes that encode TK and other proteins involved in viral DNA replication, and late (L) genes that encode viral capsids, envelopes, and proteins involved in lytic infection [17, 18]. The inherently heterochromatic and immunologically silent environment of neurons, combined with the IE promoter's affinity for repressive chromatin marks, facilitates the rapid chromatinization of HSV-1 DNA and the establishment of viral latency [23]. HSV-1 further promotes latency by expressing Latency-Associated Transcripts (LATs), non-coding RNAs that recruit host chromatin-modifying proteins to deposit repressive histone marks, thereby facilitating the formation of heterochromatin at the viral IE promoters responsible for reactivation [17, 24].

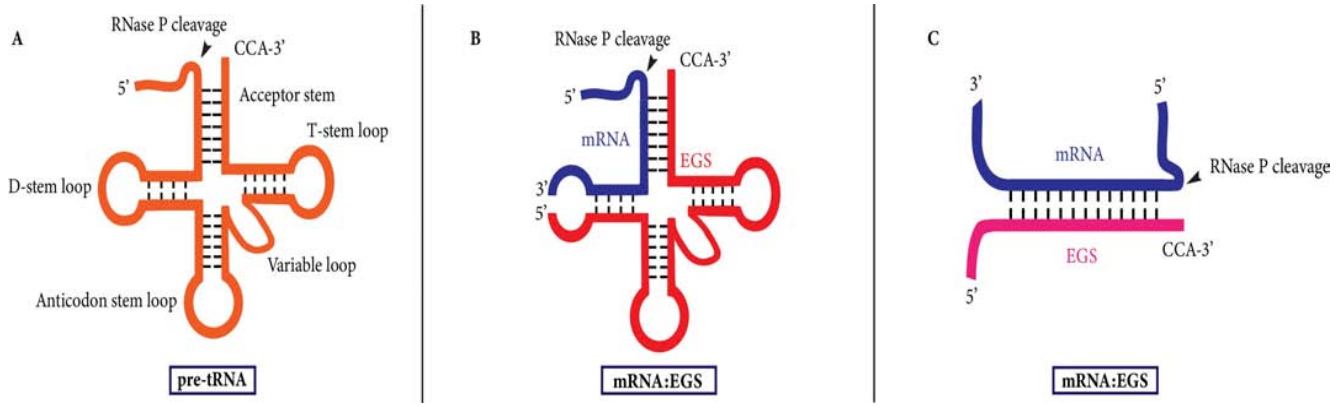

Figure 1: Substrates for bacterial RNase P and M1 RNA (A-C)

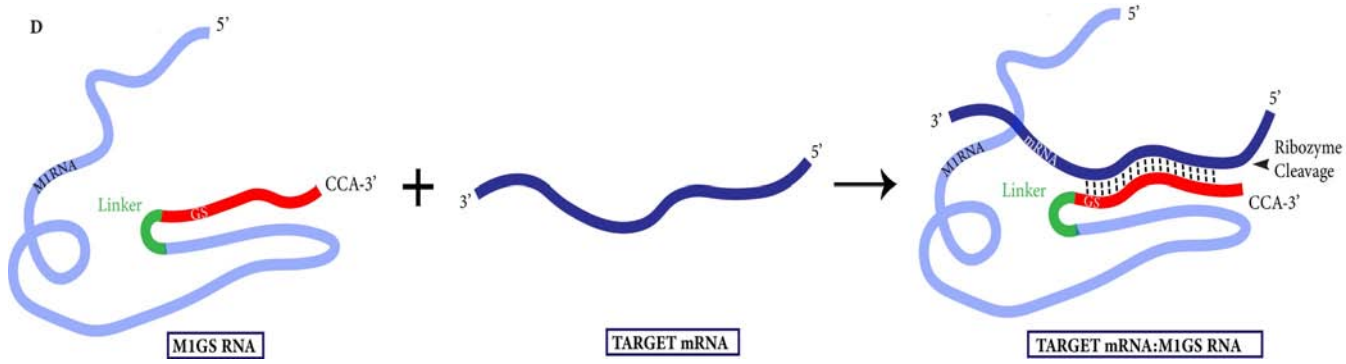

Figure 2: M1GS ribozyme binding to a target mRNA

Current antiviral treatments, such as acyclovir, only act on the lytic phases of HSV-1 infection [19]. This is clinically problematic, as reactivation is when HSV-1 is contagious and when symptoms that can be potentially severe or life-threatening take place. Unlike the latency reversal strategies used in HIV treatments, where the virus is forced into a treatable phase [25], similar approaches are not viable for HSV-1 infections, as cell destruction could result in irreversible neurological damage [19].

Efforts to treat HSV-1 latency through CRISPR-Cas9-targeted IE genes have not been very effective as the tightly packed HSV-1 DNA is inaccessible [26]. However, meganucleases have been effective in depleting HSV-1 reservoirs by targeting essential genes during latency [27]. Unlike the CRISPR-Cas9 systems, which rely on complementary base pairing, meganucleases can recognize specific DNA sequences through protein-DNA interactions in the major groove, a region potentially more accessible during latent repression [27]. Further studies are needed to investigate the effectiveness of the meganucleases and the CRISPR-Cas9-based method for anti-HSV-1 applications.

HSV-1 mutants lacking LATs have been shown to establish latency at a lower level, indicating that latency arises through multiple mechanisms [24]. HSV-1 also utilizes the host stress response to reverse its chromatin silencing [28]. HSV-1's utilization of host machinery to establish latency and reactivation makes it therapeutically challenging to distinguish viral processes from normal cellular pathways. M1GS ribozymes can achieve high specificity by acting at the mRNA level, enabling them to degrade viral transcripts without affecting host gene expression. M1GS can target reactivation at its earliest step by targeting IE mRNAs that encode essential replication proteins, preventing HSV-1 progression to subsequent steps of the lytic cycle.

III. RNASE P AND ITS CATALYTIC RNA

RNase P is a ribonucleoprotein enzyme that cleaves the 5' leader sequence from tRNA precursors [11, 29, 30] and other small RNAs such as bacterial operon RNAs [31, 32], riboswitches [33], and signal recognition particle RNAs [34]. RNase P is found in all domains of life, indicating its essential, conserved function. The catalytic activity in RNase P is in the RNA subunit rather than the protein component [35]. RNase P has one protein component in bacteria, typically 4 in Archaea, and up to 10 protein subunits in Eukaryotes [11, 14]. RNase P from *Escherichia coli* is composed of an M1 RNA subunit, which is 377 nucleotides in length, and a 14 kDa, 119 amino acid-long C5 protein subunit that assists in M1 RNA's structural stability [12]. Both the RNA and protein subunits are necessary in vivo; however, M1 RNA has been shown to cleave pre-tRNA in vitro in the absence of the protein subunit under high divalent ion concentrations (i.e., ~100 mM) [35]. This phenomenon results from the positively charged ions shielding the negatively charged phosphate backbone of the M1 RNA, thereby reducing electrostatic repulsion and allowing it to fold into its catalytically active structure without the need for the C5 protein [35-43]. Under in vitro conditions where Mg^{2+} concentrations are low, C5 protein is necessary for bacterial survival and works by stabilizing M1 RNA's structure into its catalytically active conformation, allowing M1 RNA to favor pre-tRNA over mature tRNA by improving its binding affinity to its substrates, and possibly pre-organizing metal ion binding sites [13, 35-43]. These roles have been supported by mutational and phylogenetic studies, as well as structural analysis of M1 RNA interaction with C5, using crystallography and cryo-EM [11, 12, 29, 44, 45]. Human RNase P consists of H1 RNA and at least 10 protein subunits, which are functionally analogous to the C5 subunit, but the precise functions of each subunit are not fully understood [14, 46].

IV. RNASE P SUBSTRATE RECOGNITION AND ENGINEERING OF GENE-TARGETING RIBOZYMES FROM RNASE P RNA

In *E. coli*, RNase P acts on substrates such as pre-tRNAs, 4.5S RNA precursors, and several other small RNAs [12]. These RNA substrates share structural motifs that include elements resembling the acceptor stem, T-stem/loop, and unpaired 5' leader regions (Figure 1A). These structures mimic the architecture of pre-tRNA and are what allows recognition by RNase P, reflecting RNase P's reliance on structure, rather than a specific sequence [47-49]. Early studies have shown that a shortened substrate containing the acceptor stem, T stem/loop, the 3' CCA sequence, and the 5' leader sequence can still be efficiently recognized and cleaved by RNase P and M1 RNA [48, 49], demonstrating that the full-length tRNA is not required as long as these structures are preserved (Figure 1). This study led to the development of the external guide sequence (EGS), which are short RNAs that bind to target mRNAs through Watson-Crick base pairing, reshaping them to mimic the structure of pre-tRNA (Figure 1B). This strategy leverages RNase P's conserved structure-based cleavage mechanism by converting it into a programmable, sequence-specific cleavage tool. EGSs expressed in both bacterial and human cells have been shown to inhibit the expression of the EGS-targeted mRNAs [50-56]. In one such study, EGSs were used to lower the levels of common reporter enzymes, β-galactosidase and alkaline phosphatase [51]. They suppressed the activity to 50-60% in *E. coli* expressing EGSs targeting these genes. In another study, drug sensitivity was restored in resistant strains of *E. coli* by targeting the drug resistance gene with EGS and RNase P [57]. Recent investigations indicated that EGS expressed in cultured mammalian cells inhibited the expression of the EGS-targeted mRNAs and infection of human viruses, including human cytomegalovirus (HCMV), HIV, hepatitis B virus (HBV), influenza virus, and Kaposi sarcoma-associated herpesvirus (KSHV) [58-63]. One of the main advantages of using the RNase P-EGS technology is that RNase P is expressed and active

at all stages of the cell cycle, as it is responsible for processing all tRNA molecules [11, 29, 30]. Due to the high specificity of RNase P cleavage, RNase P appears not to exhibit the off-target effects associated with other antisense strategies utilizing RNase H [64]. The limitations of EGS, including delivery and efficacy, have led to the development of M1GS RNA.

M1GS RNA addresses the issue of EGS's reliance on host RNase P by covalently linking the EGS to the catalytic M1 RNA (Figure 1C, Figure 2). The guide sequence contains an unpaired 3'-NCAA tail present in tRNA substrates that helps form the correct configuration. By tethering the GS to M1 RNA, the ribozyme forces the catalytic M1 RNA into proximity to the target site, thereby increasing cleavage efficiency and enhancing substrate binding [4, 13, 65, 66]. Researchers have shown that M1GS RNA allows for more efficient cleavage compared to unlinked M1 RNA and EGS under low Mg^{2+} conditions, which better reflect in vivo conditions [4, 13, 42, 65]. M1GS has successfully targeted HSV-1 essential genes encoding TK and ICP4 in both in vitro and in culture cell settings [13, 67]. Although the C5 protein, the cofactor of M1 RNA in *E. coli*, is absent in human cells, its role seems to be compensated for by the protein subunits of human RNase P [14]. It is believed that the C5 analogous human proteins bind to regions of M1GS RNA that are recognized by C5. Studies have shown that C5 proteins enhance M1GS activity by 30-fold compared to purified human RNase P proteins, which enhance activity by 5-fold [13, 68, 69]. Further studies on developing M1GS variants that interact more effectively with human RNase proteins may be promising for future research.

V. ENHANCEMENT OF M1GS THROUGH IN VITRO SELECTION

Through an in vitro selection process, the efficacy of RNase P ribozymes has been further improved, making it more suitable for use in clinical applications (Figure 3) [70, 71]. M1GS variants that slice an mRNA substrate more efficiently were selected by first generating a randomized M1 ribozyme pool with mutations in the M1 RNA catalytic and conserved regions [70]. They were then annealed to 5'-biotinylated mRNA substrate, such as tk46, a model mRNA substrate derived from HSV-1 TK mRNA (Figure 3). Once annealed, these RNA-RNA complexes were passed over a streptavidin column, which binds tightly to the biotinylated mRNA substrate. This step removed any M1GS ribozymes that were improperly folded or failed to bind to the mRNA substrate. After the annealed complexes were allowed to bind, Mg^{2+} ions were introduced, allowing for RNase P-mediated hydrolysis, separating the complex from the column (Figure 3). The active ribozyme complex was captured from the column and purified using gel electrophoresis. Ribozyme RNAs were reverse transcribed and amplified by RT-PCR to regenerate DNA templates for re-cloning in subsequent, more stringent rounds with a shortened annealing and incubation period. This selection process was continued until the cleavage rate ceased to increase, and the most active ribozymes were cloned and sequenced to identify the mutations responsible for the enhanced activity [70]. The high-performing variants isolated in this selection exhibited many novel mutations near the ribozyme's catalytic core [70]. For example, one selected variant, R29, exhibited a 20-fold increase in catalytic efficiency and a more than 50-fold increase in binding affinity for an HSV-1 TK mRNA substrate compared to an M1GS ribozyme (M1-TK) derived from the wild-type M1 RNA sequence [70]. In a control mutant, where the point mutation was reverted to the wild-type sequence, the binding affinity reverted to the wild-type's value, confirming that this mutation was responsible for enhanced substrate affinity. UV crosslinking showed that R29 and similar variants had enhanced crosslinking with the 3' tail sequence of the TK mRNA substrate, suggesting that these changed nucleotides strengthened the ribozyme- substrate interactions through direct interactions with the substrate [70, 72]. This study was expanded in a subsequent investigation, leading to the isolation and characterization of variants V41, M95, and M200 [71]. Among these highly active variants, V41 exhibited the greatest catalytic efficiency with a 30-fold increase in cleavage efficiency and a 25-fold increase in binding affinity compared to

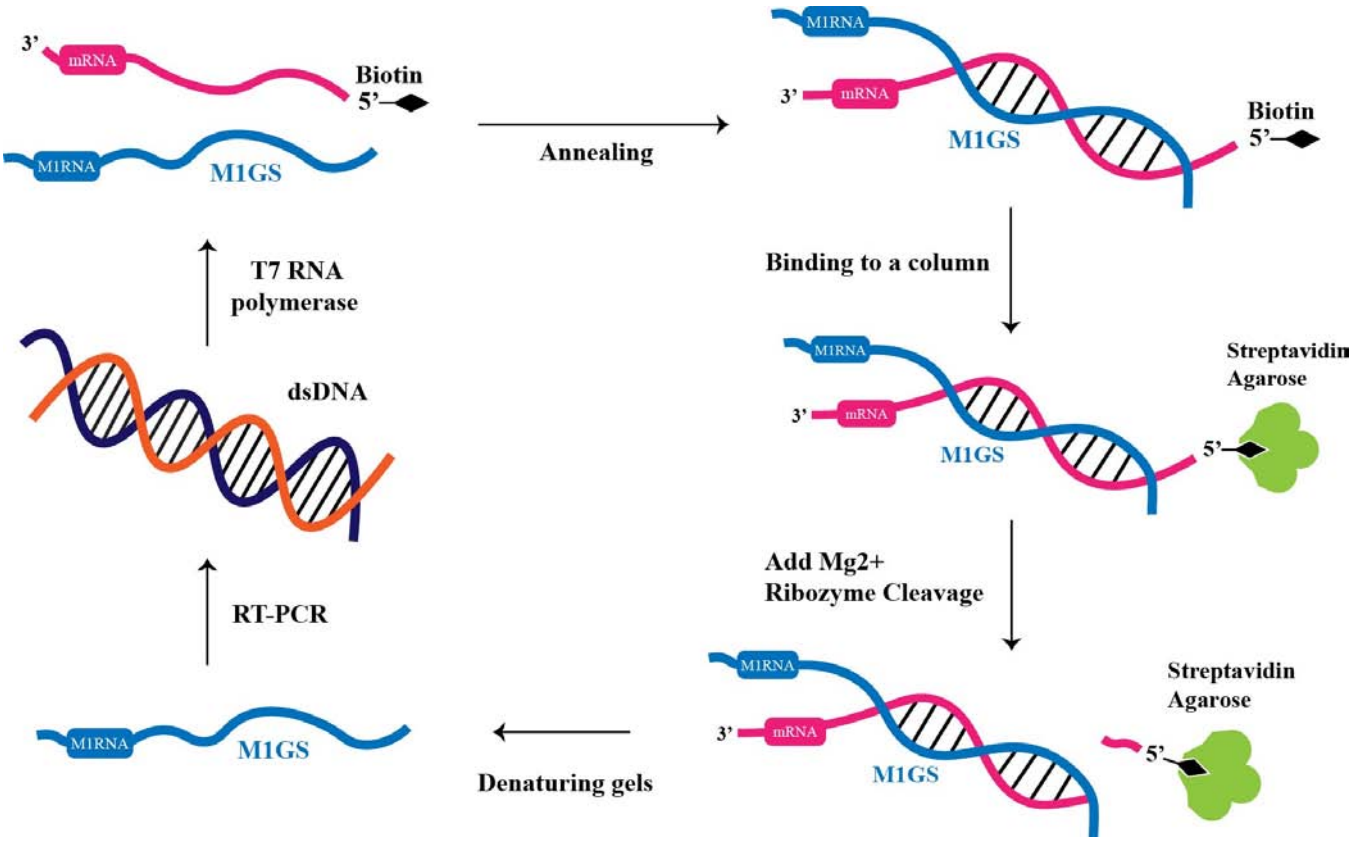

Figure 3: In Vitro Selection Procedure to Engineer RNase P Ribozyme Variants that Cleave mRNA Targets More Efficiently

the wild-type M1-TK. V41, M95, and M200 carried point mutations within the M1 RNA, which were further shown to enhance substrate binding and were responsible for the increased activity [71]. UV cross-linking and nuclease footprint analysis suggested that the point mutation was near the 3' tail sequence. These studies confirmed that the 3' tail sequence is a key interaction site for M1GS binding. Through this study, they demonstrated that catalytic activity and binding affinity can be significantly enhanced by optimizing this region to strengthen ribozyme-substrate interactions [71]. The in vitro selection process represents an excellent approach for further M1GS optimization through targeted engineering.

