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(RESEARCH ARTICLE)

In Silico Rational Design and Immunoinformatic Analysis of a Multi-Epitope Peptide Vaccine Targeting Neuroinflammatory Pathways in Migraine

Akhil Kumar Pigili [1,*], Chandra Kumar Kanemela [1], Srinivas Guntamadugu [1], Anil Kumar Babu katika [2] and kommu Yashwanth [1]

[1] *Department of Bioinformatics, Sri Venkateswara Institute of Medical Sciences, Tirupathi, India.*
[2] *Department of Biotechnology, Central Tribal University of Andhra Pradesh, Vizianagaram, India.*

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Abstract

Migraine is a major neurovascular disorder affecting millions of people globally with the current therapies which are not limited to the long-term prevention. The chronic Neuroinflammation which is a symptom of migraine. This involves pathways which are linked by the CGRP, PACAP, and TRPV which are linked to the Neuroinflammation and pain signalling. Our study aims the development of the multi epitope based peptide vaccine candidates which are protein driven primarily targeting the CGRP as the primary target. As cgrp is the key target protein in the migraine management. In this study we have targeted the key target proteins and analysed their physiochemical properties and epitopes were screened and screened epitopes were identified with the toxicity, allergenicity, antigenicity, followed by the vaccine constructs using the linkers and adjuvants. The tertiary structures were identified and the molecular docking was performed with the tlr3, tlr4, and tlr6. The final vaccine construct which was screened exhibited the high immunogenicity, stability, and non-allergenicity is observed.

Keywords: Migraine; CGRP; MEBP Vaccine; Neuroinflammation; Computational Vaccinology

1. Introduction

Migraine is an increasingly prevalent neurological condition that appears episodically and has a complex underlying pathogenesis. Recurrent, severe headaches, usually unilateral and throbbing, are accompanied by symptoms such as nausea, phonophobia, and photophobia. Transient neurological symptoms, generally visual but occasionally involving other senses and speech, precede these headaches in roughly one-third of people. It is widely accepted that migraine headaches are caused by stimulation and intensification of the trigeminovascular sensory pathway, with cortical spreading depression (CSD) providing as the neurophysiological complement of the aura. CSD is created in animals by focused stimulus of the brain's cerebral cortex and involves a silent wave of depolarization in synapses and glial cells, albeit the processes underlying its initiation and propagation are unknown.

Migraine is a fundamentally diverse condition with an estimate of heritability as high as 50%, most likely caused by a polygenic, multivariate inheritance pattern. However, the specific brain dysfunctions that cause chronic excitation of the trigeminovascular pain pathway are not well understood and are being debated. Given the disorder's significant genetic and clinical variability, it is believed that numerous processes contribute to migraine onset.

Our research is centred on designing multi-epitope-based peptide (MEBP) vaccine candidates that target the calcitonin gene-related peptide (CGRP), a critical molecule involved in the pathophysiology of migraine. In this study, we identified key target proteins and assessed their physicochemical properties.

* Corresponding author: Akhil Kumar Pigili.

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Subsequently, B-cell and T-cell epitopes were screened based on their antigenicity, allergenicity, and toxicity profiles. Vaccine constructs were formulated using appropriate linkers and adjuvants to enhance immunogenicity. The tertiary structure of the final construct was modelled, and molecular docking analyses were conducted with Toll-like receptors TLR3, TLR4, and TLR6. The optimized vaccine candidate demonstrated strong immunogenic potential, structural stability, and was predicted to be non-allergenic.

Recent advances have highlighted the involvement of specific molecular and neurovascular pathways in the onset and progression of migraine, offering new opportunities for targeted therapeutic development. Among emerging strategies, vaccine-based approaches—particularly multi-epitope-based peptide (MEBP) vaccines—have shown considerable promise due to their ability to induce highly specific and long-lasting immune responses. Engineered through computational Immnoinformatic platforms, MEBP vaccines offer several advantages, including improved safety profiles, structural stability, and feasibility for large-scale production. Although MEBP vaccines have been widely studied in the context of various infectious and non-infectious diseases, their potential application in migraine management remains underexplored. In the present study, we aimed to design a novel in silico MEBP vaccine targeting migraine-associated molecular components. Through a comprehensive Immnoinformatic pipeline, we identified potential B-cell and T-cell epitopes, constructed an optimized vaccine candidate, and assessed its immunogenicity, allergenicity, and structural stability using a suite of computational tools.

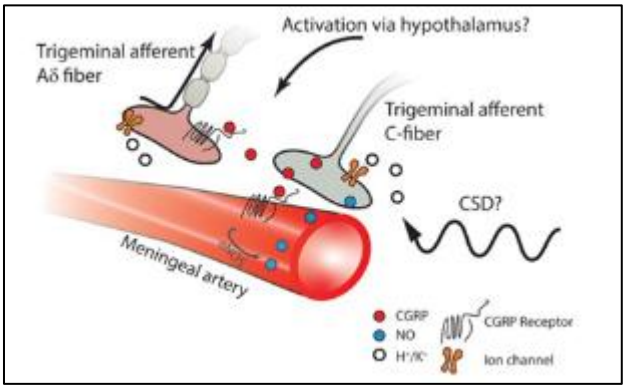

Figure 1 Central events such as hypothalamic oscillations or cortical hyper excitability, as well as peripheral phenomena like cortical spreading depression (CSD), can activate the trigeminovascular system. This leads to the release of calcitonin gene-related peptide (CGRP) from trigeminal C-fibers. CSD also triggers the release of K^+, H^+, and pro-inflammatory mediators, further promoting CGRP secretion. CGRP sensitizes Aδ fibers and induces vasodilation via endothelial nitric oxide synthase (eNOS) and nitric oxide (NO), establishing a positive feedback loop that amplifies migraine pathology

