ANTIGENIC PEPTIDE DISCOVERY AND MULTI-EPITOPE VACCINE DESIGN OF *TRICHOMONAS VAGINALIS* PATHOGEN USING IN - SILICO BIOINFORMATICS.

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Submitted to Institute of Graduate Studies in Health SciencesMaster of Science

Molecular and Medical Genetics

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- 3th august 2023

DECLARATION

I declare that this thesis is my own work and it has been completed according to the guidelines established by the faculty and has not been submitted for any other purpose, this work is submitted in partial fulfillment of the requirements for the degree of Master of Science Molecular and Medical genetics. All the references have been provided on all supporting literatures and resources.

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ACKNOWLEDGMENTS

I would like to acknowledge Assist. Prof. Dr. Dr. Ahmet Efe KÖSEOĞLU for his support and patience with me. I would like to say thank you for always being supportive comprehensive and patient, the beginning to the end of my research journey.

I would like to thank Dr. ELİF SİBEL ASLAN for her support and patient with me. She was always giving a solution when it comes to my thesis phase of my master degree.

Special thanks to my parents that have always sacrificed with me from Morocco. I will always be grateful to their efforts, prayers with me

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LIST OF ABBREVIATIONS AND SYMBOLS

6ma: N6-Methyladenine

Clrs: C-Type Lectin Receptors;

Da: Dalton

GRAVY: GRand AVerage of hydropathicitY

HIV: Human İmmunodeficiency Virus

IC50: The Half Maximal İnhibitory Concentration

I-TASSER: Iterative Threading Assembly Refinement

LG: Lipoglycan

Mb; Megabases

MHC-I: The Major Histocompatibility Complex Class 1

MHC-II: The Major Histocompatibility Complex Class 2

NCBI: National Center For Biotechnology Information

NGU: Nongonococcal Urethritis

Nlrs: NOD-Like Receptors;

PI: Isoelectric Point Value

Rlrs: ROG-Like Receptors;

STI : Sexually Transmitted Infection

Tlrs: Toll-Like Receptors

TV: Trichomonas Vaginalis

Tvmıf*: Trichomonas Vaginalis* Macrophage Migration İnhibition Factor

Tvsaplips : Trichomonas Vaginalis Aponin-Like Pore-Forming Proteins

TVV: Trichomonas Vaginalis Virus

WHO. World Health Organisation

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ABSTRACT

Trichomoniasis is the most common non-viral sexually transmitted infection (STI) worldwide, caused by trichomonas vaginalis protozoan, this latter, developed a different resistance mechanisms against the only approved and effective drug for this diseases. Therefore, it is need of the hour to develop a potential vaccine that can prevent and immunize the human being against this pathogen. Using bioinformatics and immunoinformatics approaches, different B cell and T cell epitopes were predicted to construct the vaccine design, all the physico-chemical parameters of the epitopes were analysed in order to select the most antigenic, non allergenic and non toxic epitopes,

In addition to the epitopes, adjuvant in order to increase the immunity and linkers such as EAAAK, AAY and GPGPG were combined to construct the vaccine candidate

The developed vaccine construct showing promising results in-silico and it will also show good results if it would be tested in-vitro and in-vivo.

Keywords: Trichomonas vaginalis, vaccine, epitope, ummino-informatics

Özet

Trichomoniasis, dünya çapında en yaygın viral olmayan cinsel yolla bulaşan enfeksiyon (STI), trikomonas vaginalis protozoanın neden olduğu, bu hastalıklar için onaylanmış ve etkili ilaca karşı farklı bir direnç mekanizması geliştirdi. Bu nedenle, insanı bu patojene karşı önleyebilecek ve aşılayabilecek potansiyel bir aşı geliştirmek için saat gerekir. Biyoinformatik ve immünoinformatik yaklaşımları kullanarak, aşı tasarımını oluşturmak için farklı B hücresi ve T hücresi epitoplarının tahmin edildiği, en antijenik, alerjik olmayan ve toksik olmayan epitopları seçmek için epitopların tüm fiziko-kimyasal parametreleri analiz edildi,

Epitoplara ek olarak, bağışıklığı arttırmak için adjuvan ve aşı adayını oluşturmak için EAAAK, AAY ve GPGPG gibi bağlayıcılar birleştirildi

Silico'da umut verici sonuçlar gösteren gelişmiş aşı yapısı ve in vitro ve in vivo test edilecekse iyi sonuçlar gösterecektir.

Anahtar Kelimeler: Trichomonas vaginalis, aşı, epitop, ummino-bilişim

INTRODUCTION

Trichomonas vaginalis

Trichomonas vaginalis is a protiste-flagellated parasite, estimated to be the most common non-viral sexually transmitted infection (STI) worldwide [1]. *T. vaginalis* commonly colonized in the vagina causing the trichomonasis, In 2016, a research organization of the global prevalence and incidence of urogenital infection conducted by the World health, estimates 110 million infections worldwide were due to *T. vaginalis* and much more than estimated results caused by Chlamydia trachomatis, Neisseria gonorrhoeae, and syphilis combined. [2] [3]. An article published by Dr. Yunus Emre Beyhan in 2021 estimating the infection rate of *trichomonas vaginalis* in turkey from 2002 to 2020, Overall, the mean prevalence of infection was found to be 5.94%, 2.87% in men, and 6.17% in women. The maximum prevalence rates were 42.4% in bar girls, 28.3% in patients with gynecological complaints, 28% in patients applied to gynecology clinics, and 15.37% in patients with vaginal discharge. Among the regions, the highest rate of infection was in the Black Sea (8.76%) and Eastern Anatolia (8.12%) [4].