VI. Effective Inhibition of HSV-1 Gene Expression and Growth by M1GS Ribozymes

HSV-1 has a large DNA genome that encodes numerous essential proteins controlling replication, latency, and reactivation [17, 18]. TK and ICP4 are two important viral proteins that have been used as therapeutic targets in RNase P-based strategies. TK is involved in viral replication and has been used as a model gene. In contrast, ICP4 is a major transcriptional activator that is required for the expression of viral early and late genes [67, 73].

6.1 Targeting HSV-1 TK

The first study applying the anti-HSV-1 RNase P ribozyme approach utilized an M1GS ribozyme targeting HSV-1 TK mRNA [13]. Researchers began by cloning M1GS constructs M1TK13 and ΔM1TK13 (catalytically inactive M1 region) into retroviral vectors. HSV-1-infected human cells were then transfected by these vectors, and TK mRNA and protein levels were reduced by 80% in those expressing M1TK13 [13]. Cells expressing ΔM1TK13 only exhibited a reduction of ~9% in TK mRNA and protein levels, confirming that the catalytic activity of M1RNA was necessary for effective targeting of the TK mRNA in cultured cells. The M1 RNA in this study retained the wild-type sequence and its catalytic ability, setting

the groundwork for future studies that introduced mutations in this region to enhance cleavage activity.

Follow-up studies investigated whether mutations introduced into the M1 RNA of the M1GS ribozyme could enhance its catalytic efficiency [70, 71]. These ribozyme variants were generated through mutagenesis of conserved regions in M1 RNA and selected through an in vitro process. One of the selected variants, R29 exhibited a 20-fold increase in catalytic efficiency and a more than 50-fold increase in binding efficiency compared to wild-type ribozyme (called M1-TK) activity [70]. The R29 variant was then tested in HSV-1-infected cells. Cells expressing R29 exhibited a 99% reduction in TK mRNA levels and a 98% reduction in TK protein levels [70]. These results confirmed that enhanced catalytic activity shown in vitro was also observed in a cellular context, validating the M1GS ribozyme's antiviral potential.

6.2 Targeting HSV-1 ICP4

The ability of RNase P ribozyme to inhibit HSV-1 gene expression and infection was further investigated using M1GS ribozymes that targeted the ICP4 gene [67, 73]. By targeting ICP4, researchers sought to determine whether knocking down the expression of an upstream regulatory gene could suppress the expression of the associated downstream genes. To test this, researchers used the V6-ICP4 ribozyme, which contains mutations in the M1 RNA covalently linked to a 13-nt-long GS targeting ICP4 mRNA [67]. Two controls, M1-ICP4 (wild-type M1 RNA) and C-ICP4 (no M1 RNA catalytic ability), were tested alongside the V6-ICP4 construct. V6-ICP4 was 15-fold more active than M1-ICP4 and orders of magnitude greater than C-ICP4 [67]. These ribozymes were tested in a cellular context where cells were transduced with a retroviral vector encoding V6-ICP4, M1-ICP4, and C-ICP4. These cells were then infected with HSV-1, and the levels of viral mRNAs and proteins were measured. V6-ICP4 expression resulted in a 93% reduction in ICP4 mRNA levels and a 92% reduction in ICP4 protein levels [67]. M1-ICP4 also reduced ICP4 mRNA and ICP4 protein levels by 82% and 77%, respectively. C-ICP4 had minimal inhibition of around 6-8%, solidifying that antisense base pairing alone was insufficient for suppression. Suppression of downstream gene expression was shown with V6-ICP4, which reduced TK mRNA (an early gene) by 90% and late gene products ICP35 and gB by 90% and 91% [67]. M1-ICP4 also reduced expression, but at a lower level. These findings confirmed that the inhibition of ICP4 expression also reduced the expression of viral early (β) and late genes (γ). The inhibition of viral growth in ribozyme-expressing cells was then tested and measured quantitatively through the number of plaques formed. Cells expressing V6-ICP4 had a ~4,000-fold reduction compared to the ~1,000-fold reduction in cells expressing M1-ICP4 ribozymes [67]. These results, by showcasing ICP4-targeted knockdown through M1GS ribozymes, demonstrated the viability of targeting upstream regulatory genes to suppress HSV-1 gene expression and replication [67, 73].

VII. ADVANTAGES AND DISADVANTAGES OF M1GS RIBOZYMES

M1GS ribozymes offer unique advantages over some of the other therapeutic tools. Classical antisense oligonucleotides rely on host RNase H to cleave RNA-DNA hybrids to degrade the mRNA target, but carry the risk of non-specific cleavage at non-target sites due to their tolerance of mismatches [1, 2, 74]. In contrast, M1GS ribozymes require strict Watson-Crick base pairing to form a pre-tRNA-like structure, enabling their structure-based cleavage mechanism [47, 75]. Unlike other ribozymes, such as hairpin and hammerhead ribozymes, M1GS ribozymes do not require a specific nucleotide sequence (-GUX-) in the target mRNA [4-6]. This allows M1GS to be capable of hydrolyzing almost any RNA target as long as it is accessible. M1GS ribozymes and M1RNA's catalytic activity have been shown to increase in the presence of human proteins, such as the cofactors of human RNase P, and also act irreversibly [13, 14, 68, 69]. These characteristics, along with the ribozyme's ability to hydrolyze multiple substrate molecules, make it a promising candidate for gene-targeted antiviral therapy.

Despite the many unique advantages offered by M1GS ribozymes, several concerns may need to be considered when using RNase P ribozymes for specific applications. One concern with M1GS ribozymes is the potential side effects on cellular physiology and viability resulting from the overexpression of M1GS RNA. Under high concentrations of M1GS, human RNase P and its associated pathways could be potentially disrupted [8, 9]. Additionally, M1GS RNA is prone to cellular degradation. While stability can often be enhanced through chemical modifications such as the addition of 2' hydroxyl or phosphorothioate linkages to resist endonuclease activity, these modifications could compromise M1GS's functionality [2, 76]. Due to M1GS's reliance on secondary and tertiary structure for its catalytic function, chemical modification could disrupt the precise folding required for its catalytic ability. Finally, the relatively large size of M1GS ribozymes, ~400 nucleotides, makes it challenging to deliver and synthesize. Compared to RNAi-based therapies, where delivery methods such as lipid nanoparticles and polymeric matrices are already established, M1GS RNA's large size may limit the use of these delivery mechanisms [4, 77]. This constrains M1GS delivery to alternative delivery strategies such as viral vectors or through the development of novel delivery mechanisms.

VIII. FUTURE DIRECTIONS AND CHALLENGES

Studies have shown that *E. coli*-derived M1 RNA can function in human cells [4]. However, its catalytic efficiency is limited to the human RNase P protein's ability to compensate for the absence of the bacterial C5 protein [13, 14, 68, 69]. Several issues may need to be addressed to develop M1GS ribozymes for clinical applications in treating HSV-1 infection.

First, future directions could involve enhancing the compatibility of M1GS ribozymes with human RNase P proteins. One way this could be done is by mutagenizing contact regions of the M1 RNA domain that interact with RNase P proteins. These variants could be selected through an in vitro process involving human RNase P proteins to generate constructs better adapted to the human RNase P complex. Although M1GSs are highly specific, the sustained expression of M1GS and its effects on cellular physiology are not fully understood. Overexpression may lead to unintended side effects; therefore, future studies evaluating M1GS's potential off-target effects, long-term effects, and optimal dosage will be essential in determining its safe use in therapeutic applications.

Second, the efficient and tissue-specific delivery of M1GS ribozymes to the site of HSV-1 infection in vivo presents a challenge. M1GS ribozymes' relatively large size makes them incompatible with established delivery methods for shorter RNA molecules. As a result, viral vectors remain the most practical choice for M1GS expression and delivery. HSV-1 establishes a latent infection in sensory neurons [17, 18]; this tissue specificity could be applied to the delivery mechanism of M1GS. Past studies have shown that *Salmonella* can be used as vectors for macrophage-targeted M1GS delivery in human cytomegalovirus infection [4, 78-80]. Future studies could explore neuron-specific vectors for non-invasive, sustained expression of M1GS in clinically relevant tissues and cells that are known to be infected by HSV-1 in vivo.

Finally, future studies could investigate the numerous important HSV-1 genes beyond TK and ICP4 as potential targets for M1GS. As discussed in this review, targeting ICP4, an IE gene, was highly effective in suppressing downstream gene expression and replication. Naturally, other IE genes, such as ICP0 and ICP27 [18, 20], that are critical for reactivation from the latent stage, represent promising candidates for M1GS ribozymes. Beyond IE genes, early genes such as UL5 and UL9, responsible for origin recognition and replication initiation during genomic DNA replication, are also promising M1GS targets. By disrupting the expression of these genes, viral replication can be prevented even after reactivation. Another strategy could involve M1GS -mediated destruction of latency-associated transcripts (LATs). Studies have shown that HSV-1 mutants lacking LATs still establish latency

at a lower level and remain capable of reactivation [18, 24]. Targeting LATs could induce the virus to enter the reactivation stage, albeit in a weakened state, allowing M1GS to target IE or early gene mRNAs, or with antivirals such as acyclovir. This approach could potentially address a major clinical challenge: current treatments are only effective during symptomatic reactivation [19]. M1GS-mediated controlled reactivation may provide a safer therapeutic strategy that could minimize neuronal damage and reduce the severity of symptoms, which is beneficial for immunocompromised individuals and others at a higher risk of severe HSV-1 complications. Developing M1GS ribozymes against these genes could provide a broader and layered antiviral approach. By identifying and disrupting essential proteins involved in viral reactivation, replication, or immune evasion, a combination of M1GS-mediated knockdowns may circumvent the need for a singular, universal target.

IX. CONCLUSION

In this review, we have highlighted the gene-targeting activity of the engineered RNase P ribozyme, known as M1GS RNA, and how it can be optimized through in vitro selection. We have discussed the advantages of M1GS and summarized its efficacy in targeting HSV-1 by cleaving viral mRNAs encoding the TK and ICP4 protein. We outlined the potential of M1GS as an HSV-1 antiviral by discussing its ability to intercept reactivation by targeting immediate-early (IE) genes, as well as its potential to target latency-associated transcripts (LATs) and upstream regulatory proteins. M1GS's unique specificity due to its structure-based recognition and mRNA level of attack makes it a promising candidate for future therapeutic strategies targeting both HSV-1 and other viral infections characterized by latent or transcriptionally regulated gene expression.

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Canine Chronic Inflammatory Enteropathy with Special Reference to Immunological Markers for Diagnosis

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ABSTRACT

A study on Chronic Inflammatory Enteropathy (CIE) in dogs was conducted at Madras Veterinary College Teaching Hospital. Out of 40 dogs with persistent gastrointestinal signs, 16 were diagnosed with idiopathic CIE. The condition was more prevalent in 2-5 year-old male dogs, particularly in mongrel breeds. Clinical signs included chronic diarrhoea, vomiting, and weight loss, with disease duration of 1-3 months. Gastroduodenoscopy revealed hyperemia, friability, and granularity in the stomach and duodenum, while colonoscopy showed friability, hyperemia, ulceration, and discoloration of colonic mucosa. Histopathology of duodenal biopsies showed villous stunting, mucosal fibrosis, and lymphoplasmacytic infiltration. Immunohistochemistry indicated upregulation of CD3+ cells and Ki67 antigen. A positive correlation was found between the Canine Chronic Enteropathy Activity Index (CCECAI) and endoscopic and histopathological scores.

Keywords: enteropathy, ccecai score, immunohistochemistry, cd3+positive cells.

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I. INTRODUCTION

Chronic inflammatory enteropathy (CIE) is a term describing persistent gastrointestinal signs lasting over three weeks, with histological evidence of inflammation in the small or large intestine. Diagnosis requires exclusion of extra-intestinal, infectious, and parasitic diseases, as well as intestinal diseases of known etiology (Dandrieux, 2016). CIE involves complex interactions between the host genome, intestinal barrier function, microbiota, dietary antigens, and immune system (Allenspach & Mochel, 2020). In idiopathic CIE, T cell hypersensitivity and proliferation play a key role, with CD3 and Ki-67 antigens serving as markers for T lymphocytes and cell proliferation, respectively. Immunohistochemical analysis of CD3 and Ki-67 can help determine idiopathic CIE (Karlovits *et al.*, 2019), potentially linking increased cytokine release to clinical symptoms.

II. MATERIALS AND METHODS

Sixteen client-owned dogs presented to the Referral Medicine unit of Madras Veterinary College Teaching Hospital, with the history and clinical signs of persistent or recurrent gastrointestinal signs for three weeks or longer and refractory to conventional therapies were selected for the study. These animals were subjected to detailed clinico-pathological, radiological, ultrasonographic, and special examination procedures. Gastro-duodenoscopy and colonoscopy were performed under general anesthesia by a trained endoscopist following standard operating procedures using a Veterinary Video endoscope (Karl Storz model No. 60914 PKS, Germany) with an outer diameter of 9.8 mm, biopsy channel diameter of 2.8 mm, and working length of 1400 mm.

Biopsies were taken with fenestrated, long oval cup biopsy forceps (Karl Storz) with a 2.2 mm diameter, and endoscope-aided mucosal biopsy samples were collected. Histopathology and immunohistochemistry were performed by a pathologist following standard operating procedures to confirm the diagnosis and assess immunological changes in the intestine by

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identifying specific markers (CD3/Ki67). The characterization of histologic changes in the endoscopic biopsy samples was performed according to the histopathological standards established by the WSAVA Gastrointestinal Standardization Group (Day *et al.*, 2008). Immunohistochemistry was performed following a standard procedure (Carrasco *et al.*, 2011), and the results were interpreted using the Immunoreactive Score (IRS) (Fedchenko *et al.*, 2014).

III. RESULTS

The study revealed a high proportion of Chronic Inflammatory Enteropathy (CIE) in dogs aged 2-5 years, with males being more commonly affected than females. Mongrel dogs had the highest incidence, followed by German Shepherds and Retrievers. The duration of clinical signs prior to diagnosis was 1-3 months, with chronic diarrhoea being the primary complaint, followed by vomiting, mixed signs of vomiting and diarrhoea, and weight loss (Fig.1). All dogs were fed a homemade diet and had failed previous drug therapy. Body condition scoring revealed emaciation and thin body condition (BCS 1 and 2) in 10 dogs (Table 1). Fecal scoring showed scores of 4.5 (very soft feces) and 5 (diarrhoea) in the affected dogs.

The Canine Chronic Enteropathy Clinical Activity Index (CCECAI) was used to evaluate the severity of the disease condition. The CCECAI score of the CIE dogs expressed normal attitude and appetite with no vomiting in 9 out of 16 dogs to severe vomiting in 5 out of 16 dogs. Stool consistency was watery in 43.75% (7/16), very soft feces in 25% (4/16), slightly soft feces in 12.5% (2/16), and normal in 18.75% (3/16) dogs. Stool frequency was normal in 18.75% (3/16), slightly increased (2-3/day) in 25%, moderately increased (4-5/day) in 12.5%, and severely increased (>5/day) in 43.75% of dogs.

Mild, moderate, and severe weight loss were reported in 6.25%, 31.25%, and 43.75% of dogs, respectively. Albumin levels were normal (>20g/L) in 81.25% of cases, with mild (15-19.9g/L) and moderate (12-14.9g/L) hypo-albuminemia observed in 12.5% and 6.25% of dogs, respectively. Moderate peripheral edema was seen in 6.25% (1/16) of dogs.

Dogs with CIE were classified based on the summation of individual parameters as mild (4-5) 18.75% (3/16), moderate (6-8) 31.25% (5/16), severe (9-11) 31.25% (5/16), and very severe (>12) 18.75% (3/16) of dogs.

Hematology revealed leukocytosis, neutrophilia, lymphocytosis, and monocytosis in the CIE dogs. No remarkable changes were observed in serum biochemical analysis in the CIE dogs (Table 2). Radiography revealed gas-filled intestinal loops in one dog (Fig. 2). Ultrasonography was used to measure the wall thickness of the gastrointestinal tract in the CIE dogs, which was found to be normal in all CIE dogs except one. The wall layering and echogenicity were normal in all CIE dogs. Secondary changes observed in CIE dogs included mesenteric lymphadenopathy (Fig. 3), free abdominal fluid, and distended intestinal loops in few dogs.

Gastro-duodenoscopic examination of the stomach and duodenum predominantly revealed hyperemia, increased friability, and increased granularity. Less common lesions observed included discoloration, thickening and corrugation, and increased mucus. In the colonic mucosa, the major changes observed were friability, hyperemia, ulceration, and discoloration (Fig. 4, 5, 6).