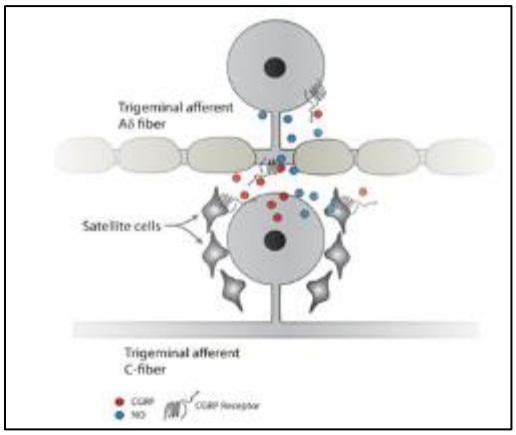

Figure 2 CGRP released by trigeminal C-fibre neurons within the trigeminal ganglion (TG) can diffuse to surrounding satellite cells, stimulating nitric oxide (NO) release. NO may then act on the originating neuron or neighbouring non-CGRP neurons, enhancing their excitability. Additionally, CGRP can directly sensitize adjacent Aδ sensory neurons expressing CGRP receptors (CGRP-R), contributing to migraine pathophysiology

2. Materials and Methods

2.1. Study Design & Target Selection

The study began by identifying the primary target proteins involved in the pathways of migraine pain. Them it was continued by retrieval of CGRP sequence from uniprot and studying its physiochemical properties and analysed the pathways in which the CGRP was involved in Migraine pain management. Further the sequence was screened for B-Cell and T-Cell epitopes then the several epitopes were identified with the antigenicity, allergenicity and other properties. The MEBP vaccine candidates were constructed using the linkers and adjuvants. The constructed vaccine candidate was developed for which molecular docking was performed with the TLR 3, 4, 6 and the Molecular Dynamics simulations were performed.

2.2. Sequence Retrieval

The protein sequence of Human CGRP Protein was retrieved from uniprot with Accession No. **P06881** named **CALCA_HUMAN Calcitonin gene-related peptid**e the selected retrieved sequence was examined with the antigenicity, allergenicity and other physiochemical properties. The retrieved sequence was elected for vaccine construction and the sequence was elucidated with the pathways involved and also in the all other disease mechanisms.

2.3. Prediction of Physiochemical properties

The physiochemical properties and computation of various physical and chemical parameters of the target Protein was screened with the PROTPARAM tool *(Gasteiger et al. 2005).*

2.4. Prediction of Linear B-cell epitopes

In the human immune system B-cells plays a key role in the long lasting immune response against the antigens. The IEDB B-cell prediction too was used to predict the linear B-cell epitopes *(Jespersen MC et al. 2017).*

2.5. Prediction of T-Cell (Cytotoxic) epitopes

The T-cell epitopes which was important in enhancing immune response. Epitopes were screened based on the frequent interactions with HLA alleles HLA-A02:01, HLA-A01:01, HLA-B07:02 and HLA-C07:01. The T-cell epitopes (MHC Class-I) were identified with the IEDB MHC Class-I prediction tool *(Andreatta M et al. 2018).*

2.6. Prediction of T-Cell (Helper) epitopes

The HLA allele sets DRB 101:01, DRB 103:01 and DRB104:01 were used as reference to predict the epitopes. The IEDB MHC-Class II binding prediction tool was used to predict the T-Helper cell epitopes. This tool was used to detect 14 mers of peptides *(Bui HH et al. 2019).*

2.7. IFN-γ-peptide prediction

IFN-γ-peptide prediction was conducted using the IL-Pred tool developed by IIIT-Delhi for the epitopes of B-cell and T-cell. *(Saha S et al. 2007)*

2.8. Toxicity, allergenicity and antigenicity profiling for screened epitopes

The toxicity, allergenicty and antigenicity was screened by the Toxinpred, Allertop and Vaxijen respectively and they are screened based on their HLA allele (Gupta et. al. 2021), *(Dimitrov et. al.)*

2.9. Population coverage analysis for selected epitopes

To make sure the vaccine works well for people around the world, it needs to trigger a strong immune response in a wide range of populations. To check this, the IEDB population coverage tool was used to see how well the selected T-helper and cytotoxic T-cell epitopes would work across different groups. Nine epitopes were tested, using both Class I and Class II options in the analysis. The chosen epitopes and their related MHC alleles were entered into the tool and submitted for the results. *(Bui H. H et. al.)*