Some patients are asymptomatic; however, symptomatic *T. Vaginalis* patients experienced various symptoms, who are usually more severe in women than in men. In women, trichomonasis has been associated with significant reproductive morbidity (eg, vaginitis, pelvic inflammatory disease, cervicitis, and increased cervical cancer risk), increased HIV acquisition risk and adverse birth outcomes [5] [6]. Along with these impacts of infection on women, transmission to men sexual partners can have important consequences. In men is traditionally considered to be benign and self limited, Moreover consequently have been overlooked [7]. However, men with this common sexually transmitted infection can be associated with nongonococcal urethritis (NGU) [8], prostatitis [9], impaired sperm function [10] and amplified human immunodeficiency virus risk in seminal fluid of HIV positive men [11] [12]. TV remain a major health threat problem among the turkish population and over the world, the lack of much advanced studies, underestimate the attention of the health public towards this disease.

However, *T. vaginalis* is a fascinating microorganism in its own right. It is a primordial eukaryote, with an anaerobic metabolism, lack of mitochondria and considered as a host of hydrogen2osomes [13]. *T. vaginalis* is considered the parasite the most studied among all the trichomonads. The size and shape of the pathogen varied from type to another, with the average

length and width being 10 and 7 μm, respectively [14]. The shape of the protozoan change according to the physicochemical conditions. The shape tends to be like a oval or pear shaped in the axenic culture (figure 1A), otherwise it showes amoeboid appearance when attached to vaginal epithelial cells (figure 1B,1C) [15]. Non devided parasite possesed four flagella, they are located in it's interior portion in addition , one flagellum integrated within the undulating membrane of the parasite, which is supported by a slender noncontractile costa [16].

Internal organelles of the organism include, a prominent nucleus that is located in the anterior portion enclosed by a porous nuclear envelope just like the other eukaryotes.the axostyle, rod like hyaline runs through the cell longitudinally [17] This structure is thought to anchor the parasite to vaginal epithelial cells. The cytoskeleton that is composed of tubulin and actin fibers. Unique energy-producing organelles, that is catalase negative. Hydrogenosomes support the metabolism [18]. The life cycle of *T. vaginalis* is simple , the transmittion of the parasite goes from trophozoite through coitus and no cyst form is known. The trophozoite divides by binary fission and, in natural infections, gives rise to a population in the lumen and on the mucosal surfaces of the urogenital tracts of humans.

Genome and gene expression

The trichomonas vaginalis genome is considered one of the largest genomes comparing to the other protozoan parasites, the size is estimated to be 160 mb in length, which encodes about 60,000 protein-coding genes [19].

At least 65% of the TV genome is repetitive which make the size enourmous. *T. vaginalis* estimated to have only 62 introns. According to Liston DR. and al, the transcription of protein-encoding genes is initiated by a gene specific regulatory elements and a concerved core promoter [21]. However, a TATA box was found to be missing from promoters of protein-encoding genes. *T. vaginalis* nonetheless does encode two TATA box-binding proteins (TBPs) which bind to initiator-binding protein 39 (IBP39)16 and to DNA as determined in electrophoretic mobility shift assays, however this binding is not specific. [22]. Epigenetic regulation determine the transcription rates, such as DNA methylation; and in trichomonas vaginalis, N6-methyladenine (6mA) was found to be the major DNA methylation mark [21] contributes in a role of the regulation of gene expression and potentially impact parasite virulence.