Histopathological examination of the gastric mucosa revealed inflammatory changes (lymphocyte and plasma cell infiltration), vacuolation or separation of focal areas of superficial epithelium, ulceration of surface epithelium and moderate form of mucosal fibrosis/glandular atrophy. Duodenal mucosal biopsy samples revealed severe morphological changes, including villous stunting and fusion, villous stricture, mucosal fibrosis, crypt epithelial hyperplasia, and inflammatory changes characterized by lymphoplasmacytic cell infiltration, which were recorded in all CIE dogs (Fig.7). In three Colonic biopsy samples inflammatory changes with mild surface epithelial

injuries like attenuation, degeneration or vacuolation and loss of focal areas of superficial epithelium, crypt hyperplasia, dilation and distortion, mucosal fibrosis were noticed.

Immunohistochemical analysis of mucosal biopsy samples revealed varying degrees of CD3+ positive cell expression, with an Immunoreactive Score (IRS) of mild (2-3) in 37.5% (6/16), moderate (4-8) in 25% (4/16), and strongly positive (9-12) in 37.5% (6/16) of cases (Fig. 8) Similarly, the IRS for Ki67 positive nuclei was recorded as mild (2-3) in 43.75% (7/16), moderate (4-8) in 25% (4/16), and strongly positive (9-12) in 18.75% (3/16) of cases (Fig. 9). The results indicated up regulation of CD3+ positive cells and Ki67 antigen in the CIE-affected dogs.

A positive correlation was observed between the Canine Chronic Enteropathy Clinical Activity Index (CCECAI) and endoscopic score, as well as between CCECAI and histopathological score. However, no correlation was found between the endoscopic score and histopathological score.

IV. DISCUSSION

Chronic Inflammatory Enteropathy was mostly reported in middle-aged dogs. Volkmann *et al.* (2017) reported that dogs between 2 to 9 years old were commonly affected, while Allenspach *et al.* (2020) noted that CIE typically presents in middle-aged dogs due to environmental influences. The current study supports these findings, with a high incidence of CIE observed in dogs aged 2-5 years.

The breed distribution in this study, with Mongrel dogs, German Shepherds, and Retrievers being commonly affected, is also consistent with previous reports. Other breeds, such as Rottweiler's and Boxers, were also represented, which is in line with the findings of Kathrani, *et al.* (2011) and Jergens *et al.* (2012). The genetic predisposition of certain breeds, such as German Shepherds, to CIE has been attributed to single nucleotide polymorphisms (SNPs) in Toll-like receptor (TLR) genes (Kathrani *et al.*, 2010).

The role of modifiable exposure factors, such as feeding practices and deworming protocols, in the development of Inflammatory Bowel Disease (IBD) has been highlighted by Hemida *et al.* (2021). Further studies are needed to investigate the impact of these factors on the development of CIE in dogs.

The findings of this study are consistent with previous reports that mixed breeds, particularly Mongrel dogs, are overrepresented in cases of Chronic Inflammatory Enteropathy (CIE) (Volkmann *et al.*, 2017; SandhyaBhavani, 2018). The clinical signs observed in this study, including chronic diarrhea, vomiting, weight loss, and decreased body condition score, are similar to those reported by Jergens *et al.* (2003) and Sattasatuchana *et al.* (2017).

The Canine Chronic Enteropathy Clinical Activity Index (CCECAI) scores revealed that the majority of dogs had moderate to very severe forms of CIE, with increased stool frequency and weight loss being the predominant changes. These findings are consistent with Volkmann *et a*l. (2017), who also reported moderate to severe forms of CIE in their study.

Gastro-duodenoscopy and colonoscopy, in combination with endoscopically guided biopsy, proved to be valuable diagnostic tools in this study, as previously reported by Jergens *et al.* (2012). The use of these techniques allowed for the visualization of gastrointestinal lesions and the collection of biopsy samples for histopathological examination, which aided in the diagnosis of CIE.

The endoscopic findings in this study are consistent with previous reports that describe various lesions in the gastrointestinal tract of dogs with Chronic Inflammatory Enteropathy (CIE). Slovak *et al.* (2014) noted that endoscopy provides a direct assessment of intestinal mucosal damage and can be used to measure disease activity index, with common lesions including erythema, friability, erosions/ulcers, and granularity. Similarly, Garcia-Sancho *et al.* (2007) reported gastric and duodenal lesions, such as mucosal erythema, granularity, friability, and erosions, in dogs with lymphocytic plasmacytic enteritis.

In the present study, the endoscopic findings revealed hyperaemia, discoloration, and friability in the gastric mucosa, while the duodenum showed predominant changes of friability, followed by hyperaemia, mucosal thickening, and corrugation, increased granularity, erosion/ ulcers, discoloration, and increased mucus. The colonic mucosa exhibited friability, hyperaemia, ulceration, and discoloration. These findings are in accordance with the aforementioned studies.

Histopathology of endoscopic mucosal biopsy remains the gold standard for diagnosing CIE, providing valuable information on the extent and severity of mucosal inflammation and damage. The combination of endoscopy and histopathology allows for a comprehensive evaluation of gastrointestinal disease in dogs with CIE.

The histopathological findings in this study are consistent with the standards established by the World Small Animal Veterinary Association (WSAVA) International Gastrointestinal Standardization Group (Day et al., 2008). The morphological changes observed in the CIE dogs, including villous stunting, fusion, epithelial injury, and fibrosis, are similar to those described by Washabau et al. (2010) as characteristic of small intestinal inflammation.

The inflammatory cell infiltrate in the CIE dogs was predominantly lymphoplasmacytic, which is in agreement with previous studies (Jergens *et al.*, 2010; Suchodolski *et al.*, 2012; SandhyaBhavani, 2018). The presence of lymphocytes and plasma cells as the primary inflammatory cells is consistent with the diagnosis of CIE.

The histopathological changes observed in this study, including gastric pit epithelial hyperplasia, glandular or mucosal fibrosis, villous epithelial injury, and villous stunting and fusion, are also consistent with the findings of Jergens and Simpson (2012), who described minimal to pronounced inflammatory cell infiltration and mucosal architectural disruption in dogs with IBD.The consistency of these findings with previous studies highlights the importance of histopathology in diagnosing CIE and understanding the underlying inflammatory processes.

The pathogenesis of Chronic Inflammatory Enteropathy (CIE) involves a complex interplay of immune cells and cytokines. T lymphocytes, particularly Th1 cells, play a major role in the development of intestinal inflammation through the secretion of pro-inflammatory cytokines such as TNF and IFNγ (Maeda *et al.*, 2013; Heilmann and Steiner, 2018). The imbalance between pro-inflammatory and anti-inflammatory responses, mediated by Th1, Th2, and Th17 cells, can lead to intestinal inflammation (Eissa *et al.*, 2019).

The immunohistochemical findings in this study revealed up regulation of CD3 and Ki67 positive cells in the intestinal mucosa of CIE-affected dogs, indicating an underlying immunological reaction against unknown etiology and luminal antigens. The presence of CD3 and Ki67 positive cells in all CIE dogs with lymphoplasmacytic cell infiltration supports the role of immune-mediated mechanisms in the pathogenesis of CIE.

The study also found a highly significant positive correlation between the clinical activity index (CCECAI) and endoscopic score, which is in contrast to the findings of Allenspach *et al.* (2007). However, the positive correlation between CCECAI and histopathological score observed in this study is consistent with the report by Allenspach *et al.* (2019). The lack of correlation between endoscopic score and histopathological score is in agreement with the findings of SandhyaBhavani (2018). These results highlight the complex relationships between clinical, endoscopic, and histopathological findings in CIE.

V. CONCLUSION

This study provides valuable insights into the clinical, endoscopic, and histopathological features of Chronic Inflammatory Enteropathy (CIE) in dogs. The findings suggest that CIE is characterized by a complex interplay of immune cells and cytokines, with upregulation of CD3 and Ki67 positive cells indicating an underlying immunological reaction. The positive correlation between clinical activity index and endoscopic score, as well as histopathological score,

highlights the importance of a comprehensive diagnostic approach in evaluating CIE. The study's findings contribute to the understanding of CIE pathogenesis and may aid in the development of effective diagnostic and therapeutic strategies for managing this condition in dogs.

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Fig. 1: Emaciation in a CIE dog

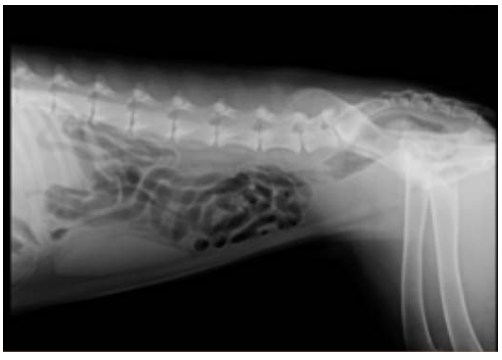

Fig. 2: Lateral Abdominal Radiograph with Gas Filled Intestinal Loops

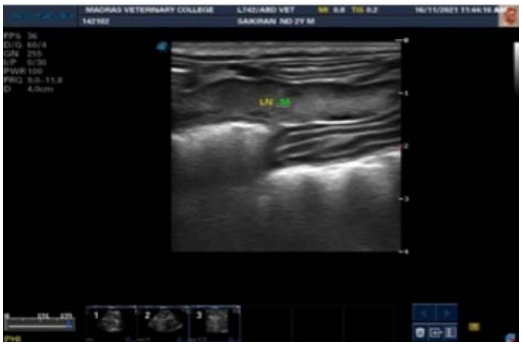

Fig. 3: Mesenteric lymph Node Enlargement in a CIE Affected Dog

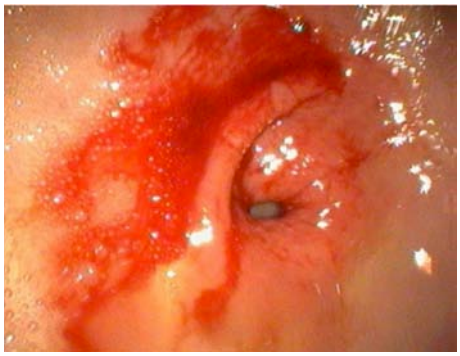

Fig. 4: Friable and Hemorrhagic Gastric Mucosa

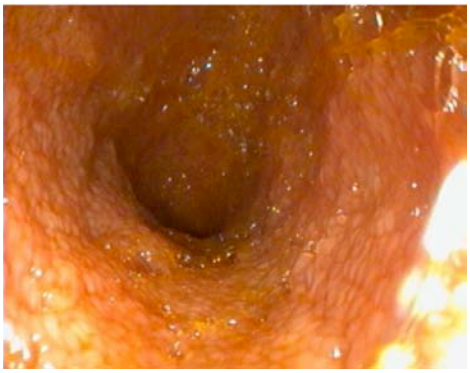

Fig. 5: Increased granularity in duodenal mucosa

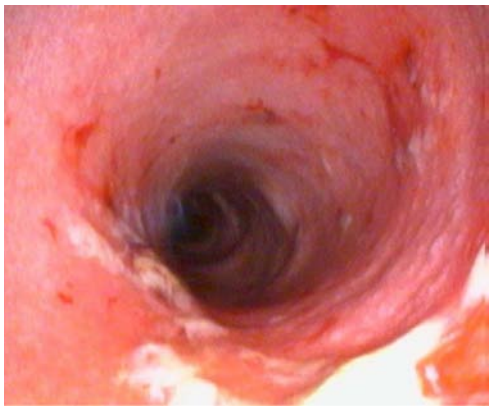

Fig. 6: Erosion/Ulcer in the Colonic Mucosa

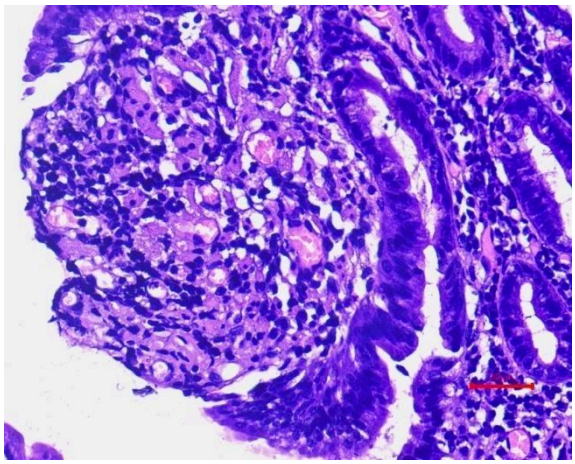

Fig. 7: Lymphoplasmacytic Infiltration in Stomach, H & E, 40X

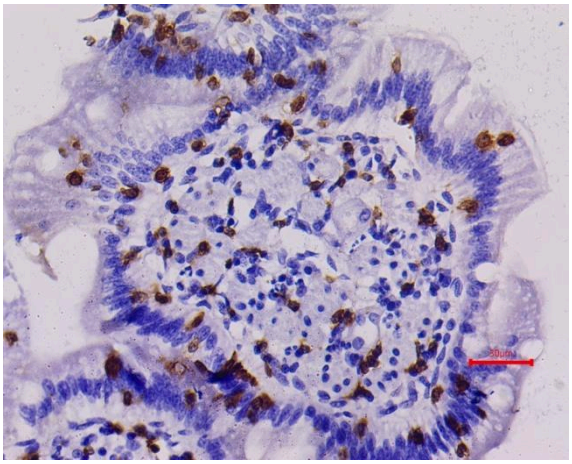

Fig. 8: Strongly Positive CD3 Cells Intraepithelial Lymphocytes of Duodenum, H & E, 40X

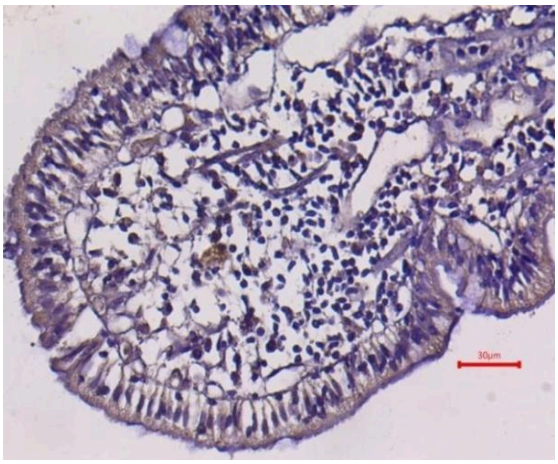

Fig. 9: Strongly Positive Ki 67 in Lamina Propria of Colon, H & E 40x

Table 1: Clinical Signs of CIE Dogs

S. No	Clinical Signs	No. of Animals (%)
1.	Diarrhea Melena Hematochezia	56.25% (9/16) 66.67(6/9) 33.33(3/9)
2.	Vomiting	18.75%(3/16)
3.	Vomiting with diarrhea	25%(4/16)
4.	Weight loss Emaciation (BCS 1) Thin (BCS 3)	75%(12/16) 31.25% (5/16) 31.25%(5/16)

Table 2: Hematology and serum biochemistry profile of CIE Dogs

S. No	Parameters	Control (n=6)	CIE affected dogs (n=16)	T value
1.	Haemoglobin(g/d1)	14.83+0.76	13.41 0.84	0.960^{NS}
2.	PCV(percent)	41+1.72	37.81+2.18	0.846^{NS}
3.	Total erythrocyte($x10^6$/cu mm)	6.55z0.27	5.8+0.35	1.040^{NS}
4.	Total leucocyte ($x10^3$/ cu mm)	8.2z0.54	17.26+2.1	2.586*
5.	Neutrophils ($x10^3$/ cu mm)	6.042+0.59	14.25a1.97	2.499*
6.	Lymphocytes ($x10^3$/cu mm)	1.02+0.1	2.32+0.31	2.507*
7.	Monocytes ($x10^3$/ cu mm)	0.27+0.04	0.75+0.08	3.226**
8.	Eosinophils ($x10^3$/cu mm)	0.05+0.02	0.18+0.08	0.938^{NS}

9.	Basophils (x10³/ cu mm)	0.01+0.02	0.00+0.00	1.706^{NS}
10.	Serum Alkaline Phosphatase(IU/L)	110.16+11.38	254.25+69.86	1.241^{NS}
11.	Total protein(g/dl)	6.4+0.30	6.58+0.27	0.270^{NS}
12.	Albumin(g/dl)	2.8+0.14	2.8+0.17	0.041^{NS}
13.	SerumCholesterol(mg/d1)	156.50+7.52	129.62+18.2	0.877^{NS}

***-Statistically highly significant(P<0.01)*

**-Statistically significant(P<0.05)*

NS-Statistically nonsignificant(P>0.05)

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Mechanisms of Cypermethrin-Induced Reproductive Toxicity

Mega Obukohwo Oyovwi, Onoriode Andrew Udi, David Atere Adedeji, Gregory Joseph Uchechukwu & Udoji Godsday Ogbutor

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ABSTRACT

Cypermethrin, a pyrethroid insecticide, is known for its effectiveness against pests but has raised concerns about reproductive toxicity, fertility issues, and developmental issues in offspring. This review explores cypermethrin's reproductive toxicological effects, identifying biological and molecular mechanisms, evaluating in vitro and in vivo studies, and highlighting potential long- term consequences on subsequent generations. A systematic literature review was conducted, focusing on peer-reviewed articles, toxicological reports, and relevant studies that explore the impact of cypermethrin on reproductive health. Databases such as PubMed, Scopus, and Google Scholar were searched using keywords related to cypermethrin, reproductive toxicity, endocrine disruption, and developmental effects. Studies selected for inclusion had to meet stringent criteria regarding experimental design, exposure levels, and outcome measures. Cypermethrin, an insecticide, has been found to disrupt reproductive health through various mechanisms.