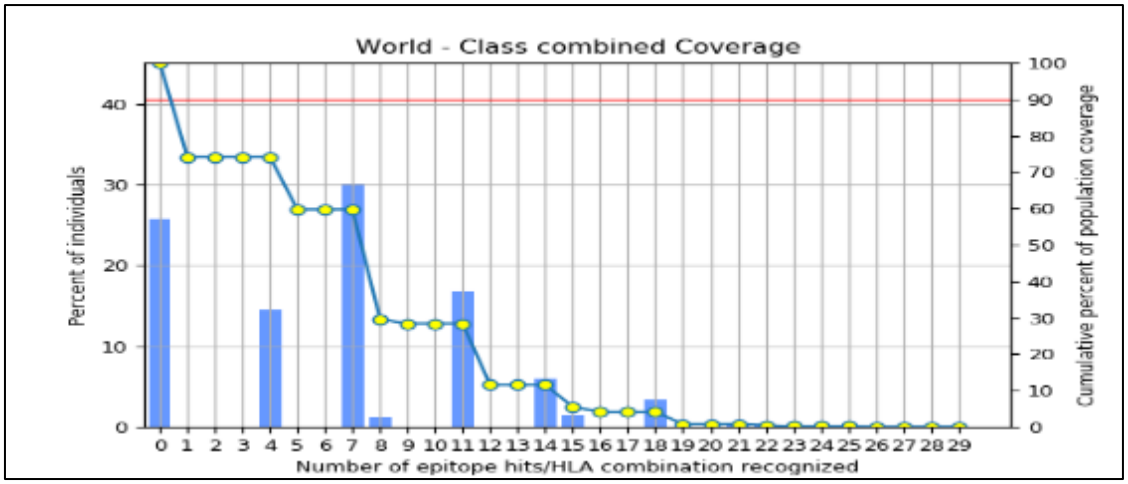

Figure 3 Population Coverage of Selected MHC-I and MHC-II Epitopes

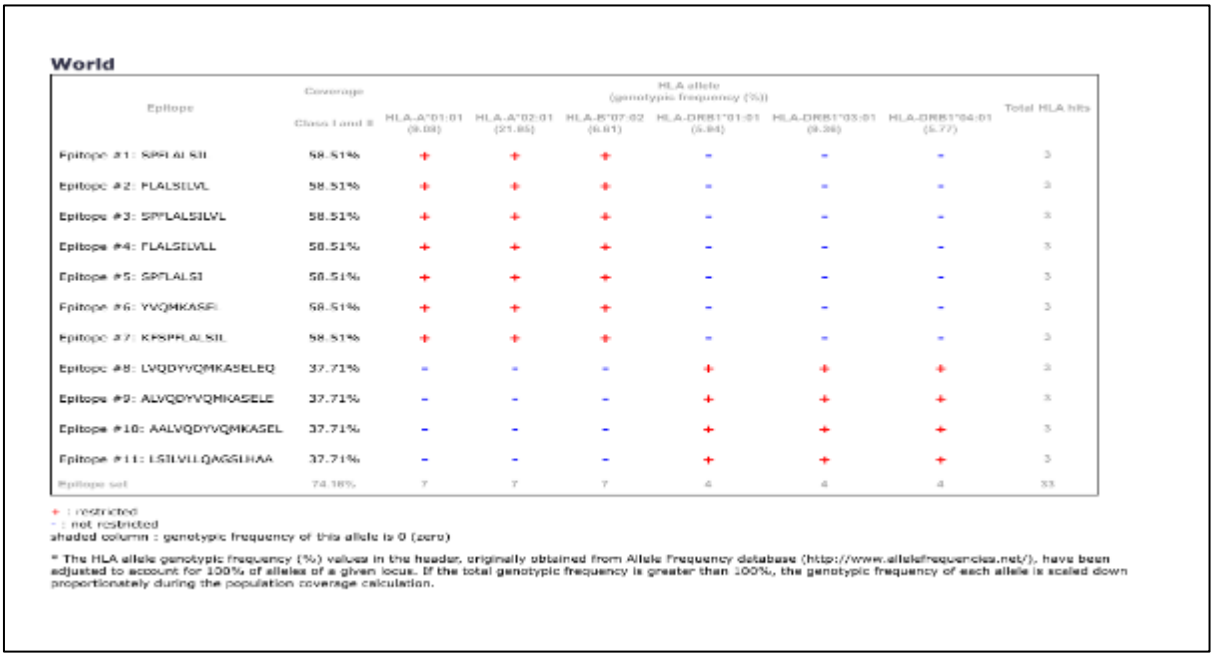

Figure 4 Interaction of epitopes with the selected HLA alleles.

2.10. Development of vaccine construct

To create the MEBP vaccine, B-cell and T-helper cell epitopes were linked using a GPGPG linker, while cytotoxic T-cell epitopes were connected with an AAY linker. To boost the immune response, β-defensin was added as an adjuvant at the N-terminal end, joined using an EAAAK linker.

2.11. Analysis of vaccine physiochemical properties

The physicochemical properties of the vaccine construct are key indicators of its antigenicity and stability. In our study, we analysed properties such as amino acid composition, GRAVY index, theoretical pI, and instability index. These analyses were performed using the ProtParam online tool *(Wilkins et al., 1999).*

2.12. Profiling of Vaccines antigenicity and allergenicity

Toxicity, allergenicity, and antigenicity of the vaccine construct was evaluated using ToxinPred, AllerTOP, and VaxiJen, respectively. The screening was performed based on their HLA alleles, following the methods described by (*Gupta et al. 2021) and (Dimitrov et al.)*

2.13. Prediction of secondary structure of vaccine construct

The secondary structure of the vaccine construct was predicted using the GOR IV online server which is based on the DPM algorithm. This algorithm predicts secondary structures using parameters defined by Chou and Fasman. *(Garnier et. al 1996).*

2.14. 3D structure prediction

The 3D structure of the vaccine construct is crucial for understanding its stability and free energy, and it also aids in studying interactions with target molecules. The structure was predicted using the Phyre2 online server and further refined using PYMOL. *(Powell HR et. al)*