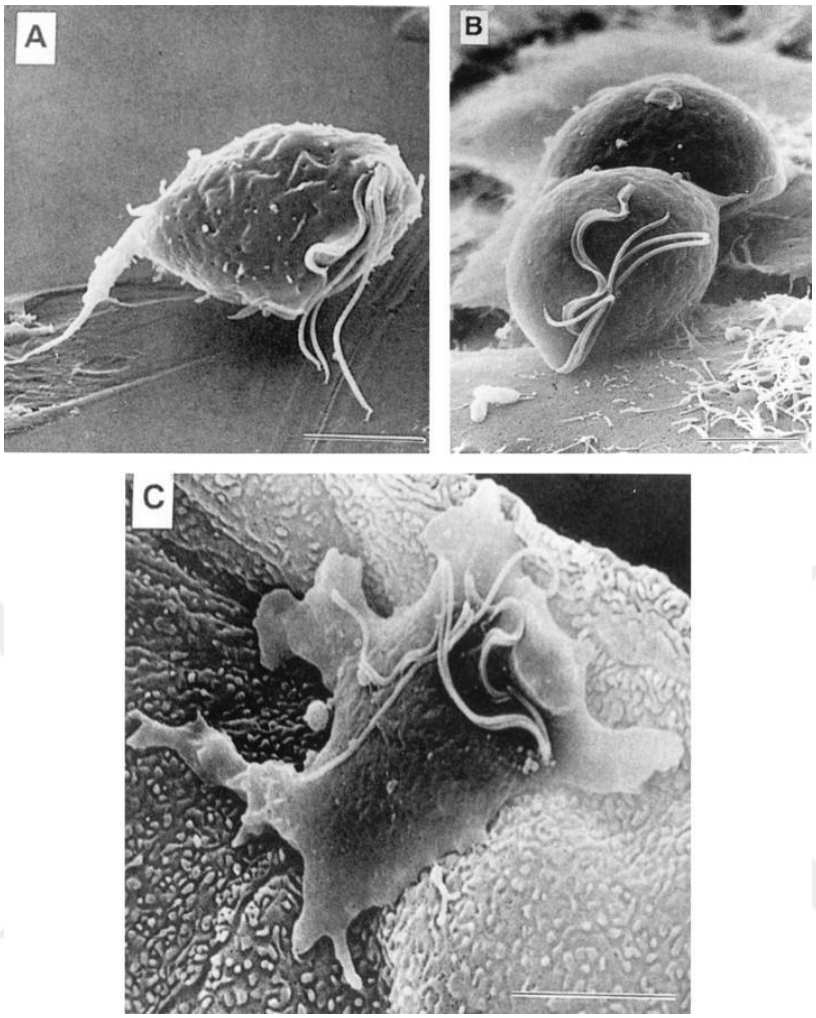

Figure 1: *T. vaginalis* parasite with the axostyle, undulating membrane, and flagella. (B) *T. vaginalis* on the surface of a vaginal epithelial cell prior to ameboid transformation. (C) Ameboid morphology of T. vaginalis as seen in cell culture. [120]

Moreover, histone acetylation is important in gene expression as a modulator. Indeed, *T. vaginalis* has a large arsenal of histone deacetylases (HDACs) of the Sir2 type and of histone acetyl transferases that regulate this acetylation [20]. Two argonaute proteins (*T. vaginalis* AGO1 and 2) required in the RNA- induced silencing complex, they constitute the central components for the presence of RNAi, that complete the process of mRNA. *T. vaginalis* AGO1 and 2 interact with sequence-specific piRNA to degrade transposase mRNAs as encoded by myriads of TEs in the *T. vaginalis* genome [21].

Cell biology and mitabolism

T. vaginalis is a primitive eukaryotic organism, similar in many aspects to the other protists, However, it diverges strongly from them in term of energetic metabolism, TV have an anaerobic

metabolism and host an unusual organelle, the hydrogenosome [18] [22]. This organelle derived from the mitochondria and carry out many of the same metabolic functions [23].

Carbohydrate and Energy Metabolism

T. vaginalis has properties that is familiar in both anaerobic bacteria as well as higher eukaryotic organisms in terms of its carbohydrate and energy metabolism. Carbohydrate metabolism is fermentative under both aerobic and anaerobic conditions because glucose is incompletely oxidized. That's because Trichomonas vaginalis lacks a complete oxidative phosphorylation system. The metabolic products include acetate, lactate, malate, glycerol, CO2, and, under anaerobic conditions, H2 [24]. Carbohydrate metabolism happens in two compartments: the cytoplasm and an organelle termed the hydrogenosome, It is 0.5 to 1.0 μm in diameter and is surrounded by a double membrane.

The hydrogenosomes are the site of fermentative oxidation of pyruvate [25], and they produce ATP by substrate-level phosphorylation, process half of the carbohydrates of the cell, produce hydrogen, , and contain homologous enzymes common to those found in both prokaryotes and eukaryotes [26] basiclly , maintain the redox balance. Hydrogenosomes shows both similarities to and differences from mitochondria, they provide the β-succinyl coenzyme A synthetase enzyme, which catalyzes ATP production by substrate-level phosphorylation and the [2Fe-2S] ferredoxins [27] found in aerobic bacteria and in mitochondria. Nonetheless, they lack cristae, cytochromes and DNA, which are typically found in mitochondria [28].

Fermentation: In the absence of oxygen, *Trichomonas vaginalis* utilizes fermentation as the primary means to regenerate NAD+ and continue glycolysis. The pyruvate produced during glycolysis is converted to various fermentation products, such as acetate, ethanol, and succinate. These fermentation products are essential for maintaining redox balance and energy production.

Amino Acid Metabolism

Studies has shown that trichomonad cell provide amino acids and they maintain the trichomonad growth and survival when the carbohydrates are limiting [29]. Since this latter is the main source of nutrients for *T. Vaginalis*. In a condition with absence of maltose, *T. vaginalis* consumes amounts od Amino acides, such as , arginine, threonine, and leucine which are used in energy generation, Thus , in a normal culture conditions, TV consumes smaller amounts of methionine for use in energy production [29]. İn conclution the Trichomonad cell maintain the concentration of aminoacids within the cell and its external environment [30]

Some studied by [31] shows that aminotransferases play a role in amino acid metabolism, In trichomonas vaginalis cells, the presence of the activity of some enzymes as aspartate/aromatic amino acid:2-oxoglutarate aminotransferase and branched-chain and aromatic aminotransferases explains why these amino acids are found predominantly with T. vaginalis.