Keywords: cypermethrin, reproductive toxicity, endocrine disruption, oxidative stress, apoptosis, developmental effects, pyrethroids, fertility, environmental health.

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Cypermethrin, a pyrethroid insecticide, is known for its effectiveness against pests but has raised concerns about reproductive toxicity, fertility issues, and developmental issues in offspring. This review explores cypermethrin's reproductive toxicological effects, identifying biological and molecular mechanisms, evaluating in vitro and in vivo studies, and highlighting potential long-term consequences on subsequent generations. A systematic literature review was conducted, focusing on peer-reviewed articles, toxicological reports, and relevant studies that explore the impact of cypermethrin on reproductive health. Databases such as PubMed, Scopus, and Google Scholar were searched using keywords related to cypermethrin, reproductive toxicity, endocrine disruption, and developmental effects. Studies selected for inclusion had to meet stringent criteria regarding experimental design, exposure levels, and outcome measures. Cypermethrin, an insecticide, has been found to disrupt reproductive health through various mechanisms. It interferes with hormone synthesis, leading to hormonal imbalances and sexual development issues. Cypermethrin exposure also results in oxidative stress, compromising gamete quality and reproductive function. Increased apoptosis in germ cells can lead to reduced sperm quantity and quality, impacting male fertility and female fertility. Cypermethrin exposure during critical growth periods can cause malformations and impaired development in offspring, posing risks to population viability and biodiversity. These mechanisms highlight the need for cautious regulation and deeper understanding of cypermethrin's impact on reproductive health. Cypermethrin, a common pest in agriculture and residential areas, poses significant reproductive health risks through endocrine disruption, oxidative stress, and cellular apoptosis, necessitating further research for safe exposure levels.

Keywords: cypermethrin, reproductive toxicity, endocrine disruption, oxidative stress, apoptosis, developmental effects, pyrethroids, fertility, environmental health.

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I. INTRODUCTION

Cypermethrin, a widely utilized synthetic pyrethroid insecticide, has garnered significant attention due to its effectiveness in pest control across various environments, including agricultural and residential settings. Its chemical formula, C22H19Cl2NO3 reveals a complex molecular structure characterized by the presence of a cyano group and a racemic mixture of isomers, each contributing to its insecticidal properties (Kumar Singh et al., 2012; Zhou et al., 2019). The IUPAC nomenclature-cyano (3-pheno-xyphenyl) methyl3- (2, 2-dichloroethenyl)-2, 2-dimethylcyclopropane-1-carboxylate-underscores its sophisticated chemical makeup, advancing our

understanding of how pyrethroids interact with pest populations (Zhou et al., 2019).

Cypermethrin's application spans a wide range of uses, from protecting crops (Dina, 1988) to controlling household pests like ants and cockroaches, making it a common component in popular insecticide brands like Raid and Ortho (Ali et al., 2022). However, its extensive use raises important questions regarding its safety and potential implications for human health and the environment.

Understanding the reproductive toxicity of cypermethrin is paramount, as research indicates alarming findings related to its impact on reproductive health in animal models. Studies have shown that exposure can result in reduced testosterone levels and structural alterations in sperm in male rats, alongside developmental delays in offspring and evidence of genetic damage through increased chromosomal abnormalities (Ikpeme et al., 2016; Alaa-Eldin et al., 2017; Abdel-Razik et al., 2021). These effects necessitate a deeper investigation into the safety profile of cypermethrin, especially given its classification as a potential human carcinogen due to its association with higher tumor frequencies in exposed animals (Ferre et al., 2018).

This review aims to meticulously analyze cypermethrin's chemical properties and classifications, evaluate its prevalence in various applications, and investigate the reproductive toxicity linked to its exposure. By synthesizing existing research, this review seeks to illuminate both the benefits and risks associated with cypermethrin, ultimately guiding safer practices in its application and informing regulatory considerations.

1.1 Search Strategy

A comprehensive search strategy was designed to explore the mechanisms underlying cypermethrin -induced reproductive toxicity, focusing on peer-reviewed studies published in scientific journals. This involved systematic searches in databases such as PubMed, Scopus, and Web of Science, using keywords like "cypermethrin," "reproductive toxicity," "mechanisms," and "endocrine disruption." By synthesizing findings from various research articles, we aimed to elucidate the pathways through which cypermethrin exerts its adverse effects on reproductive health.

II. MECHANISM OF ACTION OF CYPERMETHRIN

Cypermethrin exerts its effects primarily through interactions with ion channels and has significant implications for the endocrine system, influencing both neuronal activity and hormonal balance in various organisms.

2.1 Interaction with Ion Channels: Sodium Channel Modulation

Cypermethrin's primary mechanism of action involves its modulation of sodium channels located in neuronal cells (Kumar Singh et al., 2012; Ali, 2020). The compound binds to voltage-gated sodium channels, which are critical for the initiation and propagation of action potentials in neurons. This binding leads to prolonged activation of these channels, resulting in persistent depolarization. The consequences of this alteration include increased neuronal excitability and elevated neurotransmitter release, which can disrupt the normal signaling pathways within the nervous system. Such disruptions are problematic as they may culminate in neurotoxic effects, characterized by symptoms such as paralysis and death in target pest species (Ganguly et al., 2023). This modulation of sodium channels stands out as a core mechanism through which cypermethrin demonstrates its insecticidal properties, showcasing the compound's effectiveness against a wide range of insect pests.

2.1.1 Effects on Neuronal Signaling

The prolonged activation of sodium channels due to cypermethrin exposure can induce excessive neuronal firing. This hyperactivity not only disrupts typical neuronal signaling but may also lead to neurodegenerative effects over time. Research has indicated that cypermethrin can induce oxidative stress in neuronal cells, which contributes significantly to cellular damage and

functional impairment (Ali et al., 2020; Abd El-Moneim Ibrahim et al., 2020; Zhao et al., 2021). Such oxidative stress pathways are integral to understanding the potential long-term impacts of cypermethrin, both in terms of ecological harm and implications for human health, given the closeness of some neurological mechanisms across species.

2.2 Involvement of the Endocrine System

2.2.1 Endocrine Disruption Potential

Cypermethrin is classified as an endocrine-disrupting chemical (EDC), meaning it has the potential to interfere with hormonal functions within biological systems. This is particularly evident in its capacity to alter levels of essential steroid hormones such as testosterone and estrogen. Research has highlighted that exposure to cypermethrin can lead to significant reductions in testosterone levels, as well as impairments in spermatogenesis in male mammals (Al-Hamdani et al., 2011). Cypermethrin has been recognized as a full endocrine disruptor due to its demonstrated effects on hormone levels and reproductive health, raising concerns about its long-term implications for wildlife and human populations alike (Saillenfait et al., 2017; Guo et al., 2017; Aziz et al., 2023).

2.2.2 Impact on Hormone Levels

Chronic exposure to cypermethrin has been associated with numerous reproductive issues, such as reduced fertility and alterations in hormone levels (Liu et al., 2006). For example, studies conducted on male mice found that even low doses of cypermethrin exposure resulted in significant histopathological changes within reproductive organs, along with a decrease in serum testosterone levels (Wang et al., 2021; Al-Hamdani et al., 2011). Furthermore, cypermethrin's influence extends beyond mammals; it has also been observed to affect the hypothalamic-pituitary-gonadal (HPG) axis in fish. This underscores the compound's pervasive disruptive effects across a variety of species, illustrating the far-reaching consequences of cypermethrin exposure on reproductive health and endocrine function (Ganguly et al., 2023).

III. Effects of Cypermethrin on the Male Reproductive System

Cypermethrin is a widely used synthetic pyrethroid insecticide that has garnered attention due to its potential adverse effects on male reproductive health. Numerous studies have documented significant impairments in spermatogenesis, hormonal balance, and overall fertility outcomes linked to cypermethrin exposure, highlighting the necessity for deeper investigation into this issue.

3.1 Spermatogenesis Impairment

3.1.1 Analysis of Sperm Quality and Quantity

Research into the effects of cypermethrin on sperm quality has consistently shown a considerable decline in critical parameters such as sperm count, motility, and viability. For instance, animals subjected to cypermethrin treatment exhibit a remarkable reduction in daily sperm counts and epididymal sperm counts when compared to control groups, revealing the detrimental impact on reproductive potential (Katragadda et al., 2021; Sharma et al., 2018). Furthermore, histopathological examinations of the testes have unveiled significant damage, characterized by reduced weights of reproductive organs and altered cellular architectures within the seminiferous tubules. These alterations are indicative of impaired spermatogenesis and suggest a direct correlation between cypermethrin exposure and male reproductive dysfunction (Abd El-Hameed, A. M., & Mahmoud, 2020; Katragadda et al., 2021).

3.1.2 Histopathological Findings in Testes

Detailed histological analyses highlight pronounced degeneration of germ cells and severe impairment of spermatogenesis, attributed to cypermethrin toxicity. This degradation is often associated with oxidative stress, which leads to significant cellular damage, particularly affecting Leydig cells responsible for testosterone production. Consequently, the toxicity of cypermethrin has a cascading effect on the entire male reproductive system, interrupting the delicate balance required for normal

spermatogenesis (Solati et al., 2010; Sharma et al., 2018).

3.2 Hormonal Changes

3.2.1 Alterations in Testosterone Levels

Exposure to cypermethrin has been shown to lead to significant decreases in serum testosterone levels. This reduction can be traced back to the insecticide's antiandrogenic properties and its ability to disrupt the activity of steroidogenic enzymes, such as 3β-HSD and 17β-HSD, which are crucial for testosterone biosynthesis (Katragadda et al., 2021; Sharma et al., 2018). Additionally, lower testosterone levels correlate with decreased expression of steroidogenic acute regulatory protein (StAR), a key player in the transport of cholesterol essential for testosterone production (Katragadda et al., 2021; Sharma et al., 2018).

3.2.2 Effects on Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH)

Cypermethrin treatment has been associated with alterations in gonadotropin levels, particularly through the observation of increased LH and FSH concentrations. These changes are likely a result of negative feedback mechanisms triggered by the observed reductions in testosterone levels. Interestingly, some studies also suggest that prolonged exposure to cypermethrin may ultimately lead to decreased levels of LH and FSH as well, indicating a complex and potentially detrimental endocrine disruption (Solati et al., 2010; Sharma et al., 2018; Abd El-Hameed, A. M., & Mahmoud, 2020).

3.3 Fertility Outcomes

3.3.1 Impact on Mating Behavior

Male rats that have been exposed to cypermethrin demonstrate altered mating behaviors, which may act as a contributing factor to reduced fertility rates. The observed impairments in sexual behavior are likely intertwined with the hormonal disruptions induced by cypermethrin exposure, further compromising reproductive outcomes (Solati et al., 2010; Abd El-Hameed, A. M., & Mahmoud, 2020).

3.3.2 Consequences on Pregnancy Rates

The fertility outcomes following cypermethrin exposure are further diminished by increased rates of pre- and post-implantation losses observed in mating studies involving treated males (Katragadda et al., 2021). This finding suggests that cypermethrin impacts male reproductive health not only directly but also significantly influences the overall reproductive success rates of mating pairs, indicating a broad spectrum of reproductive challenges linked to this insecticide.

IV. Effects of Cypermethrin on the Female Reproductive System

Cypermethrin has garnered increasing attention due to its significant adverse effects on the female reproductive system, as evidenced by a growing body of research. The detrimental impacts of cypermethrin can be categorized into three principal areas: ovarian function and hormone production, effects on implantation and fetal development, and implications for reproductive longevity.

4.1 Ovarian Function and Hormone Production

4.1.1 Disruption of the Menstrual Cycle

Research has demonstrated that exposure to cypermethrin is associated with alterations in the estrous cycle of female rats, serving as a model for understanding potential implications in humans (Zhou et al., 2018; Wang et al.,, 2019). Specifically, studies have identified a dose-dependent decrease in luteinizing hormone (LH) levels, a critical hormone in regulating ovulation (Al-Hamdani & Yajurvedi, 2017; Obinna & Agu, 2019). Such hormonal disruptions can lead to abnormalities in the menstrual cycle, specifically prolonged diestrus phases, which may ultimately result in infertility issues for affected females. The intricate balance of hormones necessary for a regular menstrual cycle and successful ovulation can be significantly disturbed by exposure to this

pesticide (Zhou et al., 2018; Obinna & Agu, 2019).

4.2 Altered Ovarian Morphology and Function

Histological assessments of ovarian tissues from cypermethrin-exposed females have revealed notable morphological alterations (Singh et al., 2008; 2020). These changes include luteinization of ovarian follicles, a reduction in primordial follicular reserves, and the presence of multi-oocyte follicles, all of which suggest compromised ovarian functionality. Furthermore, cypermethrin exposure has been linked to oxidative stress in uterine tissues, contributing to a broader spectrum of reproductive and developmental problems, with profound implications for overall reproductive health (Obinna & Agu, 2016; Singh et al., 2020). This points to the pesticide's potential to instigate long-term changes in the ovarian environment.

4.3 Impacts on Implantation and Fetal Development

4.3.1 Maternal Health Effects

Maternal exposure to cypermethrin has been shown to induce adverse effects such as embryonic resorption and stillbirths in pregnant rats (Al-Hamdani, & Yajurvedi, 2017; Singh et al., 2020). These findings highlight the pesticide's detrimental impact on maternal health, particularly its ability to cause histological damage to maternal liver tissue, which may compromise essential physiological functions during pregnancy. The integrity of maternal health is vital for the development of the fetus, and disruption in this area can lead to a cascade of negative outcomes, including compromised gestational success and the health of the offspring (Al-Hamdani, & Yajurvedi, 2017; Singh et al., 2020).

4.3.2 Consequences for Fetal Outcomes

The negative ramifications of cypermethrin exposure extend beyond maternal health to affect fetal development directly (Obinna & Agu, 2019; Singh et al., 2020). Research findings indicate a concerning increase in pre-implantation loss and a significant reduction in litter sizes among the offspring of cypermethrin-exposed mothers (Singh et al., 2020). This suggests a disturbing trend where the health and viability of newborns are adversely compromised. Furthermore, instances of perinatal exposure have been connected to long-term reproductive dysfunction in female offspring of the first filial generation (F1), indicating potential transgenerational effects that could unfurl across multiple generations, thereby exacerbating the impact of this environmental toxin on reproductive health (Obinna & Agu, 2019; Singh et al., 2020).

4.3.3 Impact on Reproductive Longevity

Cypermethrin's adverse effects on reproductive longevity are particularly alarming, as studies have indicated its capacity to induce irreversible changes in ovarian activity at specific dosage levels (Al-Hamdani, & Yajurvedi, 2017; Marettova et al., 2017). Research has shown that even exposure to low doses of cypermethrin can culminate in infertility over time, signaling a concerning trend whereby long-term reproductive consequences may follow exposure to this pesticide (Al-Hamdani, & Yajurvedi, 2017; Marettova et al., 2017). The cumulative nature of hormonal disruption, coupled with morphological changes in the ovaries, poses a significant risk for the reproductive lifespan of females. This decline in reproductive potential underlines the need for careful consideration of cypermethrin usage and exposure, especially in contexts related to female reproductive health and fertility (Al-Hamdani, & Yajurvedi, 2017; Marettova et al., 2017). The evidence presented emphasizes the urgent need for further investigation into the mechanisms underlying cypermethrin's harmful effects and the potential establishment of more stringent regulations regarding its use to safeguard female reproductive health.

V. EXPERIMENTAL EVIDENCE OF CYPERMETHRIN'S INDUCED REPRODUCTIVE TOXICITY

Given its widespread application, there has been a growing concern regarding its potential impact on human health, particularly in relation to

reproductive toxicity. Numerous studies across various biological models have sought to elucidate the effects of cypermethrin on reproductive health. This review aims to provide a detailed overview of existing experimental evidence derived from animal studies, in vitro experiments, and human exposure assessments.

5.1 Animal Studies

5.1.1 Rodent Models

In many reproductive toxicity studies, rodent models, particularly rats and mice, are employed due to their genetic, biological, and behavioral similarities to humans. For example, a pivotal study that investigated perinatal exposure to cypermethrin in female F1 generation rats revealed noteworthy findings. While the exposure did not significantly alter the estrous cycle or most serum sex hormone levels-except for a marked decrease in luteinizing hormone (LH)—it led to persistent phases of diestrus in these animals. Such prolonged periods of diestrus can disrupt the normal reproductive cycle, potentially culminating in infertility (Obinna & Agu, 2019). This highlights the need for further investigation into the long-term effects of cypermethrin exposure on reproductive capabilities.

Another critical study assessed the impact of cypermethrin on pregnant female albino rats. Results indicated a significant reduction in neonatal birth weights, suggesting suboptimal development in utero. Furthermore, increased rates of embryonic resorption—a condition where embryos fail to implant or are reabsorbed by the body—and stillbirths were noted among the treated groups (Obinna & Agu, 2016). These alarming indicators of reproductive toxicity raise concerns regarding the implications of cypermethrin exposure during sensitive developmental windows.

5.1.2 Effects on Fertility and Reproductive Parameters

Investigations into the fertility effects of cypermethrin highlight its role as an endocrine disruptor. In male rabbits subjected to cypermethrin exposure, significant toxico-pathological changes were documented, which negatively impacted semen quality and overall testicular health (Ahmad et al., 2012). Such findings underscore the potential for cypermethrin to disrupt male reproductive function, which could have far-reaching implications for fertility.