2.15. 3D structure refinement and its validation

To validate the refined 3D structure of the vaccine construct, the ProSA web tool was used. ProSA calculates the overall quality score for the input protein structure. *(Wiederstein and Sippl, 2007)*

2.16. Immune response profiling of the vaccine construct

The immune response to the vaccine construct was assessed using the C-immSim immune stimulator. This tool utilizes the PSSM algorithm to predict immunogenic epitopes and how they interact with the immune system. A simulation was conducted with default settings to assess the antibodies generated. *(Castiglione et al., 2021)*

3. Results

3.1. Retrieval of the Protein Sequence

The CGRP protein sequence was analysed to identify potential B-cell and T-cell epitopes. Antigenicity prediction was performed using VaxiJen v2.0 (threshold: 0.4, virus model), which assigned the protein a score of 0.5793, indicating probable antigenicity. Furthermore, AllerTOP v2.0 classified the protein as non-allergenic, and ToxinPred analysis confirmed its non-toxic nature.

3.2. Prediction of B-cell epitopes and T-cell epitopes (MHC Class-I and MHC Class-II epitopes)

The identified epitopes underwent rigorous screening for antigenicity and allergenicity. Following these criteria, three B-cell epitopes were chosen due to their high antigenic scores, while three MHC-II (Helper T-cell) and three MHC-I (Cytotoxic T-cell) epitopes were selected based on their strong binding affinity (low IC50 scores). The antigenic potential of each epitope is detailed in Table 1.Since IFN-γ plays a vital role in suppressing viral replication, IL6-Pred analysis confirmed that the selected epitopes can effectively induce IFN-γ production, making them suitable candidates for vaccine development

Table 1 Epitopes screened for the vaccine construct

Sl. No.	Types of Immune Cells	Epitopes	Antigenicity
1	B-Cell	APFRSALESSPADPATLSED SGGVVKNNFVPTNVGSKAFGRRRRD	0.3737 1.0034
2	T-Cell (MHC-I)	SPFLALSIL FLALSILVL SPFLALSILVL FLALSILVLL SPFLALSI YVQMKASEL KFSPFLALSIL	0.8480 1.0225 0.7757 0.8306 1.0062 1.1452 1.1752
3	T-Cell (MHC-II)	LVQDYVQMKASELEQ ALVQDYVQMKASELE	0.4487 0.6132

		AALVQDYVQMKASEL LSILVLLQAGSLHAA	0.5756 0.6809

3.3. Characterization of the Vaccine construct

3.3.1. Formulation of MEV construct against Migraine

To enhance immune stimulation, β-defensin was incorporated as an adjuvant at the C-terminal end. The adjuvant was fused to the B-cell epitopes using an EAAAK linker for stable conjugation. Additionally, GPGPG linkers were strategically employed to connect Epitopes. This modular design ensures optimal spacing and structural flexibility for effective antigen presentation and immune recognition. The various linkers used in the vaccine construct are shown in Table 2

Table 2 Various linkers used in the vaccine construct *(Kumar KMet.al 2021)*

S.no	Linkers
1	Adjuvant (Beta-defensin 4A)
2	EAAAK (adjuvant to B-cell epitopes)
3	GPGPG (B-cell epitopes, B-cell epitopes to MHC-II binding epitopes, MHC-II binding epitopes, and MHC-II binding epitopes to MHC-I binding epitopes)
4	AAY (MHC-I binding epitopes)

3.3.2. Antigenicity and allergencity

The immunogenic safety and efficacy of the designed vaccine were validated through comprehensive evaluations. The final vaccine construct demonstrated non-allergenic properties and strong antigenic potential, achieving an antigenicity score of 0.7632 (exceeding the threshold of 0.4) when analysed using the virus-based prediction model in VaxiJen. These results confirm its suitability for further immunological studies.

3.3.3. Population coverage

A total of 74.16% of the world population exhibited an immune response to the vaccine construct, according to the IEDB population coverage online tool. The population coverage was calculated based on the interaction of selected epitopes with the corresponding HLA alleles. This affirmed that the vaccine design was operational for most populations worldwide.

Table 3 Population coverage

population/area	Class combined		
	coverage	average_hit	pc90
World	74.16%	6.47	1.55
Average	74.16	6.47	1.55

3.3.4. Secondary structure prediction

The secondary structure analysis of the vaccine construct performed using GOR IV revealed a predominant alpha-helical conformation (52.59%), accompanied by random coil (31.90%) and extended strand (15.52%) structures, Figure 5 provides a graphical representation of the probability distribution for these secondary structural elements along the protein sequence, demonstrating their potential contribution to the overall stability and functionality of the vaccine construct.