Nucleotide Metabolism

T. vaginalis requires adenine and guanine or their nucleosides for growth, in addition to thymidine, cytidine, uracil, and/or uridine, whereas, *T. vaginalis* has not the ability to synthesize pyrimidines and purine, thus it must resort to salvage pathways to generate nucleotides [32]. Phosphoribosyltransferases and nucleoside kinases are able to recover pyrimidines. While purine salvage is mediated by nucleoside phosphorylases and kinases [33]. This protozoan can scavenge the purines from the host enviroment, and that using the two separate carriers for nucleotide transport [34]. One carrier is believed to accommodate adenosine and pyrimidine nucleosides, while the second shows a high affinity for uridine and a separate site for guanosine transport.

Pathogenesis of trichomoniasis

In recent years, the pathogenesis of trichomoniasis is receiving the attention it deserves. Studing the parasite in all terms, from host response to the virulence factors. Some studies appear that the pathogenesis of *T. vaginalis* is very complicated, and involves many pathogenic mechanisms such as the adhesion, soluble factors, cell detaching factors and hemolysis. For its survival in the vaginal environment, the parasite interact with members of the resident vaginal flora, and induce advance immune evasion strategies that causes stress responses.

Due to the response of the host cells to the *T. vaginalis*, cytokines is producted by the immune cells at the site of infection. Mainly interleukin 1 (IL-1), IL-6, IL-8, and IL-17 [35] are induced and this is defined as a **pro-inflammatory response**. The levels of cytokine upgrade after the exposure to *T. vaginalis* in the host enviroment. IL-6 was reported to induce polarization of THP-1–derived macrophages into M2-type macrophages [36] and IL-1 production is linked to activation of the NLRP3 inflammasome in macrophages resulting in processing of precursor IL-1β to bioactive IL-1. IL-1, in turn, can induce pyroptotic cell death in macrophages [37]. Furthermore, a Toll-like receptor 2 (TLR2), triggered also by *T. vaginalis*, is increased which cause some immune mediators such as p38, ERK, and p65 NF-κB that were found to be phosphorylated to a lesser extent after stimulation with *T. vaginalis* [38] . However,

intraepithelial dendritic cells and regulatory T cells were shown to react to one of *T. vaginalis* major antigens, actinin-2, with the expression of IL-10, which is an anti-inflammatory cytokine [39]. *T. vaginalis* secretes a homologue human macrophage migration inhibition factor (TvMIF) causes the increase survivability under serum starvation several-fold76, this latter contains amino acids, lipids, and precursors of nucleotides for the parasite's nutrition [40].

The surface of the trichomonad cell is a mosaic of adhesins, receptors in order to host extracellular matrix proteins, and carbohydrates, which provide the basis for ligand-receptor binding [41]. **Adherence** of *T. vaginalis* to host epithelium is an interesting event in trichomoniasis. During this process, the host's surface proteins galactin-1,-3 [42] [43] bind to lipoglycan (LG) on the parasite's surface [42]. Moreover, those galectins dime the host response to *T. vaginalis*, and TvLG can further tune this [43]. Many other cell surface–associated factors involved in the promotion of adherence to TV such as cadherin-like protein, actinin-2, and triosephosphate isomerase [44] [45] [46]. glycolytic protein also is involved in the association with parasite's cell surface and bind to fibronectin and laminin. Finally, palmitoylation of proteins was found to be positively affect adherence of *T. vaginalis* to host cells [47].

Basically, after the adherence comes the **cell lysis**. Some of parasite's proteases such as metalloprotease TvMP50 or cysteine proteinase 2 (CP2) [48] induce the damage of the host cells. *T. vaginalis* cysteine proteases do also degrade the anion channel CFTR on the host cell surface, causing the elevation of intracellular Cl^- concentrations and induction of NF-κB signaling [49].Also, the rhomboid protease TvROM1, exerts an indirect effect by cleaving *T. vaginalis* substrate proteins, resulting in enhanced attachment and damage to host cells [50]. Moreover, some recent researches identify the saponin-like pore-forming proteins (TvSaplips) in the *T. vaginalis* genome [51] and one of these, TvSaplip12, the expression of one of the TvSaplip12 comes on contact with host cells and has a strong lytic activity against bacteria and HeLa cells.

Another mechanism of the *T. vaginalis* pathogenesis, the parasite drope some extracellular vesicles (EVs) carrying cargo to interact with the host cells, consequently, it promotes the infection process [52]. Two types of *T. vaginalis* EVs have been identified: exosomes (50–150 nm) and microvesicles [53], which are considerably larger (100–1000 nm).The vesicles contain tetraspanins or BspA-domain proteins involved in mediating adherence, and arguably

in tuning the host's response. The image of pathogenesis of trichomoniasis is still incomplete and complex but the gaps are being filled at an increasing pace.