In contrast, female rats demonstrated severe toxicity manifestations following exposure to varying doses of cypermethrin. Observable symptoms included substantial reductions in body weight along with compromised reproductive health parameters, indicating a clear negative impact on female fertility and reproductive potential (Shuklan et al., 2023). Collectively, these findings emphasize the potential risks associated with cypermethrin exposure for both genders.

5.2 In Vitro Studies

5.2.1 Impact on Gamete Viability and Function

In vitro research has revealed that cypermethrin has a detrimental impact on gamete viability and function. Specifically, studies indicate that exposure to this insecticide can lead to impaired sperm motility and alterations in motility patterns. Such impairments suggest that cypermethrin exposure may have significant implications for reproductive outcomes, potentially reducing the likelihood of successful fertilization (Shuklan et al., 2023).

5.2.2 Effects on Reproductive Cell Lines

Investigations involving reproductive cell lines have highlighted the cellular mechanisms through which cypermethrin may exert its toxic effects. Notably, exposure to cypermethrin has been shown to induce oxidative stress within these cells, leading to cellular damage and impairments in reproductive function (Shuklan et al., 2023). Moreover, the antiandrogenic effects of pyrethroids have been observed in various assays, implicating disrupted hormonal signaling pathways that are essential for normal reproductive processes (Shuklan et al., 2023). These findings underscore the need for continuous monitoring of cypermethrin's effects at the cellular level.

5.3 Human Exposure Studies

5.3.1 Epidemiological Data

Epidemiological studies have been pivotal in linking chronic exposure to pyrethroids, including cypermethrin, with adverse reproductive outcomes in humans (Koureas et al., 2012; Burns & Pastoor, 2018; Wang et al., 2020). Although establishing direct causal relationships is challenging due to the presence of confounding factors, a discernible association has been noted between pyrethroid exposure and altered hormone levels in adult males. Such hormonal changes could serve as indicators of potential fertility issues, warranting further investigation into the long-term effects of cypermethrin exposure on reproductive health in humans (Shuklan et al., 2023).

5.3.2 Case Studies and Reported Outcomes

Numerous case studies have documented significant reproductive health concerns among individuals exposed to cypermethrin through occupational or environmental routes (Obinna & Agu, 2016; Ullah et al., 2018). Reported issues include decreased fertility rates and increased instances of pregnancy complications, although comprehensive data remains limited. The variability in exposure levels and individual susceptibility complicates the establishment of a clear causative link, underscoring the need for additional research to elucidate the full scope of cypermethrin's impact on human reproductive health (Obinna & Agu, 2016).

VI. MECHANISMS OF CYPERMETHRIN-INDUCED CELLULAR AND MOLECULAR TOXICITY

6.1 Oxidative Stress and Its Implications

6.1.1 Generation of Reactive Oxygen Species (ROS)

Cypermethrin exposure is characterized by an elevation in the production of Reactive Oxygen Species (ROS), which play a pivotal role in triggering oxidative stress across various cell types, including but not limited to macrophages and hepatocytes. The excessive generation of ROS initiates a cascade of oxidative damage that is closely linked to significant cellular injury through mechanisms such as lipid peroxidation and protein oxidation. These events can severely disrupt cellular functions and compromise cellular integrity, resulting in a range of detrimental effects on cell viability and health (Huang et al., 2016; Elblehi et al., 2023; Hussain et al.,2023).

6.1.2 Cellular Damage and Apoptosis

The accumulation of ROS not only leads to oxidative stress but also results in substantial DNA damage and the initiation of apoptosis. Research has established that cypermethrin can induce apoptotic pathways through signaling mechanisms involving Jun N-terminal Kinase (JNK) and Extracellular signal-Regulated Kinase (ERK). Notably, the inhibition of these pathways has shown potential in partially reversing the apoptotic effects induced by cypermethrin. The morphological features of apoptosis are evident in affected cells, displaying characteristics such as nuclear fragmentation and chromatin condensation, indicative of the cell undergoing programmed cell death (Huang et al., 2016; Ashafaq et al., 2023).

6.2 Inflammatory Responses

6.2.2 Cytokine Release and Its Effects on Reproduction

Cypermethrin exposure is strongly associated with an increase in the levels of pro-inflammatory cytokines, including Interleukin-1 beta (IL-1β), Interleukin-6 (IL-6), and Tumor Necrosis Factor-alpha (TNF-α) (Elblehi et al., 2023; Hussain et al.,2023). Elevated levels of these cytokines have been shown to disrupt reproductive health by altering hormonal balances and impairing various reproductive processes. The implications of the inflammatory response are not confined to local tissues but may extend systemically, contributing to broader reproductive health concerns and issues (Elblehi et al., 2023; Hussain et al.,2023).

6.3 Genetic and Epigenetic Effects

6.3.1 DNA Damage and Repair Mechanisms

Cypermethrin has been documented to inflict significant DNA damage across a variety of organisms, including zebrafish and mammalian models (Jin et al., 2011; Paravani et al., 2018, 2019). Such damage can overwhelm cellular repair mechanisms, consequently leading to mutations and potential carcinogenic outcomes. The inability of cells to effectively repair DNA lesions instigated by cypermethrin exposure raises concerns regarding long-term genetic stability and the risks of oncogenesis (Jin et al., 2011).

6.3.2 Epigenetic Changes Influencing Reproductive Health

The exposure to cypermethrin may induce epigenetic modifications that significantly influence gene expression patterns related to reproductive health (Irani et al., 2022; Song et al., 2022; Hussain et al., 2023). These modifications can alter the development and functionality of germ cells, giving rise to substantial long-term reproductive complications (Hussain et al., 2023). The ramifications of these epigenetic changes underscore the necessity for further investigation into how environmental toxins like cypermethrin can alter genetic expression and consequently affect reproductive outcomes over successive generations (Hussain et al., 2023).

6.4 Signaling Pathways Affected by Cypermethrin

6.4.1 Role of Apoptosis in Reproductive Toxicity

The activation of apoptotic pathways, primarily driven by oxidative stress through ROS generation, is critical in understanding cypermethrin's reproductive toxicity. The dysregulation of these apoptotic pathways can lead to compromised germ cell viability and functionality, raising vital questions about the long-term implications of such toxicity on reproductive health (Huang et al., 2016; Ashafaq et al., 2023).

6.4.2 Alterations in Gene Expression Related to Reproduction

Cypermethrin exposure is implicated in the alteration of gene expression associated with critical reproductive processes (Elblehi et al., 2023; Hussain et al.,2023). These alterations may disrupt normal processes such as spermatogenesis (the development of sperm) and oocyte maturation (the maturation of eggs), significantly affecting overall fertility and reproductive success (Elblehi et al., 2023; Hussain et al.,2023).

6.5 Impact on Germ Cell Development and Function

6.5.1 Effects on Spermatogenesis

Research has indicated that cypermethrin adversely influences spermatogenesis, primarily through the induction of oxidative stress and subsequent apoptosis in Sertoli cells, which are essential for the proper development of sperm (Ashafaq et al., 2023). The implications of such effects extend to the fertility potential of males exposed to this insecticide, highlighting critical concerns regarding reproductive health in male populations (Ashafaq et al., 2023).

6.5.2 Effects on Oocyte Maturation

Similarly, cypermethrin exposure has been shown to substantially impair oocyte maturation, predominantly via mechanisms associated with oxidative stress. These impairments can directly affect fertility outcomes, suggesting that exposure to cypermethrin poses a profound risk to female reproductive health and the quality of eggs produced (Jin et al., 2011).

VII. SEX DIFFERENCES IN CYPERMETHRIN TOXICITY

Research indicates that there are significant *sex differences* in the toxicity of cypermethrin, a widely used pyrethroid insecticide. Studies have demonstrated that male and female subjects exhibit different responses to cypermethrin exposure, particularly concerning physiological and biochemical parameters. This variation is

crucial for understanding the safety and environmental implications of cypermethrin use.

7.1 Physiological Effects

In various animal studies, cypermethrin exposure has shown to result in differing impacts on body and organ weights between the sexes. For example, male rats have demonstrated markedly more pronounced reproductive toxicity, which includes lower testosterone levels and impaired spermatogenesis following exposure to cypermethrin. This response is notably more severe in males compared to females, highlighting potential susceptibility differences associated with sex (Grewal et al., 2010). It has been observed that male animals often suffer from additional physiological stressors that further exacerbate the toxic effects of cypermethrin, leading to a heightened risk of reproductive issues.

7.2 Biochemical Responses

Significant differences in biochemical markers have been identified based on sex. For instance, levels of liver enzymes such as aspartate aminotransferase (AST) and alanine amino-transferase (ALT), which are indicative of liver function and damage, tend to fluctuate in response to cypermethrin exposure. Alongside these markers, indicators of oxidative stress, including malondialdehyde levels, as well as metrics for DNA damage, such as micronuclei formation, also vary. Males typically exhibit a greater tendency towards higher susceptibility to oxidative stress and genotoxic effects. This greater vulnerability suggests a heightened risk of organ damage and reproductive impairment as a consequence of cypermethrin exposure (Seven et al., 2022; Grewal et al., 2010). These biochemical alterations not only reflect the immediate toxicological impact but also provide insight into long-term health risks.

7.3 Genotoxicity

The genotoxic effects of cypermethrin have been extensively documented, revealing that it can induce chromosomal abnormalities in both male and female mice; however, the frequency and severity of these chromosomal aberrations can diverge based on sex. Research indicates that male mice are more prone to exhibit higher rates of chromosomal aberrations in bone marrow cells in comparison to their female counterparts, thereby suggesting an increased risk of genetic damage arising from exposure to cypermethrin in males (Seven et al., 2022; Grewal et al., 2010). This distinction underscores the need for sex-specific evaluations in toxicological studies involving cypermethrin.

VIII. ROLE OF GENETIC AND HORMONAL FACTORS

The differences in sensitivity to cypermethrin toxicity between males and females can be attributed to several *genetic* and *hormonal factors* that significantly shape the response to toxic exposures.

8.1 Hormonal Influence

Hormonal levels significantly affect how each sex metabolizes toxins like cypermethrin. For instance, testosterone has been shown to influence the metabolism of various xenobiotics, leading to increased susceptibility in males (Abd El-Hameed & Mahmoud, 2020). This increased vulnerability may result from relatively lower detoxification capabilities compared to females, who typically have higher levels of estrogen. Estrogen has been suggested to provide protective effects against certain toxins, potentially contributing to the observed differences in sensitivity to cypermethrin (Elser et al., 2020; Ganguly et al., 2023). Therefore, hormonal context plays a pivotal role in defining the toxic thresholds and responses in males versus females.

8.2 Genetic Factors

Beyond hormonal influences, genetic predispositions also play a crucial role in determining individual sensitivity to toxic substances. Variations in genes responsible for the metabolism of pyrethroids can result in differing toxicity levels observed in males versus females. For example, genetic polymorphisms in cytochrome P450 enzymes, which are essential for detoxification processes, may exhibit sex-specific

expression patterns. These variations in enzyme activity can influence overall susceptibility to cypermethrin, with implications for health outcomes following exposure (Ganguly et al., 2023; Han et al., 2024). This aspect emphasizes the importance of considering genetic background when assessing risk and vulnerability to toxic substances on a sex-specific basis.

8.3 Intergenerational Effects

Moreover, exposure to cypermethrin during critical developmental periods can yield lasting impacts on offspring, with maternal exposure affecting fetal development in ways that differ based on the sex of the offspring. Studies have indicated that prenatal exposure can disrupt neurodevelopmental pathways, with significant differences noted between male and female offspring. This observation suggests that the hormonal environments present during gestation could influence these developmental outcomes, potentially leading to sex-specific vulnerabilities that persist beyond prenatal stages (Elser et al., 2020 ; Han et al., 2024).

IX. ENVIRONMENTAL AND ECOLOGICAL IMPLICATIONS OF CYPERMETHRIN TOXICITY

Cypermethrin presents significant environmental and ecological challenges, particularly with respect to wildlife reproduction and the regulatory framework governing its use. As its prevalence in agricultural practices continues to rise, understanding the extensive implications of cypermethrin toxicity becomes increasingly vital.

9.1 Impact on Wildlife Reproduction

9.1.1 Reproductive Toxicity

Research reveals that exposure to cypermethrin can lead to substantial reproductive toxicity across various animal models. For instance, studies conducted on pregnant rats have demonstrated that cypermethrin significantly reduces litter weight and adversely affects fetal development. This occurs likely due to disruptions in placental function and nutrient uptake, which are critical during gestation (Obinna & Agu, 2016; Sharma et al., 2018). Furthermore, the administration of cypermethrin has resulted in decreased weights of vital reproductive organs, including the testes and epididymis, alongside diminished sperm counts and motility (Sharma et al., 2014; 2018). These detrimental effects imply that chronic exposure to cypermethrin could severely compromise reproductive success in wildlife populations, potentially leading to declines in species numbers and disruptions in local ecosystems.

9.1.2 Effects on Hormonal Profiles

Cypermethrin has also been linked to significant alterations in hormone levels that are essential for reproduction. In studies involving male rats, exposure to cypermethrin led to decreased levels of testosterone and other critical reproductive hormones, which are necessary for normal spermatogenesis (Sharma et al., 2014). The resulting hormonal disruptions may have cascading effects on population dynamics, leading to reduced fertility rates among affected species. The implications of these alterations extend beyond individual organisms, threatening the stability and resilience of entire populations.

9.1.3 Broader Ecological Consequences

The impact of cypermethrin toxicity is not confined to direct reproductive effects; it can also disrupt behavioral patterns within wildlife populations. For instance, altered reproductive behaviors stemming from neurological impacts may lead to decreased mating success, further jeopardizing population stability (Grewal et al., 2010). The cumulative ramifications of these disruptions could result in long-term ecological imbalances, particularly within ecosystems that heavily depend on vulnerable species. As key players in food webs, the decline of specific wildlife can trigger ripple effects throughout the ecosystem, affecting plant diversity, predation dynamics, and overall habitat health.

9.2 Considerations for Pesticide Regulation and Usage

9.2.1 Regulatory Challenges

Given the alarming evidence of cypermethrin's toxicity to wildlife, there is an urgent need to address regulatory challenges surrounding its use. While cypermethrin has received approval for agricultural applications, the ongoing potential for chronic exposure via food chains necessitates a critical reevaluation of current pesticide regulations (Sharma et al., 2014; Sharma et al., 2018). Regulatory agencies must take into account not only the immediate effects stemming from pesticide application but also the extensive long-term ecological impacts on non-target species. Failure to adequately assess these risks could precipitate further declines in sensitive wildlife populations and disrupt the ecological balance.

9.2.2 Usage Guidelines

In an effort to mitigate the risks associated with cypermethrin usage, it is crucial to establish comprehensive guidelines that emphasize integrated pest management (IPM) strategies. These strategies should minimize reliance on chemical pesticides by promoting a diverse range of pest control methods, including biological control agents, crop rotation, and organic farming practices (Shuklan et al., 2023). Additionally, public awareness campaigns can play a significant role in educating farmers and agricultural stakeholders about the potential ecological consequences of pesticide usage. By fostering a culture of sustainability and responsible pesticide application, the agricultural sector can shift toward practices that preserve biodiversity and maintain ecological integrity.

9.2.3 Research and Monitoring

Ongoing research into the ecological impacts of cypermethrin is paramount for safeguarding wildlife and the environments they inhabit. Longitudinal studies that monitor wildlife populations in regions with high pesticide utilization can provide critical data regarding the long-term effects of exposure. Furthermore, establishing stricter monitoring protocols for pesticide residues in agricultural products can help protect both human health and environmental integrity (Grewal et al., 2010; Shuklan et al., 2023). By generating robust data and maintaining vigilance regarding pesticide application, stakeholders can better inform regulatory frameworks and promote agricultural practices that are more attuned to ecological health.

X. MITIGATION STRATEGIES OF CYPERMETHRIN-INDUCED REPRODUCTIVE TOXICITY

10.1 Antioxidants

10.1.1 Vitamin C, E & Curcumin

Numerous scientific studies have demonstrated that Vitamin C and Curcumin has a significant mitigating effect on the reproductive toxicity that can be induced by cypermethrin (Obinna & Agu, 2016; Ziada et al., 2020}, particularly in pregnant rat models. The administration of Vitamin C resulted in a notable decrease in the rates of embryonic resorption and stillbirths, with concurrent improvements observed in neonatal birth weights when compared to untreated control groups (Obinna & Agu, 2016). These findings suggest that antioxidants such as Vitamin C may be effective in counteracting the oxidative stress that arises from exposure to cypermethrin, thereby offering potential therapeutic benefits during pregnancy. Studies have shown that Cypermethrin exposure leads to a marked increase in oxidative stress levels, as evidenced by elevated concentrations of malondialdehyde (MDA) in both serum and tissue samples of affected rats. MDA serves as a biomarker for lipid peroxidation, indicating that cellular membranes are being compromised due to oxidative damage (Ziada et al., 2020). Concurrently, the activities of key antioxidant enzymes, crucial for mitigating oxidative damage, such as superoxide dismutase (SOD) and catalase, were notably diminished, further emphasizing the detrimental effects of Cypermethrin on oxidative balance. In light of these findings, the protective roles of Vitamins C and Curcumin have gained attention as potential

countermeasures against oxidative stress induced by pesticide exposure (Ziada et al., 2020). When administered in combination, these antioxidants significantly reduced MDA levels, signifying a decrease in lipid peroxidation and an overall improvement in oxidative status. The enhancement of antioxidant enzyme activity observed with the combination treatment, particularly in SOD and catalase, suggests a reinvigoration of the body's natural defense mechanisms against oxidative stress. The biochemical improvements following the combined administration of Vitamins C and Curcumin underscore their protective effects against Cypermethrin-induced toxicity. Parameters disrupted by the toxic effects of the pesticide showed normalization, indicating a restoration of physiological balance (Ziada et al., 2020). This reveals a promising avenue for therapeutic interventions aimed at ameliorating the oxidative damage and biochemical disruptions caused by pesticide exposure, emphasizing the importance of antioxidants in safeguarding health against environmental toxins.