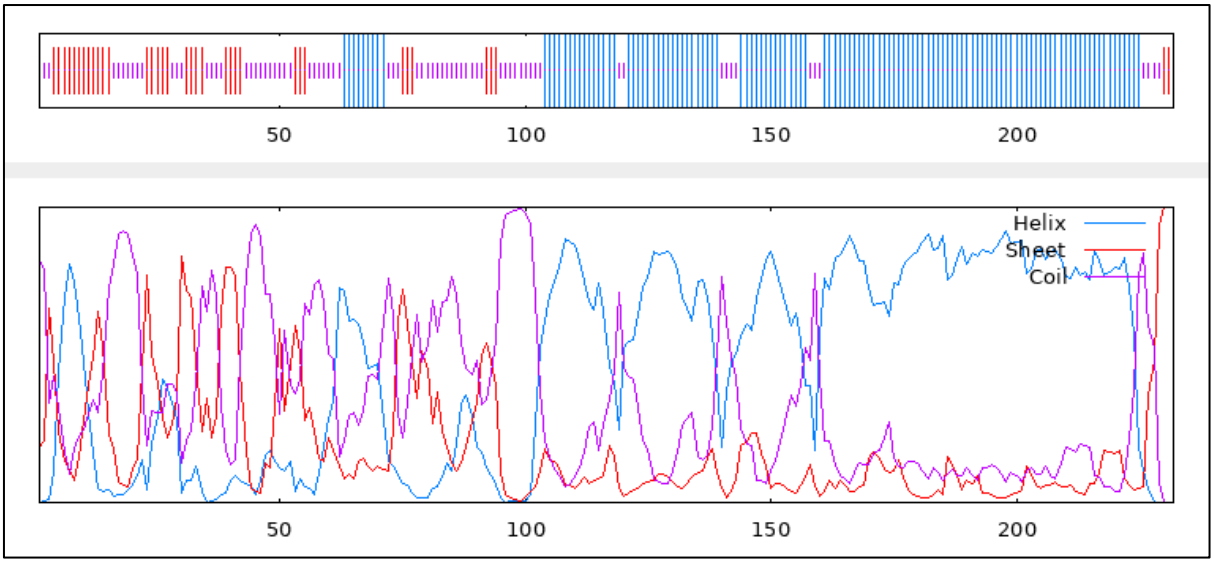

Figure 5 Graphical representation of the probability distribution for these secondary structural elements along the protein sequence

3.4. 3D structure prediction and Validation

The three-dimensional structure of the vaccine construct was modelled using Phyre2, which generated contact-based distance matrices with values ≤8Å. The analysis revealed detailed structural properties, including the distribution of helices, coils, beta-turns, bridges, bends, as well as ordered/disordered regions and solvent accessibility. Further refinement was performed

Using PyMOL to optimize the structural conformation. The final refined 3D model, visualized in PyMOL, is presented in Figure 6, demonstrating the well-defined architecture of the vaccine construct.

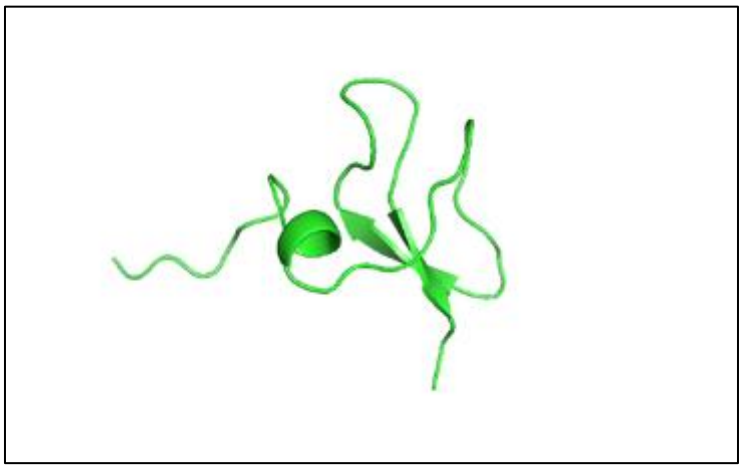

Figure 6 3D structure of the vaccine construct formed by Phyre 2

3.4.1. 3D Structure Validation

The 3D structural validation was performed using ProSa, which confirmed the model's reliability. The overall quality of the structure fell within the NMR-determined range, suggesting a biologically plausible conformation. For local quality assessment, a 40-residue sliding window was applied, revealing that most residues exhibited negative energy values—a hallmark of a stable, well-folded structure.

The Z-score of the refined model was −4.75, aligning closely with values typical for native proteins of comparable size (see Figure 7a). Additionally, a free energy profile was generated, mapping energetic contributions across the amino

acid sequence (Figure 7b). In this visualization, high-energy regions (indicating potential instability) are highlighted in red, while low-energy regions (reflecting structural stability) are marked in blue (Figure 8).

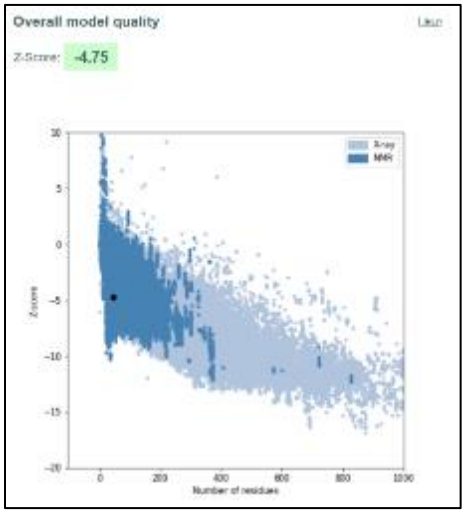

Figure 7a Z-score plot of the refined structure representing the quality of the vaccine construct in the NMR region.

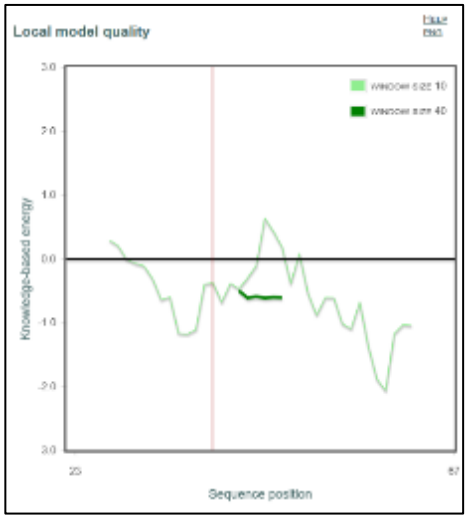

Figure 7b Local model quality representing the energies as a function of amino acid sequence position.