Diagnosis

Microscopic and culture techniques:

The first studies of the diagnosis of trichomoniasis was described by Donné in 1836 [54] by observing the mortility of the protozoa in vaginal or cervical excretions using the microscope. Based on the mortility the parasite is differenciated, the sensitivity of this technique varied betweed 38% and 82%. However, this technique is the most cost-effective diagnostic test, and because of the low sensitivity due to the loss of distinctive motility after after the protozoan has been removed from body temperature, the results are far to reliable. The broth culture method is key for the diagnosis of trichomoniasis by the fact that it's simple to interept and requires for the initiation of the culture as few as 300 to 500 trichomonads/ml of inoculum [55]. Nonethless, there are some limitations of this culture, such as the periode of incubation that was between 2-7 days to identify the parasite, during which time infected patients may continue to transmit the infection. The inPouch system utilised to prevent this inconvenient although this system in significantly much sensitive [56]. An other culture technique has been used based on the cell lines to recover *T. Vaginalis* from clinical specimens such as McCoy cells [57], the advantage was that this technique was able to detect the concentration of *T. Vaginalis* as low as 3 organisms/ml. However, cell culture is not routinely performed, because it is expensive and not convenient for rapid diagnosis.

The limitations of culture and microscopy methods for the detection of *T. vaginalis* notified the advance of the more sophisticated methods which can detect antigen, antibody, or nucleic acids in urethral or vaginal exudate.

Antibody-Based Techniques

Using the mmunoblot analysis, a variety of antigenic markers are seen in *T. Vaginalis*, [58]. Various techniques including agglutination, complement fixation, indirect hemagglutination, gel diffusion, fluorescent antibody, and enzyme-linked immunosorbent assay have been used to demonstrate the presence of antitrichomonal antibodies [59]. The inconvinient is that the

serum or local response to a pathogen depends on many factors, like, the nature of the antigen or pathogen, its live or inactivated form, its local concentration, and the frequency and length of immune system stimulation. Moreover, an antibody response is not observed either because the system is too insensitive to detect low levels of specific antibodies or because the serum humoral response has not yet been elicited.

DNA Techniques

Several Dna techniques have been used in clinical laboratories to improve the specificity and sensitivity of *T. vaginalis* diagnosis. PCR methods helps detect nonviable organisms and also has the ability to detect cells and target sequences in clinical samples that have undergone fixation or partial degradation [60]. The commercially available Affirm VP system (MicroProbe Corp, Bothwell, Wash.) uses synthetic oligonucleotide probes for the detection of both *Gardnerella vaginalis* and *T. vaginalis* from a single vaginal swab [61].

The dot-blot hybridization technique, which uses a 2.3-kb *T. vaginalis* DNA fragment as a probe, can detect *T. vaginalis* DNA from vaginal exudate. However, because of the instability of the probe and the special care needed in the handling and disposal of the radioactive material, the technique has significant drawbacks [62]

The usefulness of these techniques for identifying asymptomatic carriers needs to be evaluated.

Treatment

Througout the last six decades, the 5-nitroimidazole drug metronidazole has remained the mainstay in the treatment of anti-trichomonadal chemotherapy [63] it was defined as the ‘’essential medecines’’ by WHO. It was developed in 1959. However, the resistance has become a worrying issue increasingly, and that due to a complex phenomenon affecting numerous enzymatic pathways in *T. Vaginalis* [64].

Some studies on a gene expression in metronidazole-sensitive and -resistant strains [65]. Flavin reductase 1 (FR1) is one of the genes expressed in three metronidazole-resistantstrains, that enzyme produces hydrogen peroxide, that latter has been identified as a mediator of metronidazole resistance and also found to be downregulated [66]. The failures with the metronidazole treatment it’s not always due to the *T. vaginalis* resistance, the treatment regimens such as the dosage, the frequency and duration of treatments can affect the treatment outcome [67], Moreover , it’s is necessary to test alternative treatments within the drug class of 5-nitroimidazoles, such as secnidazole, or novel derivatives [68]. Auranofin is also effective

against *T. vaginalis* and can cure experimentally infected mice [69]. Thioredoxin reductase has been proposed to be the main target of auranofin, but a lot of complimentary studies is needed.

Numerous other candidate drugs, were also evaluated,including proteasome inhibitors such as carmaphycin-17 [70] zinc sulfate and zinc complexes [71], bisbenzimidazole analogues [72], boric acid [73], and tetracycline [74]. In conclusion, it is very important to emphasize that assay conditions can be important during the evaluation of the efficacy of established and novel anti-trichomonadal drugs.

Abraham et al. have conducted some studies [75] to induce immunity to *T. vaginalis* in the mouse model, in order to develop a vaccine against the trichomoniasis. Immunity has been difficult in the model in vivo, and because in human, repeated infections with the parasite do not confer immune protection [76].