In addition to their antioxidant properties, vitamins C and E has also demonstrated a remarkable ability to prevent apoptosis, a form of programmed cell death that can be detrimental to cellular health (Bhardwaj et al., 2018). The administration of these vitamins significantly reduces caspase-3 activity, an essential executor of apoptosis, thereby suggesting that they play a protective role in cellular survival. Histological analyses indicate that supplementation with vitamins C and E helps maintain the structural integrity of testicular tissues, effectively preventing the degeneration of spermatogonial cells (Bhardwaj et al., 2018). This preservation of testicular architecture is pivotal for the overall health and functionality of the male reproductive system. Ultimately, the protective effects of vitamins C and E against oxidative stress, particularly in the context of cypermethrin (CYP)-induced toxicity, can have profound implications for male fertility (Bhardwaj et al., 2018). By safeguarding spermatogonial cells from oxidative damage and subsequent loss, these vitamins contribute to the maintenance of reproductive health. Thus, their supplementation may serve as a valuable strategy for enhancing male fertility and providing a countermeasure against oxidative stress-induced reproductive disorders.

Molavi et al.'s 2016 study revealed that cypermethrin significantly affects ovarian health in rats, particularly follicular atresia. The study found an increase in atretic follicles in rats exposed to cypermethrin, with the early antral and antral stages being the most affected. This raises concerns about the reproductive implications of pesticide exposure, particularly disrupting normal ovarian function. The study also revealed significant biochemical alterations, with reduced serum estradiol levels, indicating impaired ovarian function. However, the introduction of vitamin E in conjunction with cypermethrin showed a protective role, reducing the incidence of follicular atresia and improving serum estradiol levels, suggesting restoration of normal ovarian function. The protective effects of vitamin E may be attributed to its role in energy metabolism, counteracting oxidative stress induced by cypermethrin. Future research could explore interventions targeting oxidative damage and energy homeostasis to mitigate reproductive toxicity associated with pesticide exposure.

10.1.2 Curcumin and Quercetin

Sharma et al.'s 2018 study found that curcumin and quercetin can protect against reproductive system impairment caused by synthetic pyrethroid insecticides, specifically cypermethrin and deltamethrin, in male Wistar rats. The antioxidants reduced reproductive toxicity and oxidative damage, leading to increased sex organ weights, improved sperm count, and elevated levels of sex hormones. The study also revealed that curcumin and quercetin upregulated essential steroidogenic enzymes, particularly 3β-HSD and 17β-HSD, which play a crucial role in testosterone synthesis. The antioxidants also restored disrupted testicular architecture, indicating a direct cytoprotective effect on tissues. While curcumin showed marginally greater protective effects compared to quercetin when administered alone, the combination of both

antioxidants provided superior protection. This suggests the potential for synergistic effects when used together, suggesting new therapeutic interventions for combating reproductive toxicity induced by environmental pollutants.

10.1.3 Resveratrol

Resveratrol, a naturally occurring polyphenolic compound found in various plants, has been investigated for its protective properties against testicular damage associated with cypermethrin exposure. Research indicates that resveratrol can enhance sex hormone levels while concurrently reducing markers of oxidative stress. Notably, treatment with resveratrol led to improvements in sperm quality parameters as well as bolstered antioxidant defense mechanisms in male rats exposed to cypermethrin (Sharma et al., 2014). This suggests that incorporating resveratrol into dietary interventions may provide significant protective effects against reproductive impairment resulting from toxic exposures.

10.1.4 L-carnitine-Loaded Nanoparticles

A study by Alyasari and Selman (2023) found that L-carnitine-loaded nanoparticles can protect against cypermethrin, a common pesticide, in adult male rats. Cypermethrin exposure led to reduced testosterone levels and adverse effects on sperm parameters, highlighting the potential risks. Treatment with L-carnitine-loaded nanoparticles restored testosterone levels, improved sperm count and motility, and reduced morphological abnormalities in sperm cells. The enhanced bioavailability of L-carnitine facilitated by the nanoparticles promotes improved cellular energy metabolism and reduces oxidative stress in testicular tissues. This suggests that L-carnitine-loaded nanoparticles may safeguard reproductive functions and represent a promising therapeutic approach for countering pesticide-related reproductive toxicity.

10.1.5 Astaxanthin

Astaxanthin, a potent xanthophyll carotenoid, is known for its unique red pigmentation and antioxidant properties. Although it does not exhibit pro-Vitamin A activity in humans, it may surpass other carotenoids in biological activity, particularly in protecting against cellular damage and enhancing immune function. A study by Sun et al. (2023) revealed that exposure to cypermethrin (CYP), a widely used pyrethroid insecticide, significantly diminishes porcine oocyte maturation rates due to increased reactive oxygen species (ROS) and a decrease in glutathione, leading to oxidative stress. DNA damage was observed in oocytes subjected to CYP, and disruptions in endoplasmic reticulum function were observed. However, when treated with astaxanthin, porcine oocytes exposed to CYP showed a marked improvement in maturation rates and embryo development compared to those exposed only to CYP. Astaxanthin's antioxidant properties play a crucial role in alleviating oxidative stress and repairing DNA damage caused by the insecticide.

10.1.6 L-DOPA

Baghel and Prasad's 2021 study explores the protective effects of L-DOPA against the reproductive toxicity caused by cypermethrin in Japanese quail. Cypermethrin negatively impacts reproductive function in non-target organisms, particularly avian species. The research highlights the need for effective mitigative strategies to address these toxicological concerns. L-DOPA, a precursor to dopamine, was found to alleviate reproductive toxicity associated with cypermethrin exposure. The study found significant improvements in reproductive performance among quails treated with L-DOPA compared to those treated with cypermethrin (Baghel and Prasad, 2021). L-DOPA also restored disrupted hormonal levels, suggesting its role in maintaining endocrine balance. Histopathological assessments confirmed the protective effects, with L-DOPA treatment causing less severe damage to reproductive organs. These results suggest the potential use of L-DOPA as a therapeutic agent in mitigating reproductive toxicity in avian species.

10.1.7 Glutathione

The study by He et al. (2023) investigates the harmful effects of beta-cypermethrin (β-CYP) on porcine oocytes, highlighting its potential to

induce meiotic defects. The research emphasizes the importance of understanding the impact of pesticides on animal husbandry and ecological integrity. The study also found that glutathione (GSH) can counteract the adverse effects of β-CYP exposure by reducing the incidence of meiotic defects (He et al., 2023). GSH regulates reactive oxygen species (ROS) levels, which can lead to oxidative stress and disrupt meiotic processes (He et al., 2023). This suggests that antioxidants can protect oocyte quality in toxic environments. The research emphasizes the need for therapeutic interventions that use natural compounds like glutathione to combat environmental pollutants' negative effects on reproductive functions.

10.1.8 Date Fruit (Phoenix Dactylifera)

Ubah et al.'s study investigates the impact of date fruit (Phoenix dactylifera) on sperm cell morphology and reproductive hormonal profiles in Wistar rats with cypermethrin-induced male infertility (Ubah et al., 2021). Cypermethrin, a pesticide, has been linked to detrimental reproductive effects, with significant decreases in sperm motility, viability, and mass activity in the cypermethrin-only group. However, date fruit extracts alone showed promising results, improving sperm motility and viability. The combined treatment of date fruit extracts with cypermethrin resulted in better outcomes, indicating the potential of date fruit as a supportive treatment. The study also found lower testosterone and follicle-stimulating hormone levels in the cypermethrin-only group, raising concerns about hormonal balance. The study's statistical analysis revealed significant differences between treatment groups, highlighting the potential of date fruit in mitigating reproductive health challenges posed by environmental toxins.

10.2 Other Nutritional Supplements

10.2.1 Selenium-Enriched Spirulina

In addition to the aforementioned antioxidants, the incorporation of selenium-enriched spirulina has emerged as a promising avenue for research concerning its protective effects against reproductive toxicity (Lu et al., 2021).. A study focusing on zebrafish exposed to beta-cypermethrin revealed that spirulina supplementation could mitigate the adverse effects on reproduction. These findings indicate that nutritional interventions utilizing specific supplements might have broad applicability across different species, potentially enhancing reproductive health and resilience against pesticide-related toxicity (Lu et al., 2021).

10.2.2 Selenium Nanoparticles (SeNPs)

The study by Hozyen et al. (2020) found that selenium nanoparticles (SeNPs) can mitigate reproductive toxicity caused by environmental contaminants like deltamethrin in male rats. The SeNPs-treated group showed significant improvements in reproductive parameters, including increased sperm quality and overall reproductive performance compared to the DLM-only group. Hormonal balance was restored, as elevated testosterone levels were restored. SeNPs also reduced malondialdehyde levels, indicating oxidative damage, and increased antioxidant markers, demonstrating their protective role against DLM-induced oxidative stress. Histological examination revealed that SeNPs preserved the normal architecture and function of testicular tissue, whereas the DLM-only group showed disruptions in testicular morphology, potentially leading to long-term reproductive issues. The study suggests SeNPs could be a potential therapeutic approach to combat reproductive toxicity.

10.3 Regulatory Recommendations and Safety Guidelines

10.3.1 Exposure Limits

It is imperative for regulatory agencies to establish strict guidelines regarding allowable limits of cypermethrin exposure, especially for particularly vulnerable populations, which include pregnant women and children (Roberts et al., 21012). This call for stringent regulations is grounded in the growing body of evidence that underscores the reproductive toxicity associated with cypermethrin exposure. By enacting and enforcing lower exposure limits, we can better protect these at-risk groups from potential harm.

10.3.2 Safety Guidelines

Risk Assessment

It is essential to conduct regular assessments of both environmental and occupational exposure levels of cypermethrin, particularly in agricultural environments where this pesticide is frequently utilized (Ullah et al., 2018; Behnami et al., 2021; Taheri et al., 2023). These risk assessments can provide critical data that can inform safety practices and regulatory decisions, aiming to minimize exposure risks among workers and the surrounding community.

Public Awareness Campaigns

The implementation of public awareness campaigns is vital in educating communities about the potential health risks associated with cypermethrin exposure (Thammachai et al., 2022; Ullah et al., 2018). Such educational efforts can highlight safer alternative pest management strategies and empower individuals to make informed choices regarding exposure, thereby serving to mitigate health risks associated with this pesticide.

10.3.3 Research and Monitoring:

Continued research into the long-term effects of cypermethrin on reproductive health is crucial for understanding the broader implications of its use in agriculture and pest control (Farag et al., 2021). In parallel, robust monitoring programs should be established to track the impacts of cypermethrin exposure on both wildlife and human populations. By fostering ongoing research and effective monitoring, we can better assess the cumulative effects of cypermethrin, ensuring that necessary adjustments to guidelines and practices are made promptly to safeguard reproductive health.

XI. FUTURE RESEARCH DIRECTIONS OF CYPERMETHRIN-INDUCED REPRODUCTIVE TOXICITY

11.1 Gaps in Current Knowledge

11.1.1 Mechanisms of Action

While numerous studies have demonstrated that cypermethrin is capable of inducing reproductive toxicity, the precise molecular mechanisms underlying this phenomenon remain inadequately explored. Notably, the pathways through which cypermethrin disrupts hormonal balance and reproductive functions have not been sufficiently elucidated. Current findings suggest that cypermethrin may interfere with the hypothalamic-pituitary-gonadal (HPG) axis, a crucial network involved in reproductive hormone regulation (Ye et al., 2017; Gan et al., 2023). However, the intricate details of these mechanisms, including specific cellular pathways and molecular interactions, continue to elude researchers, highlighting a critical area for further investigation.

11.1.2 Long-Term Effects

The majority of existing research tends to concentrate on the acute or short-term impacts of cypermethrin exposure. However, there exists a substantial knowledge gap regarding the long-term reproductive effects and potential cumulative toxicity that may arise from chronic exposure to low doses of this pesticide. This is particularly important for at-risk populations, such as pregnant women and developing fetuses, who may be more susceptible to the adverse effects of cypermethrin. Comprehensive studies aimed at elucidating the long-term reproductive consequences of cypermethrin exposure are essential for a more complete understanding of its safety profile (Obinna & Agu, 2016).

11.1.3 Species Variability

A significant portion of the current literature is grounded in research conducted on specific animal models, such as rats and zebrafish. Despite the insights gained from these studies, there is an urgent need for investigations across a broader array of species, particularly human populations, to evaluate the translational relevance of findings related to cypermethrin's reproductive toxicity. Understanding species-specific differences in response to cypermethrin exposure can provide valuable information for risk assessments and regulatory decisions (Sharma et al., 2014).

11.1.4 Protective Agents

Although some preliminary studies have investigated the potential protective effects of antioxidants such as resveratrol and vitamin C against cypermethrin-induced toxicity, the exploration of various protective agents remains limited. A comprehensive evaluation of different antioxidants, as well as hormonal therapies that may mitigate the toxicity associated with cypermethrin exposure, is essential. Such investigations could lead to the identification of effective strategies for minimizing reproductive risks in exposed populations (Sharma et al., 2014).

11.2 Suggestions for Mechanistic Studies and Long-Term Exposures

11.2.1 In Vitro Studies

In vitro research utilizing human-derived cell lines represents a promising avenue for dissecting the cellular mechanisms affected by cypermethrin. Such studies can facilitate the identification of specific gene expressions and signaling pathways involved in the manifestation of reproductive toxicity, providing foundational knowledge that could enhance our understanding of the pesticide's impact on human health.

11.2.2 Longitudinal Animal Studies

The implementation of long-term animal studies designed to simulate chronic exposure to cypermethrin could yield critical insights into its cumulative effects on reproductive health over time. These studies should encompass assessments of reproductive outcomes across multiple generations, allowing researchers to evaluate potential transgenerational effects that may arise from parental exposure to cypermethrin (Obinna & Agu et al., 2016; Lu et al., 2021).

11.2.3 Focus on Endocrine Disruption

Delving into the interactions between cypermethrin and endocrine systems can yield important insights into its role as an endocrine disruptor. Researchers should pay particular attention to cypermethrin's effects on estrogen and testosterone signaling pathways, as well as its impact on fertility-related hormones such as follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Lu et al., 2021; Aziz et al., 2023). This focus could illuminate the broader implications of cypermethrin exposure on reproductive health.

11.2.4 Environmental Impact Studies

Research that explores the effects of environmental concentrations of cypermethrin on wildlife and ecosystems is also crucial. Specifically, studies should focus on aquatic species, which may serve as sensitive indicators of broader ecological impacts resulting from pesticide exposure (Abd El-Hameed & Mahmoud, 2020). Understanding these dynamics can inform regulatory practices and promote sustainable agricultural practices.

11.2.5 Development of Biomarkers

There is a need to establish biomarkers for the early detection of reproductive toxicity associated with cypermethrin exposure. Identifying such biomarkers could greatly facilitate monitoring and intervention strategies in both animal models and human populations that are exposed to this pesticide. Early detection of reproductive effects could lead to timely interventions and potentially improve health outcomes for vulnerable populations impacted by cypermethrin exposure.

XII. CONCLUSION

Cypermethrin, a pyrethroid insecticide, has been linked to reproductive toxicity due to hormonal disruption, oxidative stress, and genetic damage. Hormonal disruption can lead to developmental abnormalities, changes in reproductive organ function, and impaired fertility. Oxidative stress can cause cellular damage and inflammation, further affecting reproductive tissues. Cypermethrin's genetic effects may induce DNA damage, leading to mutations and potential long-term consequences for reproductive success and viability. Understanding these pathways is crucial for assessing environmental risks associated with cypermethrin exposure. To mitigate these risks, more comprehensive research is needed to investigate the full extent of

its reproductive effects across various species, life stages, and exposure scenarios. This will help clarify dose-response relationships and the mechanisms at play, ultimately informing regulatory policies and risk management strategies.

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Impact of the Transplant Acceleration Program (PAT-MA) Phase I on Transplant Indicators in Maranhão, Brazil: A Retrospective Study

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ABSTRACT

Introduction: In the context of public health, the efficiency of transplant programs is crucial to improving outcomes for patients on waiting lists. This study evaluated the impact of implementing the Transplant Acceleration Program (PAT-MA) at the Transplant Center of Maranhão, Brazil.

Objectives: The aim of this study was to assess the impact of PAT-MA implementation on key performance indicators of the Maranhão Transplant Center, with a focus on improving the organization and effectiveness of the regional transplant system through structured management methodology.

Methods: We conducted a retrospective observational study analyzing data from the Maranhão Transplant Center before and after the first phase of PAT-MA as a quality improvement initiative, comparing the years 2022 and 2023. The indicators collected included: number of transplants performed, waiting list size, notifications per million population, effective donors per million, organ transplants per million, corneal transplants per million, and number of active CIHDOTTs and OPOs.

Keywords: organ transplantation, quality improvement, management program, PAT-MA, health systems, Maranhão, Brazil.