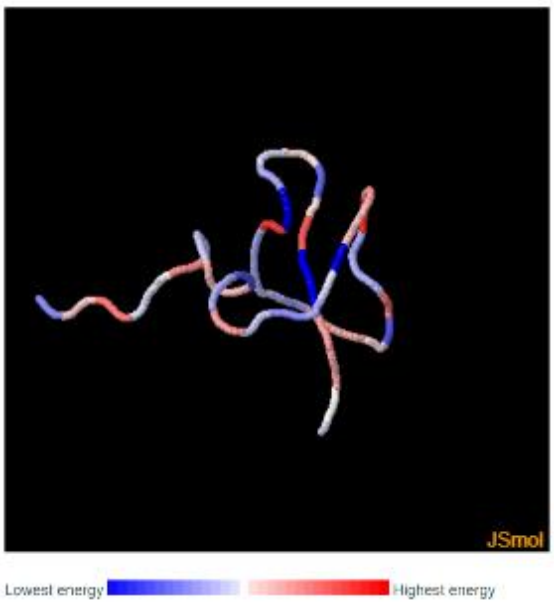

Figure 8 3D structure of the vaccine construct with energy representation.

3.4.2. Molecular docking of the vaccine construct with TLR receptors

The three-dimensional structures of TLR3 and TLR9 were computationally modelled using Phyre2, with subsequent secondary structure analysis conducted via PDBsum. The predicted models demonstrated stable conformations and favourable structural properties, making them suitable candidates for molecular docking studies. These TLR models were specifically selected based on their high-ranking scores and low energy values, indicating optimal structural reliability. To investigate potential immune interactions, docking simulations between the vaccine construct and TLR3/TLR9 were performed using the HADDOCK 2.2 server. Post-docking, the TLR-vaccine complexes underwent secondary structure evaluation through PDBsum to validate the stability and binding compatibility of the docked conformations.

Table 4 Haddock scores of TLR 4

Cluster No	HADDOCK Score	Z-Score
1	116.8 +/- 26.9	-2.1
2	193.6 +/- 7.5	1.1
3	159.3 +/- 4.9	-0.3
4	165.6 +/- 14.3	-0.0
5	139.5 +/- 5.7	-1.1
6	194.6 +/- 4.8	1.2
7	178.4 +/- 14.3	0.5
8	162.3 +/- 23.3	-0.2
9	196.3 +/- 9.3	1.2

3.5. Immune Simulation

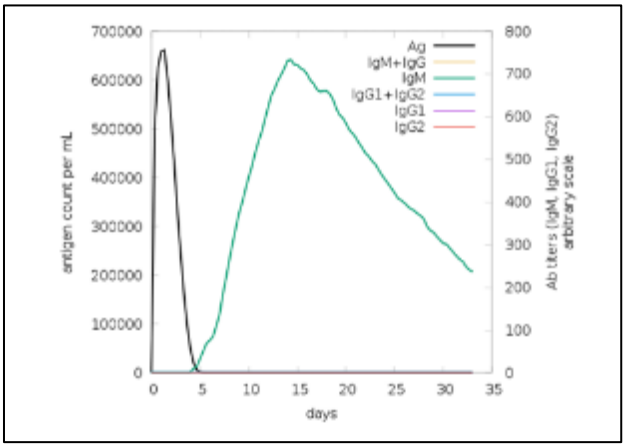

Figure 9 The virus, the immunoglobulins and the immunocomplexes *(s.succi.et.al 1997)*

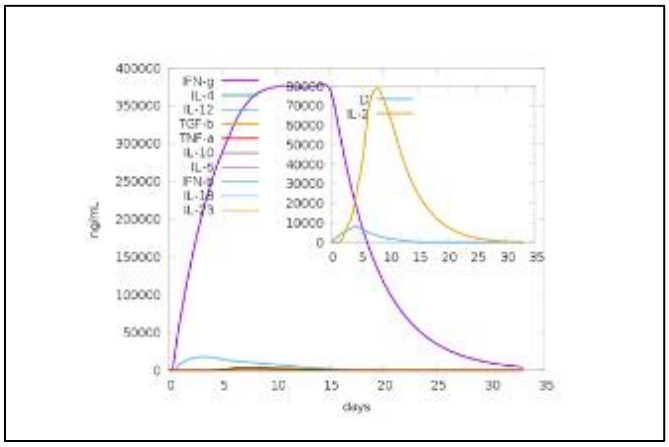

Figure 10 Concentration of cytokines and interleukins. Inset plot shows danger signal together with leukocyte growth factor IL-2. *(s.succi.et.al 1997)*

The immune response elicited by the vaccine candidate was computationally modelled using C-immSim with a single antigen dose administered for HLA alleles. The simulation tracked the dynamic immune interactions over 35 days, generating comprehensive profiles of both humoral and cellular responses. Analysis of the humoral response revealed

antigen clearance kinetics and antibody production, with detailed quantification of B-cell populations including memory B-cells and plasma B-cells secreting IgM, IgG1, and IgG2 isotypes. The simulation further characterized B-cell states (active, presenting, duplicating, and anergic) and their antigen processing dynamics. Cellular immunity assessment demonstrated robust activation of CD4+ T-helper cells, CD8+ cytotoxic T-cells, and regulatory T-cells, along with their respective memory populations and state transitions. Innate immune components including natural killer cells, dendritic cells, macrophages, and epithelial cells were monitored for antigen presentation efficiency. The cytokine and interleukin profiles provided additional insights into the immune signalling cascade. These computational results collectively predicted a strong and balanced immune response to the vaccine construct.