Since vaccine are on of the most cost-effective pharmaceuticals and in many studies prouved to be applicable and prevent and/or treat the diseases for over two centeries. As immunization against infectious agents activate the humoral and cellular immunity rsulting a decrease in mortality and morbidity rates also improved human life [77], since the classic modalities of vaccination can take up to 5-15 years to develop a propre vaccine, a new method in the postgenomic era is chalenging by the determination of antigenic regions or epitopes in order to boost the stimulation of the immune response [78]. Computational in silico immune-informatics can aid the vaccine development by rational design in a time- and cost-effective manner.

In silico multiepitope- epitope vaccine design

The epitope-based vaccines have been developed to deliver both prophylactic and therapeutic effects on pathogen specific immunity, with promising outcomes. And itis reported to be the therapeutic solution with less side effects. This modality of vaccine provides several advantages, including : elimination of undesirable immune responses by designing specific constructs; generation of prolonged immunity with required responses; finally it’s cost- and time-effectiveness [79].In reverse technologie,

The main notion of the multi-epitope based vaccine is to stimulate the immune response of the pathogen and that’s by the prediction of B cells and Tcells, Thus, the most significant part in the vaccine development is the identification of the appropriate B cell and Tcell epitopes. To

induce both the humoral and cellular immune responses. As known, for the construction of a vaccine, Linkers and adjuvants must be added. The fusion of all this componends is important as well as the arrangement of them, resulting in the efficient stimulation of the immune response. [80]. The multiepitope based vaccine is concidered safe, However , the possible emergence of a cytokine storm should be taken into account, as reported for other immu notherapies. The rational steps for in silico epitope-based vaccine design is represented in Figure 2.

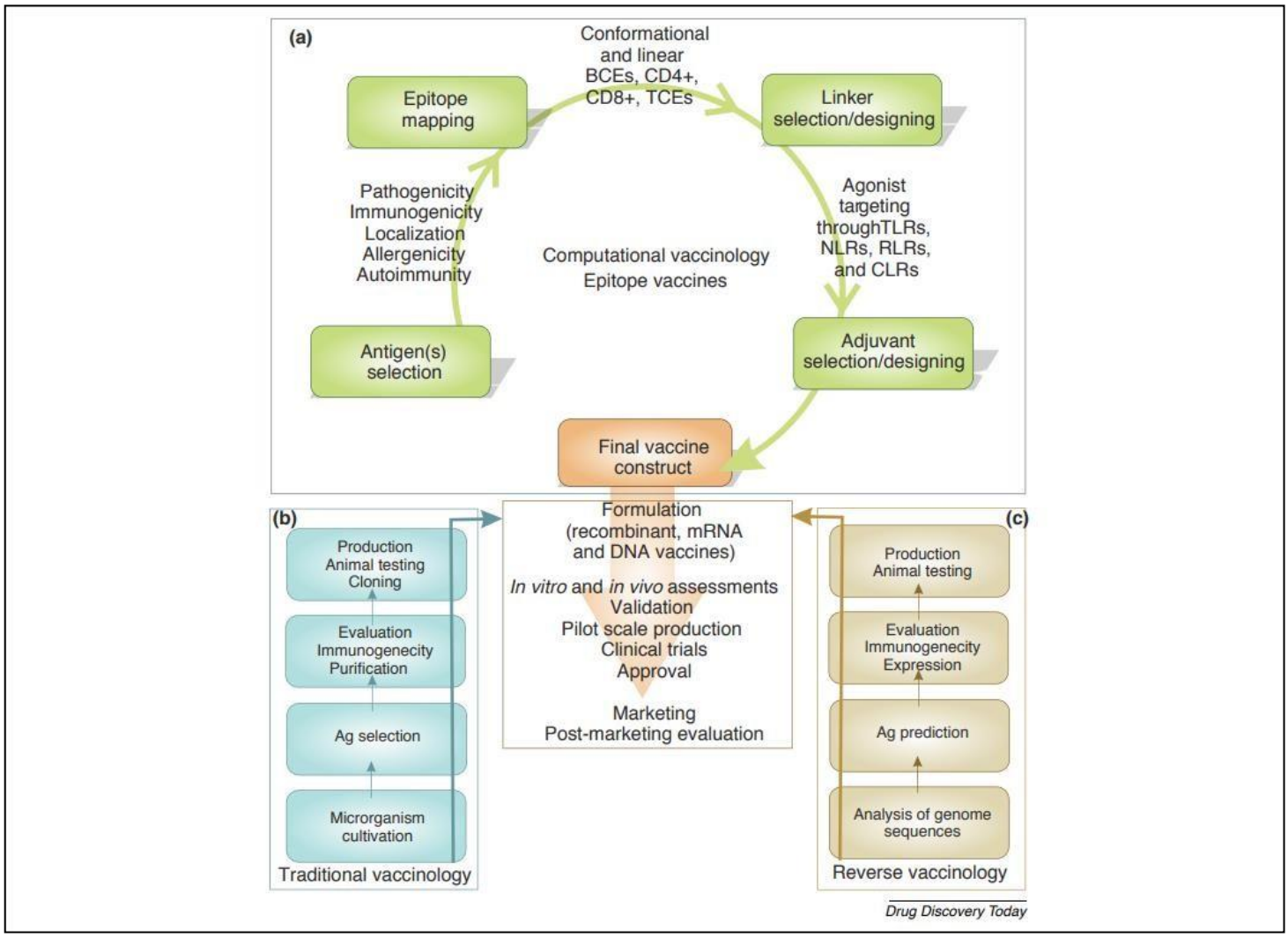

Figure 2: Steps for in silico epitope-based vaccine design and validations. The rational design of the vaccine **(a)** In silico epitope vaccine design and development. **(b)** Traditional approach for vaccine development. **(c)** Reverse vaccinology approach.