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ABSTRACT

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Results: Following PAT-MA implementation, there was a reduction in the family refusal rate (from 67% to 63%), a decrease in the rate of cardiac arrests before procurement completion (from 28% to 26%), an increase in notifications per million (from 172 [24/million] to 327 [46/million]), effective donors per million (from 12 [1.7/million] to 28 [3.9/million]), organ transplants per million (from 27 [3.9/million] to 62 [8.7/million]), corneal transplants per million (from 133 [18.6/million] to 255 [35/million]), and number of active CIHDOTTs and OPOs (from 3/0 to 10/1). These findings demonstrate marked growth and improvement in the regional transplant system.

Conclusion: This study demonstrates that the implementation of the Transplant Acceleration Program (PAT-MA) had a positive impact on key performance indicators at the Maranhão Transplant Center. The results underscore the importance of strategic management approaches to optimize transplant program outcomes.

Keywords: organ transplantation, quality improvement, management program, PAT-MA, health systems, Maranhão, Brazil.

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I. INTRODUCTION

Organ transplantation is a vital component of modern public health, offering life-saving treatment for patients with end-stage organ failure. The effectiveness of transplantation programs directly influences patient survival rates, quality of life, and healthcare system efficiency, particularly for those awaiting transplantation. In

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Brazil, regional disparities and operational challenges frequently hinder optimal transplant outcomes. To address these challenges, the State of Maranhão launched the Transplant Acceleration Program (PAT-MA) with the objective of reorganizing and enhancing the local transplant system through evidence-based management strategies.

II. OBJECTIVES

This study aimed to evaluate the impact of PAT-MA implementation on critical indicators within the Maranhão Transplant Center, specifically focusing on program organization, process effectiveness, and overall transplant system performance.

III. METHODS

A retrospective, comparative study was conducted using data collected from the Maranhão Transplant Center in the years immediately preceding (2022) and following (2023) the initial phase of PAT-MA implementation. PAT-MA was introduced as a comprehensive quality improvement program encompassing organizational restructuring, process optimization, and targeted professional training. The following indicators were analyzed:

- Number of transplants performed (organs and corneas).
- Size of the transplant waiting list.
- Number of notifications per million population Number of effective donors per million population.
- Number of organ transplants per million population.
- Number of corneal transplants per million population.
- Number of active Intra-Hospital Donation and Transplant Commissions (CIHDOTTs) and Organ Procurement Organizations (OPOs).
- Family refusal rates.
- Rate of cardiac arrests before organ procurement completion.

All data were collected and validated by the central transplant coordination team. Comparative analyses were performed using descriptive statistics.

IV. RESULTS

Following the implementation of PAT-MA, there was a notable improvement across all evaluated indicators. The family refusal rate decreased from 67% to 63%. The incidence of cardiac arrests before procurement completion declined from 28% to 26%. Notifications per million increased from 172 (24/million) to 327 (46/million). The number of effective donors per million rose from 12 (1.7/million) to 28 (3.9/million), while organ transplants per million grew from 27 (3.9/million) to 62 (8.7/million). Corneal transplants per million increased from 133 (18.6/million) to 255 (35/million). The number of active CIHDOTTs increased from 3 to 10, and active OPOs increased from 0 to 1.

These findings highlight the positive impact of PAT-MA on the organizational capacity and effectiveness of the regional transplant system.

V. DISCUSSION

The implementation of PAT-MA in Maranhão resulted in significant improvements in transplant activity and system indicators. The observed reduction in family refusal rates and cardiac arrests prior to procurement completion suggest enhanced donor management and family engagement processes. Substantial increases in donor and transplant rates per million population demonstrate the effectiveness of targeted quality improvement and organizational strategies. Expansion of active CIHDOTTs and OPOs reflects greater institutional engagement and operational reach.

VI. CONCLUSION

The Transplant Acceleration Program (PAT-MA) significantly improved key performance indicators of the Maranhão Transplant Center. These results reinforce the crucial role of structured management programs and continuous quality improvement strategies in optimizing transplant system outcomes. Further studies are warranted to assess the long-term sustainability of these improvements and their impact on patient outcomes across different regions.

Prevalance of HPV Infection in Women Living with HIV Attending Waibargi Specialist Hospital in Myanmar

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ABSTRACT

Cervical cancer remains a significant public health concern, particularly for women living with HIV (WLWH), due to their increased susceptibility to persistent human papillomavirus (HPV) infections.

This study aims to assess the prevalence of high-risk HPV (hrHPV) infections in WLWH attending Waibargi Specialist Hospital in Myanmar in the year 2024. The cross-sectional analysis involved 234 WLWH, tested with Cobas 4800 system HPV DNA test. Colposcopy directed biopsies were conducted among the participants to detect the presence of cervical intraepithelial neoplasia (CIN) and invasive carcinoma.

Keywords: cervical cancer, CIN, HIV, HPV, screening, WLWH, ART, colposcopy.

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ABSTRACT

Cervical cancer remains a significant public health concern, particularly for women living with HIV (WLWH), due to their increased susceptibility to persistent human papillomavirus (HPV) infections.

This study aims to assess the prevalence of high-risk HPV (hrHPV) infections in WLWH attending Waibargi Specialist Hospital in Myanmar in the year 2024. The cross-sectional analysis involved 234 WLWH, tested with Cobas 4800 system HPV DNA test. Colposcopy directed biopsies were conducted among the participants to detect the presence of cervical intraepithelial neoplasia (CIN) and invasive carcinoma.

The results revealed a high HPV prevalence of 29.9% among the study population, with 70 women tested positive for one or more high-risk HPV types. Among those, single infection with HPV 16 was found in 9 women (3.8%), HPV 18 in 8 (3.4%) while 35 (15%) of them were infected with other high-risk HPV types. Additionally, 18 women (7.7%) had mixed infections involving multiple hrHPV types.

The study also identified that women on ART for more than three years exhibited significantly lower HPV infection rates, as did those with undetectable viral loads, suggesting that effective HIV management can mitigate HPV-related cervical cancer risk.

In this study, 41 women (70.7%) out of 58 HPV-positive cohort diagnosed with CIN I or more severe histological abnormalities after colposcopy directed biopsy. Notably, 86.7% of patients with HPV 16, 18, or mixed infections had positive histology results, compared to 53.6% in those with other hrHPV.

In conclusion, this study highlights the high prevalence of hr HPV infections in WLWH compared to general population (29,9% vs 4 - 11%)in Myanmar as well as the association between specific HPV genotypes and the development of cervical pre cancer and cancer among WLWH. The study also emphasizes the need for integrated cervical cancer screening and vaccination programs within HIV frameworks along with the importance of proper HIV treatment. However, since it is a hospital-based study, further research is required to cover the broader populations, particularly in rural and underserved areas of Myanmar.

Keywords: cervical cancer, CIN, HIV, HPV, screening, WLWH, ART, Colposcopy.

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I. INTRODUCTION

The incidence of cervical cancer has been changed in various parts of the world, after the knowledge of persistent infection with Human Papillomavirus (HPV) is the main cause of this deadly cancer. This noble finding leads to the development of HPV tests as well as various triage strategies to detect premalignant lesions of cervix and various treatment strategies.

Although cervical cancer is a largely preventable disease, it is still one of the leading causes of cancer death of women in developing countries. In Myanmar, according to GLOBOCAN 2022, cervical cancer becomes the second most common cancer among women with estimated age standardized incidence rate of 21.4 per 100,000 female population per year (Ferlay et al,2024). According to that data Myanmar ranked third after Indonesia and Maldives in cervical cancer incidence and mortality. Although Myanmar started to implement cervical cancer prevention and control program in National Health Plan since 2018, there are still some difficulties and limitations to screen and give proper management to those high risk populations.

Since the progression to precancerous lesion of cervix in HPV infected person largely depends on their immune status, women living with HIV (WLWH) have higher risk of HPV infection along with development of high-grade intraepithelial lesions and cervical cancer than the general population. Moreover, the lifespan of WLWH has increased after introduction of Highly Active Anti-Retroviral Therapy (HAART); yet there is a prolonged risk of exposure to HPV putting them at higher risk for cervical cancer.

However, for WLWH, access to the cervical cancer screening and treatment facilities are relatively out of reach compared to the general population around the world. In Myanmar, there are very limited data related to the prevalence of HPV infection as well as cervical cancer screening facilities for women living with HIV.

For low-to middle-income countries (LMICs), the World Health Organization (2014) recommends HPV testing as the preferred choice for cervical cancer screening. By detecting the presence of HPV infection among WLWH, it would prevent rapid progression to cervical cancer in those high risk women.

Therefore, for WLWH, HPV based cervical cancer screening is an essential measure for their health care which should be implemented in Myanmar while collecting the HPV positive and cervical pre cancer rates are the fundamentals beforehand.

This study aims not only to assess the prevalence and subtypes of HPV infection and to evaluate the presence of cervical precancerous lesions among WLWH in Myanmar but also to inform and enhance the national cervical cancer screening programme.

II. AIM AND OBJECTIVES

The study was conducted to determine the prevalence of HPV infection in women living with HIV attending Waibargi Specialist Hospital. This study identified the social and demographic characteristics of study population and also determined the rate of HPV positive cases and precancerous lesion of cervix in those women. Precancerous lesions of the cervix among those HPV positive cases of study population were also identified.

II. STUDY DESIGN AND METHOD

It was a hospital based cross sectional study which carried out in Waibargi Specialist Hospital, Cervical cancer screening clinic, colposcopy clinic and pathology department of Central Women's Hospital, Yangon, Myanmar.

Study population was all eligible women with HIV infection in 25 to 55 years' age group attended the Waibargi Specialist Hospital in the year 2024. Women who were eligible in term of the inclusion and exclusion criteria were recruited and explained about study procedure in details.

Written consent and relevant history were taken from the participants. Altogether 234 eligible WLWH were included in this particular study.

Self-collected samples for HPV genotyping were taken with FOLQSwabs after thorough explanation and demonstration of the collection method. Collected samples were sent to the laboratory and analysed with Cobas 4800 system. HPV positive cases were examined with colposcopy and directed cervical biopsy in colposcopy clinic of Central Women's Hospital, Yangon to detect the presence of cervical precancerous lesion. All colposcopy directed biopsy tissue samples were sent to pathology department, CWH for histological confirmation.

Women who had precancerous lesion were treated mainly with LLETZ surgery while more advance cases were treated with hysterectomy, radical hysterectomy and/or radiotherapy.

III. RESULTS

Table 1: Demographic Characteristics of Study Population (N= 234)

Demographic Characteristics	Frequency	Percent
Age		
21 to 30 years	44	18.8
31 to 40 years	88	37.6
41 to 50 years	92	39.3
51 to 60 years	10	4.3
Education		
Illiterate	10	4.3
Primary	38	16.2
Middle	61	26.1
High	71	30.3
Up to collage/ University	8	3.4
Graduate	46	19.7
Smoking		
No	212	90.6
<5 /days	19	8.1
≥5 /days	3	1.3
Age at sexual exposure		
13 to 19 years	72	30.8
20 to 29 years	126	53.8
30 to 39 years	33	14.1
≥40 years	3	1.3
HPV vaccination		
Yes	4	1.7
No	230	98.3
Total	234	100
History of screening		
Yes	19	8.1
No	215	91.9
Total	234	100

Age distribution of the study population ranged from 25 to 55 years, with a mean (SD) age of 39 (8.09) years and a median age of 39 years. The most common age group was 41 to 50 years, representing 39.3 percent (92/234), followed by 31 to 40 years, which accounted for 37.6 percent (88/234). Only 4.3 percent (10/234) were over 50 years.

The participants had diverse educational backgrounds. Among them, 4.3% (10 out of 234) were illiterate, while 3.4% (8 out of 234) had attended but not completed college or university. Notably, 19.7% (46 out of 234) were university graduates.

Regarding smoking habits, 90.6% (212/234) of the participants were non-smokers. A smaller

portion, 8.1% (19/234), smoked fewer than 5 cigarettes per day, while 1.3% (3/234) smoked 5 or more cigarettes per day.

The age of first sexual exposure among participants ranged from a minimum of 13 years to a maximum of 41 years, with a mean (SD) age of 23.03 (5.55) years. A significant portion, 30.8% (72/234), experienced sexual exposure between the ages of 13 and 19 years. The majority, 53.8% (126/234), were exposed between 20 and 29 years. Those exposed between 30 and 39 years accounted for 14.1% (33/234), while only 1.3% (3/234) were exposed at 40 years or older.

Concerning the HPV vaccination, only 4 participants (1.7 percent) had vaccinated HPV vaccine. Majority were not vaccinated for HPV infection. Only 8.1% (19/234) of study population reported to have cancer screening experience.

Table 2: Distribution Participants Regarding the HIV Infection, Treatment and Cervical Cancer Prevention

	Frequency	Percent
Viral Load		
Detected	24	10.3
Not detected	171	73.0
Not done	39	16.7
Total	234	100
ART		
Yes	*207*	*88.5*
No	*6*	*2.6*
Defaulter	*21*	*9.0*
Total	*234*	*100*
Duration of ART		
<1 year	2	0.9
1 year	43	18.9
2 years	37	16.2
3 years	45	19.7
>4 years	101	44.3
Total	228	100

Regarding the viral load, 24 participants (10.3 percent) still had detectable virus in their blood, while 171 participants (73 percent) had an undetectable viral load. The remaining 39 participants (16.7 percent) had not yet been examined for viral load.

Among the 234 participants, 207 (88.5 percent) were on ART, and 21 (9 percent) were defaulters. The remaining 6 participants (2.6 percent) had not yet started ART. Among the 228 participants on ART, about half were treated with ART for more than 4 years and only 2 participants (0.9 percent) were less than one-year duration.

Table 3: Distribution of HPV Genotype

HPV Genotype	Frequency	Percent
Negative	164	70.1
16 positive	9	3.8
18 positive	8	3.4
other high risk group positive	35	15.0
Mixed infection	18	7.7
Total	234	100.0

Table 4: Findings of Overlapping HPV Genotypes

HPV Genotype	Frequency	Percent
Negative	164	70.1
16 positive	9	3.8
16 positive+18 positive	1	0.4
16 positive+18 positive + other high risk group positive	1	0.4
16 positive + other high risk group positive	9	3.8
18 positive	8	3.4
18positive + other high risk group positive	7	3.0
other high risk group positive	35	15.0
Total	234	100.0

The distribution of HPV genotypes among the 234 women in the study shows that the majority were tested negative for HPV, with 164 women falling into this category. A smaller group of 9 women tested positive for HPV 16, and 8 women tested positive for HPV 18. Additionally, 35 women were found to be positive for other high-risk HPV types, and 18 women had mixed infections involving multiple HPV genotypes.

When looking at mixed infections, one woman had both HPV 16 and 18, while another had a combination of HPV 16, HPV 18, and other high-risk HPV types. Nine women had HPV 16 in conjunction with other high-risk types, and seven women had HPV 18 along with other high-risk types. In this study out of 70 HPV positive cases, 35 women (50%) were infected with HPV subtypes other than 16/18.

Table 5: Distribution of Histology Result after Colposcopy

Histology Result	Frequency	Percent among Total	Percent Among Colposcopy Done
Negative (non-malignant)	17	7.3	29.3
CIN I (LSIL)	16	6.8	27.6
CIN II (HSIL)	14	6.0	24.1
CIN III(HSIL)	6	2.6	10.3
Carcinoma in situ	2	0.9	3.4
Invasive	3	1.3	5.2
Not done	176	75.2	
Total	234	100.0	

Out of 70 women with positive HPV results, 58 underwent colposcopy. Among these, 17 women (7.3%) had negative or non-malignant results. Low-grade lesions (CIN I) were found in 16 women (6.8%), while CIN II and CIN III (HSIL) were detected in 14 women (6.0%) and 6 women (2.6%) of the total population, respectively. A smaller percentage were diagnosed with carcinoma in situ (2 women, or 0.9%) and invasive cancer (3 women, or 1.3%). Among the 58 women who had colposcopies, a notable proportion were diagnosed with HSIL, with 24.1% having CIN II and 10.3% having CIN III.

Table 6: Demographic Characteristics of and HPV Genotype (n=234)

Demographic Characteristics	HPV Negative n (%)	HPV Positive n (%)	OR (95%CI)	P value
Age				
21 to 40 years	90 (68.2)	42 (31.8)	0.81	0.469
41 to 60 years	74 (72.5)	28 (27.5)	(0.46,1.43)	
Education				
Middle school & below	78 (71.6)	31 (28.4)	1.14	0.646
High school & above	86 (68.8)	39 (31.2)	(0.65,2.00)	
Smoking				
Yes	16 (72.7)	6 (27.3)	1.15	0.776
No	148 (69.8)	64 (30.2)	(0.43,3.08)	
Age at sexual exposure				
13 to 19 years	49 (68.1)	23 (31.9)	0.87	0.651
≥20 years	115 (71.0)	47 (29.0)	(0.48,1.59)	

Table (6) presents the demographic characteristics of the study participants in relation to their HPV genotype status (n=234). Among different age groups, 31.8% of women aged 21–40 and 27.5% of those aged 41–60 tested HPV positive, with no significant difference observed (OR 0.81, 95% CI: 0.46–1.43, p=0.469). Educational level, smoking status, and age at sexual debut also showed no statistically significant association with HPV positivity. Overall, none of the demographic variables demonstrated a significant correlation with HPV infection in this study population.