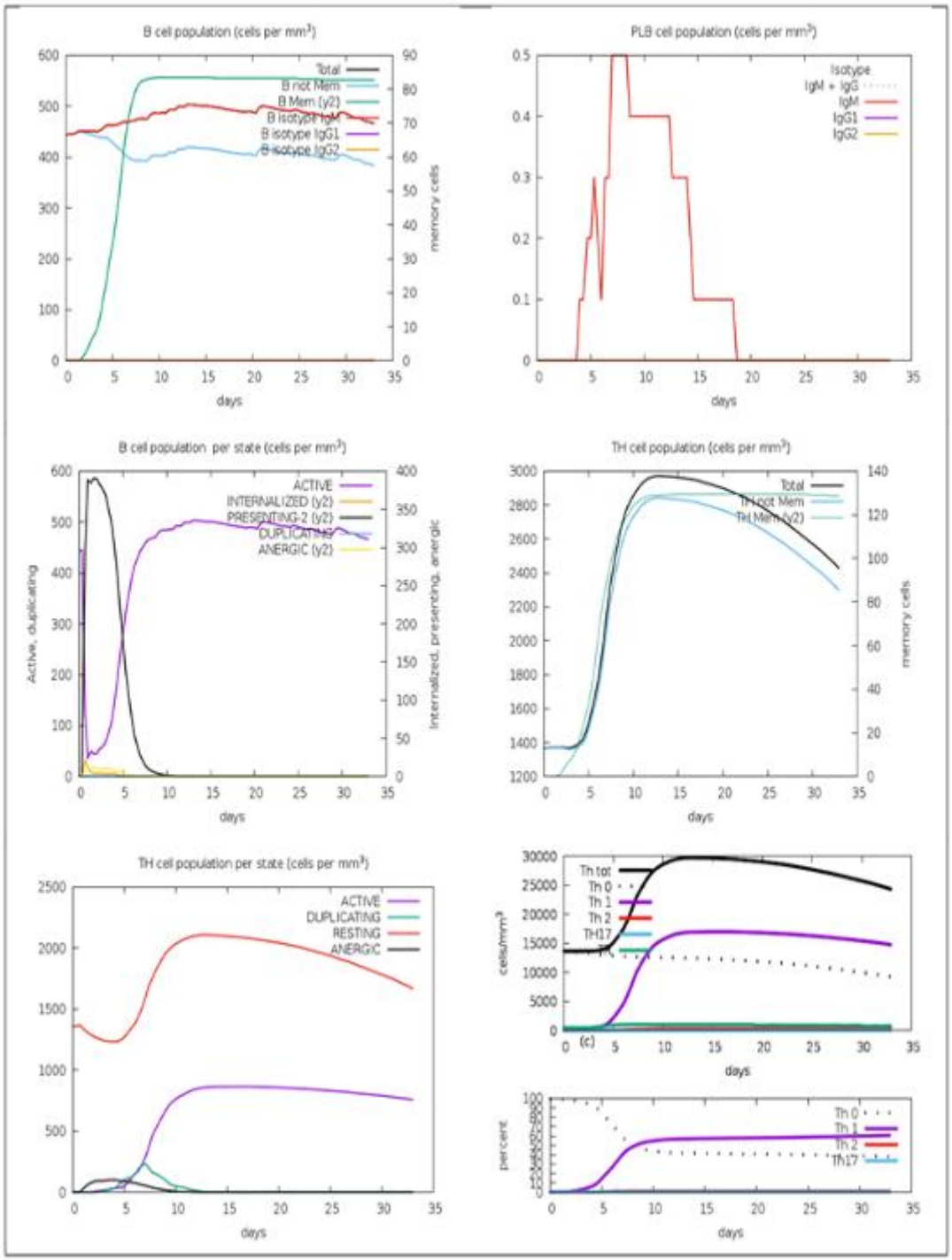

Figure 11A Different Cell counts shown in Immune System by the Vaccine Response

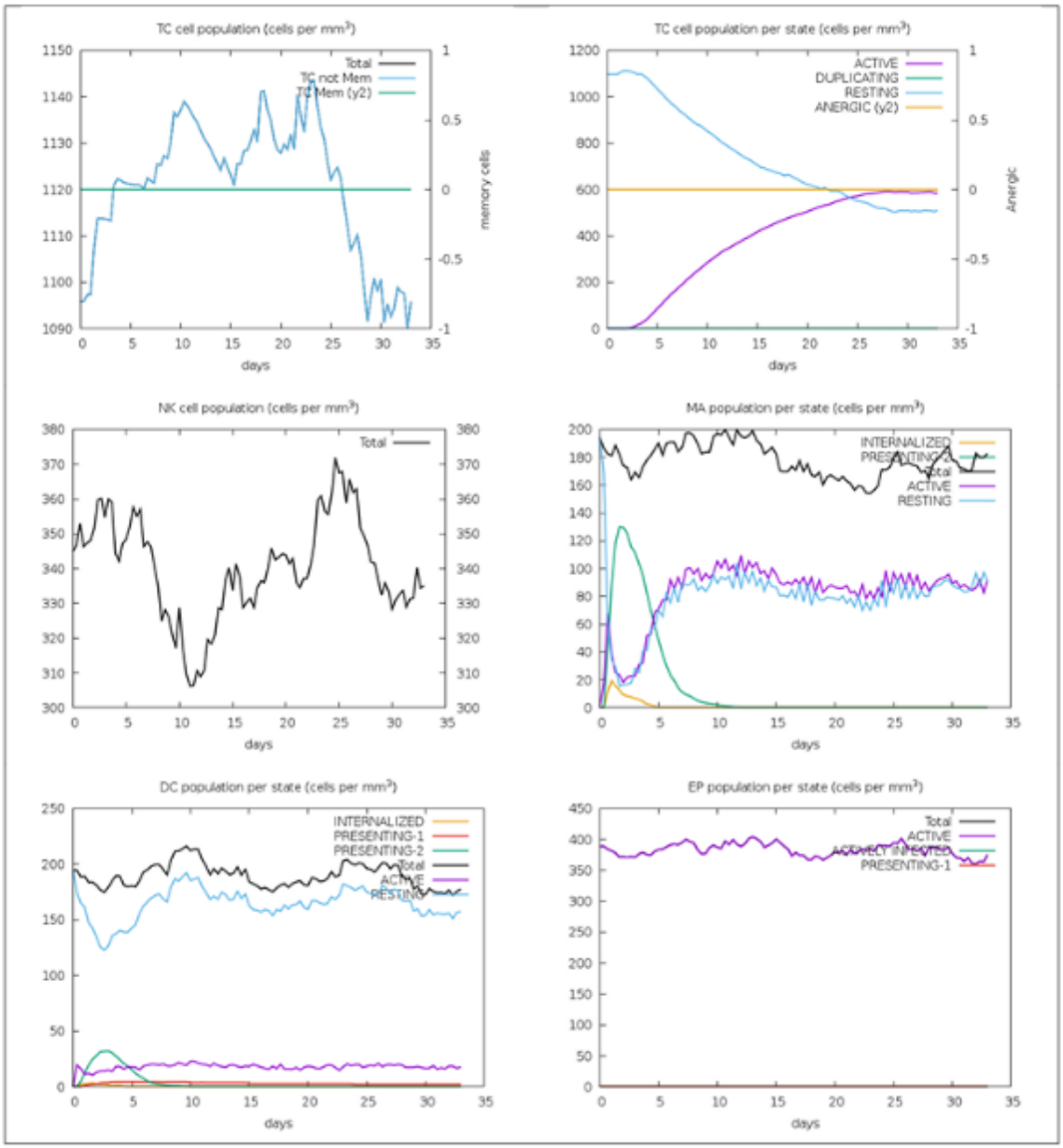

Figure 11B. Different Cell counts shown in Immune System by the Vaccine Response

4. Conclusion

In this study, we adopted an innovative computational approach combining reverse vaccinology and immunoinformatics to design a sophisticated multi-epitope peptide-based vaccine against migraine. Our comprehensive strategy involved systematically identifying and combining optimal B-cell and T-cell epitopes capable of eliciting robust and specific immune responses. Through extensive computational analysis, we demonstrated that this carefully engineered vaccine construct possesses the remarkable ability to simultaneously activate both humoral (B-cell mediated) and cell-mediated (T-cell dependent) immune pathways, thereby creating a powerful defensive mechanism against migraine triggers within the human body.

To ensure the highest standards of vaccine efficacy, safety, and population coverage, we employed an array of cutting-edge bioinformatics tools and in silico methodologies throughout our research process. Our computational pipeline included: detailed epitope mapping and prediction to identify immunodominant regions; thorough antigenicity and allergenicity profiling to guarantee safety; comprehensive population coverage analysis to assess global applicability; advanced structural bioinformatics for secondary and tertiary structure prediction; and sophisticated molecular

modelling techniques for 3D structure refinement and validation. Furthermore, we conducted immune simulation studies to model and predict the vaccine's performance in a human biological environment.