B-Cell and T-Cell Epitopes:

Epitopes is a specific regions of an antigen where antibodies bind, thus it is recognized by the immune system. Those epitopes can belong to self-proteins or to foreign proteins. B cell epitopes can be recognized and bound to antibodies. They are typically composed of amino acids Moreover can be linear and Conformational. B cell epitopes effectively kill pathogenic agents by the help of receptors present on their bodies.T cell epitopes actually bound to major histocompatibility complex, two types are involved, MHC-I and MHC-II [81]. There are some viruses termed immune dificiency viruses, as they avoid recognition by T-Cell epitopes and thus encodes their structural proteins in the host. The process is achieved by the molecular interaction between the specific region and the target molecule [82]. B cells and T cells epitopes are used in advanced technologies and immunoinformatics approaches for the fact that they acquire a potential to trigger the immune response in 15 individual. Constructing vaccine design using these epitopes is the new advanced technique and proved effective in different aspects [83].

Adjuvant Selection:

Adjuvants are added to the vaccine design construction in order to increase and maximize the efficiency of the devolloped vaccine. They are generally defines as molecular complexes that amplify an immune response when managed within a vaccine construct

Peptide-based vaccines can intrinsically result in poor immunogenicity even with optimal BCEs and TCEs. The strength and duration of the immune response to the vaccine are also vital. Thus,the adjuvant must be well selected so the Ags are enhanced, inclusion of which might enhance the immune response. Furthermore, the addition of adjuvants into epitope-based vaccines provides several advantages, including (i) indirectely extension of the vaccine long-term memory; (ii) expansion of the Ab repertoire; (iii) refreshing the stunned immune system in older patients; and (iv) opportunity for dose-sparing [84]

Linker Selection:

Linkers, also termed ‘spacers’, are key entities in designing and constructing protein vaccines. Their role defines as the stability of the vaccine’s structure , interdomain interactions, and functionality of the vaccines [85]. Three types of linkers are involved, the one linking BCEs, TCEs, and intramolecular adjuvants. The fusion of an inappropriate linker in the vaccine

construction can lead to undesirable effects, such as a decreased quantity in production, misfolded protein structures, and/or weakened bioactivity [86]. Several properties should be taking into consideration when it comes to selection of a linker, including length, arrangement, hydrophobicity, secondary structure, sensitivity to proteases, and potential interaction with other components of the immunogenic construct.

Parvizpour, S and all [87] mentioned that SynLinker server is the only up-to-date webserver that facilitates the selection of suitable linker(s) and modeling of a fusion protein, it contains repository of 2150 naturally occurring peptide linkers plus 110 artificial or empirical linkers. Moreover, The server suggests potential linker candidates based on user-specified criteria, like, linker length, composition, solvent accessibility, and other features that might affect linker flexibility and function.

linker candidates based on user-specified criteria, including linker length, aa composition, solvent accessibility, and other features that might affect linker flexibility and function.

MATERIALS AND METHODS

This thesis is realized using in-silico immunoinformatics and reverse vaccinology approaches. The type of thesis is descriptive and prospective; The antigenic peptides discovered and the vaccine molecules designed within this study can be published in journals with high impact factor in vaccine development studies. Moreover, it can be carried out both in turkey and the world. The study was carried out in Biruni university Molecular biology and Genetics department from September 2023 to June 2023;

The data sample analyzed in the thesis is created from the NCBI (https://www.ncbi.nlm.nih.gov) database and the data collection tools are the programs used in bioinformatics analysis. The details of the methods and materials used are explained below. Ethics committee approval is not required as the data used is obtained from public biological databases.

The approaches used in this study has been used in antigen discovery and vaccine design in many researches and studies on other pathogens [88]. In this thesis, we targeted the most prominent secretion and surface proteins defined in the literature of *T. vaginalis* or found in NCBI database. (https://www.ncbi.nlm.nih.gov) [89].The proteins are presented in table 1

1. Sequence Retrieval

National Centre for Biotechnology Information (NCBI) was used to retrieve the sequence data of the 15 secretory and surface proteins reported in previous literature of Trichomonas vaginalis. We used the accession number noted in the table 1.