Table 7: Association Between Clinical Characteristics Regarding HIV Infection, Treatment, Screening History and HPV Positive Result

Clinical Characteristics	HPV Negative n (%)	HPV Positive n (%)	OR (95% CI)	P Value
Duration of treatment				
No/ Defaulter	15 (55.6)	12 (44.4)	3.75(1.48,9.52)	0.003
≤3 years	74 (63.8)	42 (36.2)	2.66 (1.38,5.14)	
>3 years	75 (82.4)	16 (17.6)	Ref	
VDRL				
Positive	29 (69.0)	13 (31.0)	0.94	0.871
Negative	135 (70.3)	57 (29.7)	(0.46,1.94)	
Viral load (n=195)				
Not detected	127 (74.3)	44 (25.7)	2.44	0.040
Detected	13 (54.2)	11 (45.8)	(1.02,5.85)	
Previous screening history				
Yes	12 (63.2)	7 (36.8)	0.71	0.491
No	152 (70.7)	63 (29.3)	(0.27,1.89)	

Compared to ART treatment of longer duration (>3 years), treatment of shorter duration was 2.66 times more likely to result in HPV infection, and patients who received no treatment or were defaulters were 3.75 times more likely to have an HPV infection, with a p-value of 0.003. There was no significant association between VDRL test results, history of previous screening, and HPV results.

Out of 234 patients, 195 were examined for viral load. Among the 24 patients with detectable viral load, 11 (45.8%) had a positive HPV result, while among the 171 patients with undetectable viral load, 44 (25.7%) had a positive HPV result. There was a significant association between viral load and HPV results, with a p-value of 0.040. Patients with detectable viral load were 2.44 times more likely to get an HPV infection than those with undetectable viral load.

Association between demographic characteristics of study population and HPV positive result was calculated in this study but there was no significant association between age, education, smoking status and HPV result.

Table 8a: Association between Distribution of HPV Genotype and Histology Result (N=58)

HPV Genotype	Negative	CIN I to Invasive	Fisher's exact test	P Value
16 positive	1 (14.3)	6 (85.7)	7.023	0.058
18 positive	1 (16.7)	5 (83.3)		
other high risk group positive	13 (46.4)	15 (53.6)		
Mixed infection	2 (11.8)	15 (88.2)		
Total	17 (29.3)	41 (70.7)		

Table 8b: Association between Distribution of HPV Genotype in Group and Histology Result (N=58)

HPV Genotype	Negative	CIN I to Invasive	OR (95% CI)	P value
Other High-Risk Group Positive	13 (46.4)	15 (53.6)	5.63 (1.55,20.43)	0.009
16+, 18+, mix Infection	4 (13.3)	26 (86.7)		
Total	17 (29.3)	41 (70.7)		

Among the 30 WLWH with HPV type 16, type 18, or mixed infections, 26 (86.7%) had positive histology results (CIN I and greater). However, among the 28 patients with other high-risk group infections, 15 (53.6%) had positive histology results. Women in HPV type 16, 18, or mixed infections group were 5.63 times more likely to have positive histology results than those with other high-risk group infections. There was a significant association between HPV genotype and histology results among HIV-positive patients, with a p-value of 0.009.

Table 9a: Association between Distribution of Individual HPV Genotype and Histology Result (N=58)

HPV Genotype	Negative/CIN I n (%)	CIN II to invasive n (%)	Fisher's exact test	P value
16 positive	3 (42.9)	4 (57.1)	7.410	0.055
18 positive	2 (33.3)	4 (66.7)		
other high risk group positive	21 (75.0)	7 (25.0)		
Mixed infection	7 (41.2)	10 (58.8)		
Total	33 (56.9)	25 (43.1)		

Table 9b: Association between Distribution of Individual HPV Genotype and Histology Result (n=58)

HPV Genotype	Negative/CIN I n (%)	CIN II to Invasive n (%)	OR (95% CI)	P Value
other high-risk group positive	21 (75.0)	7 (25.0)	4.5 (1.46,13.86)	0.007
16+, 18+, mix infection	12 (40.0)	18 (60.0)		
Total	33 (56.9)	25 (43.1)		

Among the 30 women with HPV type 16, type 18, or mixed infections, 18 (60 %) had CIN II to invasive histology results. However, among the 28 patients with other high-risk group infections, 7 (25.0%) had CIN II and above lesions. Women in HPV type 16, type 18, or mixed infections positive group were 4.5 times more likely to have CIN II to invasive histology results than other high-risk positive group. There was a significant association between HPV genotype and histology results among HIV-positive patients, with a p-value of 0.007.

IV. DISCUSSION

Cervical cancer is the second most prominent gynaecological cancer endangering life of Myanmar women caused primarily by the human papilloma virus which is more prevalent in immunocompromised individuals. Sample collections took place in Waibargi Specialist Hospital, Yangon among eligible women living with HIV aged between 25 to 55 years and altogether 234 of them were recruited for the study. Their sociodemographic characteristics especially risk factors for the development of cervical cancer, HIV infection and treatment related variables were studied as well.

This study, conducted at Waibargi Specialist Hospital in Yangon, involved 234 HIV-positive women aged 25 to 55 years. It examined their sociodemographic characteristics, risk factors for cervical cancer, and aspects related to HIV infection and treatment. Around 40% of participants were in their 40s, and nearly half had completed high school or higher education, which could suggest a relatively informed population regarding health issues. However, despite a low prevalence of smoking and other typical risk factors, one-third of the participants had early sexual debut, a known risk factor for HPV and cervical cancer.

Alarmingly, the uptake of preventive measures was very low: only 8.1% had ever undergone cervical cancer screening and a mere 1.7% had received the HPV vaccine. Vaccination coverage of this study was much lower than that of the Puerto Rico study where 14.7% of WLWH were vaccinated (Soto-Salgado et al, 2025). This finding highlights a significant gap in the implementation of cervical cancer prevention for those high-risk population in HIV care services.

In terms of HIV management, the majority (88.5%) were on antiretroviral therapy (ART), and 73% had undetectable viral loads within the past year, indicating effective treatment adherence. Many had long-term ART experience, with some on treatment for nearly two decades. This reflects a commendable level of HIV care and engagement.

Although the study population demonstrates strong engagement with HIV care, including high ART adherence and viral suppression, the uptake of cervical cancer preventive measures remains relatively low. This suggests a need for further enhancement in the integration of cervical cancer screening within existing HIV clinics with excellent care frameworks. Despite being engaged in regular healthcare for HIV, these women are not being adequately screened or protected against HPV and cervical cancer. The lack of preventive measures among an otherwise health-engaged population suggests that barriers may be more structural (such as lack of service integration like provider education, community outreach, streamlined services and financial

assistance) than personal. These findings indicate that expanding access and awareness of cervical cancer prevention within HIV care settings could be beneficial. Strengthening coordination between HIV clinics and reproductive health services would support a more holistic approach to women's health, particularly in high-risk populations.

As regards to HPV infection among study population, 70 (29.9%) out of 234 HIV positive women tested positive for one or more of high risk HPV infection with Cobas HPV test. HPV 16 was found in total 20(8.4%) women while 9 of them had mixed infection with other hrHPV, one woman was 16 and 18 positives and another one was tested positive for 16, 18 and other hrHPV infection. HPV 18 was found in 16 (6.8%) cases including 8 mixed infections. The rest of the positive women i.e. 35 (15%) cases had other high risk HPV infections. Mixed infection was found in 18 women (7.7%) out of 234 total study participants.

According to hospital records, the HPV positivity rates among women attending the Cervical Cancer Screening Clinic at Yangon Central Women's Hospital were 10.15% in 2017, 11.02% in 2018, and 11.32% in 2019 (YCWH statistics,2017,2018,2019). In comparison, meta-analyses have estimated the prevalence of HPV infection among the general female population aged 18–65 years in community-based settings in Myanmar to be approximately 4% (95%CI: 3–5%) (Win et al, 2025). The HPV positivity rate among women living with HIV in the present study appears to be substantially higher than that of the general population in Myanmar.

Since women living with HIV (WLWH) have a substantially higher risk of acquiring HPV infection compared to the general adult female population (Clifford et al., 2005; Grulich et al., 2007; Bratcher and Sahasrabuddhe, 2010), the prevalence of HPV infection in WLWH is approximately seven times greater than in HIV-negative women (Womack et al., 2000). So also in this study, HPV positive rate was nearly 30% which was three times higher than that of hospital statistics (29.9% vs round about 11%) in Yangon Central Women's Hospital while it was seven fold greater than general population (29.9% vs 4%).

In the SHADE study conducted in Denmark in 2011, high risk HPV infection was positive in 26.4% out of 334 WLWH, in contrary to women in general population where only 16.6% were HPV positive cases. That study also revealed higher number of multiple or mixed infection compared to general population (38.55% versus 25.7%) with HPV 58 (7.1%) being the highest followed by 52 (5.4 %), and 16 (4.8 %) in WLWH (Thorsteinsson et al, 2016).

Therefore, HPV positive rate of WLWH attending Waibargi Specialist Hospital seems not much higher than the Denmark study (29.9% versus 26.4%). However, the study population did not represent all HIV positive women throughout the whole country since this present study delineate more of the urban population though health care facilities for WLWH might be limited in hard to reach or border areas where high risk behaviour were more common.

In an Egyptian study published in 2024, the overall prevalence of HPV infection was 13.5% for all women whereas 24.4% among WLWH denoting those women are 6 times at greater risk of developing cervical cancer. Moreover, hrHPVs other than genotype 16 and 18 were isolated from 71% of infected women (Ashry et al, 2024). Another analytical cross-sectional study from Ghana revealed as the prevalence of hr-HPV among WLWH was 44.4% and 46.8% of positive women found to have multiple HPV genotypes (Agyare Gyane et al,2024).

In the current study, all the HPV positive cases were arranged to do colposcopy examination in the Cervical Cancer Screening Clinic of Yangon Central Women's Hospital. Unfortunately, 12 women failed to show up for colposcopic examination for various reasons. Therefore, those women were arranged to visit their corresponding township hospitals to undertake visual assessment tests with VIA and/or VILI tests and treated according to national guideline (MRH,

2018). After colposcopy and directed biopsy of 58 HPV positive women, CIN I was diagnosed in 16 women (6.8%), CIN II in 14 (6.0%), CIN III in 6 (2.6%), in situ carcinoma in 2 (0.9%) and invasive carcinoma in 3 (1.3%) of the total study population.

In this study, association between HPV positive results and sociodemographic characteristics of the participants, clinical characteristics including marital status and number of pregnancies as well as HIV infection and treatment conditions were analysed. There was no statistically significant association between sociodemographic factors and the HPV infection in WLWH attending Waibargi Hospital. Age of first sexual exposure, marital status, number of marriages, number of pregnancies, previous screening histories and presence of other sexually transmitted infections were also not associated to the presence of HPV infection.

Duration of anti-retroviral therapy and HIV viral load were the significant associated factors for HPV infection in WLWH. HIV positive women who have been taking ART for more than 3 years has significantly lower HPV infection than those with ART of less than 3 years. Similarly, HIV viral load has significant association for HPV infection showing significantly lower HPV positive rate in women with undetectable HIV viral load i.e. 25.7% vs 45.8%, OR 2.44 (1.02-5.8) with p value 0.040. This means patients with detectable viral load were 2.44 times more likely to get an HPV infection than those with undetectable viral load.

In this current study, not only the CIN II+ disease but also all the CIN I cases were regarded as positive lesions for treatment since those women have had HIV infection which may lead to a rapid progression of the disease. Moreover, many of the participants seems not affordable or possible for further repeated follow up visits to YCWH. Therefore, all the HPV positive cases and CIN I and higher cases were offered screen, triage and treat option as well as screen and treat option to reduce the burden of repeated follow up visits according to the WHO guideline (WHO, 2021).

Association between the different groups of positive HPV genotypes and positive histology results were also analysed in this study. There was no significant association between the presence of histologically confirmed premalignant cervical lesions, considering both CIN I+ or CIN II+ and specific HPV genotypes individually. However, in women with hrHPV 16, 18 and mixed infection group, CIN I+ lesions were found in 85%, 83.3 % and 88.2% respectively whereas 53,6% of other high risk group had CIN I+ cases (p-0.058). On account of higher grade pre cancer for different genotype groups, CIN II+ lesions were reported in 57.1%, 66.7%, 25.0% and 58.8% for HPV 16, 18, other high risk and mixed infection groups respectively (p- 0.055)..

The results indicated that a relatively higher proportion of histologically confirmed abnormal lesions occurred in women who were positive for HPV 16 or 18 as well as a statistically significant difference was observed when comparing the prevalence of both CIN I+ and CIN II+ lesions between those with other high-risk HPV types and those with HPV 16, 18, or mixed infections. Further analysis of CIN grade distribution revealed that 43.1% of HPV-positive women had CIN II or more severe lesions. Specifically, 60% of women with HPV 16, 18, or mixed infections presented with CIN II to invasive carcinoma, compared to 25% of those with other high-risk HPV types.

Although these results still denote the risk of premalignant lesion and malignant lesion are higher with HPV 16 or 18 infection, the possibility of disease in other high risk group is not negligible. Among 70 HPV positive women, 35 (50%) of them found to be infected with other hrHPV without 16 nor 18. CIN I+ lesions were reported in 15 women (36.58%) from other hrHPV positive group out of total 41 CIN I and above lesions as well as 9 women (33.33%) out of 27 CIN II and above cases were from other high risk group. Two cases of carcinoma insitu were diagnosed from those other hrHPV positive group too. These findings high light the changing trend of HPV infection and occurrence of premalignant lesion of cervix in recent years.

HPV types 16 and 18 are well established as the primary causes of cervical cancer, responsible for approximately two third of cases, while nearly all cervical cancers (around 99%) are associated with HPV infection (Bruni et al., 2010). In this study, there was no statistically significant difference indicating that precancerous lesions were more likely to be associated with isolated HPV 16 or 18 infections (table 6a and 7a), although the proportion of CIN I and above lesions was lower in the group with other high-risk HPV types (53.6%) while it was over 80% in HPV 16, 18, or mixed infections group.

In Southeast Asia, including Myanmar, approximately 3% of women in the general population are estimated to carry cervical HPV types 16 or 18 at any given time, and these two types are responsible for around 70.4% of invasive cervical cancer cases (Bruni et al, 2023). Conversely, in this study WLWH have much higher HPV positive rate along with different trend of HPV genotype other than 16/18 which prompt to consider separate screening and treatment plans for precancerous lesions as well as vaccination programs for WLWH.

In Myanmar, according to HIV/AIDS data hub for the Asia- Pacific region, in 2023 about 280,000 people were estimated to have HIV infection while 216,757 (77%) of them were under ART coverage. There might have been many more undiagnosed cases in the vulnerable groups who are out of access to the health care facilities. HPV infection and presence of precancerous lesions may be much higher in those women without antiretroviral treatment because of the findings of the study indicates that duration of ART and the undetectable viral loads are the significant protective factors for HPV infection.

Women living with HIV have an increased likelihood of acquiring high-risk HPV 16 and 18, as well as multiple HPV infections compared to HIV-negative women (Sun et al., 1997). Mixed infections were found in 18 women (7.7%) in this current research and one woman had had multiple infection i.e.16, 18 along with other hr HPV and she was diagnosed as CIN III disease.

In 2022, Seyoum et al carried out a meta-analysis for prevalence and genotype distribution of high-risk human papillomavirus infection among sub-Saharan African women. Their study revealed that the pooled distribution of HPV 16, 52, 18, 56, and 58 genotypes have been slightly different in different regions. In South Africa, HPV 58 was the most commonly detected type, followed by HPV 52, 45, 16, and 18. Similarly, in Mozambique HPV 52 is the most common genotype, followed by HPV 35 and 16. Furthermore, Mayaud et al (2003) also stated that in women living with HIV, HPV58 has been proven to be the second leading cause of cervical cancer after HPV 16.

In this study, among the 70 HPV-positive cases, 35 (50%) were infected with high-risk HPV types other than genotypes 16 and 18. Therefore, for women living with HIV, prompt interventions including referral for colposcopy and timely treatment should be provided even when non-16/18 high-risk HPV types are detected. Additionally, these findings should be reflected in vaccination strategies for this vulnerable population, including consideration of the use of the vaccines which could cover more HPV subtypes.

This study for HPV genotyping was performed with Cobas 4800 system, which categorizes results into HPV 16, HPV 18, and other high-risk groups. Future research should focus on tests capable of identifying individual high-risk HPV genotypes. As this was a hospital-based study, similar studies are warranted in community settings, particularly among high-risk groups of women like WLWH, to better understand the prevalence of HPV infection. Additionally, this study adopted self-collection technique to get the vaginal samples, demonstrating satisfactory performance and supporting the potential for broader use of this method to enhance screening coverage in community-based programs.

V. CONCLUSION

In Myanmar, given the significant number of HIV positive women, there is also high burden of cervical cancer. So, it is important to identify HPV infection early through targeted screening among

HIV positive women. In Myanmar, data on this topic is limited; however, figures collected at Waibargi Hospital show a much higher HPV positivity rate of 29.9%, compared to 4–11% in the general population.

Among HPV positive women, about half of them had precancerous lesions. Moreover, high risk HPVs other than 16 and 18 account for 50% of positive cases and also caused a noticeable percent of high grade lesions. However, since this was a hospital based cross sectional study, it does not indicate the information for the general population and thus further studies should be done especially for high risk groups in the society. These findings underscore the urgency of integrating HPV-based screening into existing HIV care programs and revising the preventive measures to consider high- risk HPV types beyond 16 and 18.

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