A particularly significant aspect of our investigation involved molecular docking studies to examine the crucial interactions between our vaccine construct and key Toll-like receptors (TLR3 and TLR9). These computational analyses revealed strong binding affinities and stable molecular interactions, providing compelling evidence for the vaccine's potential to effectively stimulate the innate immune system. The successful engagement of these pattern recognition receptors further supports our vaccine's capacity to trigger appropriate immune signaling cascades, ultimately leading to the development of lasting immunological memory against migraine-associated targets.

Through this multifaceted computational approach, we have developed a promising vaccine candidate that not only demonstrates excellent immunological properties but also shows strong potential for further development through experimental validation and clinical studies. Our findings represent a significant step forward in the field of computational vaccinology for neurological disorders, offering new possibilities for migraine prevention and treatment through immunological intervention.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Credit authorship contribution statement

- Chandra Kumar K: Writing – original draft, Software, Investigation, Data curation.
- Srinivas G: Writing – review & editing, Writing –original draft, Validation, Software, Investigation.
- Anil Kumar Babu K: Writing – review & editing, Supervision, Conceptualization.
- Yashwanth K: Writing – original draft, Validation, Investigation, Formal analysis.
- Pigili Akhil Kumar: Writing – review & editing, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Data Availability statement

All data and findings generated in this study are available within the manuscript and its supplementary materials. For additional information, please contact the corresponding author.

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