2. Structural analysis

2.1 Antigenicity estimation

We will use Vaxijen v2.0 (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) program for the antigenicity prediction either for proteins or the predicted epitopes [90]

2.2 Estimation of physicochemical parameters and secondary structures

For the prediction of physiochemical parameters of selected proteins, **ExPASy-Protpram** online tool from ExPASy bioinformatics resource portal was utilized [91]. This program

provides the molecular weight, hydrophilicity (solubility), aliphatic index, stability and half-time. Regarding the secondary structure-related predictions, we used **SolPro** and **GOR IV** programs to predict alpha helix and random coil of the proteins. [92]

Table 1 Proteins targeted within the study on *T. vaginalis pathogen*

Protein	NCBI Accession number
adhesin protein AP33-1	AAC48337.1
adhesin protein AP33-3	AAC48339.1
adhesin protein AP51-2	XP_001303981.1
adhesin protein AP51-3	XP_001584099.1
AP65-1 adhesin	AAA87406.1
AP65-2 adhesin	AAA87407.1
AP65-3 adhesin	AAA91133.1
putative TBP-associated protein	AAX62619.1
cytotoxic cysteine proteinase	ABX56032.1
surface immunogen P270	AAD40228.1
putative surface protein	AAM51159.1
surface protein (LP07818P1 family)	XP_001330776.1
surface protein (calponin-homology domain, CH-domain family)	XP_001326135.1
surface protein (hypothetical protein TVAG_2v0540870)	XP_001326045.1
surface protein (myeloid leukemia factor family)	XP_001325068.1

2.3 Signal peptide prediction

Signal peptide detection includes four different detection modes, Sensitivity, Specificity, Balanced prediction and Cleavage site. We used **signal –BLAST** program [93].

2.4 Prediction of BetaWrap motifs

Beta Wrap parameter is an important factor, the estimation will be performed with BetaWrap program [94]

2.5 Estimation of subcellular localization and number of transmembrane helix

The prediction of the subcellular localization will be estimated by **Euk-mPLoc** program [95]. For the estimation of the transmembrane helix number, we will use TMHMM Server v. 2.0 [96]

2.6 Allergenicity estimation

Allergenicity will be estimated by the program Algpred (http://crdd.osdd.net/raghava/algpred/) using the MEME/MAST motif and IgE epitopes approach (Saha and Raghava, 2006).

3. Prediction of B cell epitopes

IEDB Linear Epitope Prediction Tool v2.0 (http://tools.iedb.org/bcell) was used for the prediction of B-Cell epitopes [97]. This tool is considered the better comparing to the other B-Cell epitope prediction tools,for the reason that it's accuracy and its authencity , the tool uses epitope data from crystallized structures [98].Following the B cell prediction , the antigenicity value of each epitope will be determined by the Vaxigen 2.0 program as described above. Finally, the epitopes whose antigenicity is supported by Vaxigen 2.0, and which are predicted to be hydrophilic in the exposed or accessible regions of the protein will be considered as B cell epitopes studied [99].

4. Prediction of MHC-I and MHC-II epitopes

MHC-I binding predictions

IEDB MHC-I binding prediction tool was used for the determination of MHC class I binding predictions of the selected most antigenic, non-allergenic and soluble epitopes [100]. HLA supertype allele set up for the prediction of the MHC-I epitopes, twelve different MHC-I alleles was choosed among the 27 alleles that covers more than 97 % of the population (**A01.01, A02.01, A03.01, A24.02, A26.01, B07.02, B08.01, B27.05, B39.01, B40.01, B58.01, and B15.01)** . Selection of all the alleles present there was done at the length of 10-m; The prediction method was ANN 4.0 and the MHC source and host species was selected as human. Regarding the output format was selected as XHTML table in a reason that All the predictions will be saved for further analysis so the epitopes will be screened on the basis of of their antigenicity and IC50 values. All the other parameters will be kept as default. The antigenicity of each epitope with a low "IC50" value as well as a low "Percentile Rank" will be checked with Vaxigen 2.0 and MHC-I or II epitopes will be determined.

MHC-II binding predictions

Estimation of MHC-II epitopes will be performed by IEDB (https://www.iedb.org/) [101]. The Sequence submission of the most antigenic, non-allergenic and soluble proteins was done in FASTA format and Net MHCII pan 4.1 BA prediction method was used. All species and locus selected human HLA-DR, seven alleles were selected, **DRB1.03.01, DRB1.07.01, DRB1.15.01, DRB3.01.01**, **DRB3.02.02, DRB4.01.01 and DRB5.01.01** with 15-m length. All parameters were kept as default with XHTML table format. Outputs were sorted according to IC50 value defining the half maximum inhibitory concentration and percentile rank values. Moreover, the epitopes with low IC50 value as well as the percentile rank were further evaluated for the antigenicity and allergenicity using VaxiJen v2.0. Based on the outputs of these servers, epitopes nonallergenic, soluble and provide highest antigenicity value were selected for each protein.

5. Population Coverage Analysis

IEDB's Population Coverage Analysis tool (http://tools.iedb.org/population) determines the population that can be covered by the selected epitopes by processing HLA class I and II alleles separately or combined [102]. This tool calculate the population covered by the following vaccine construct in order to have a better idea about the immune gene coverage before proceeding further. It defines a target population at four levels: world, geographic regions, countries and ethnicities. The areas worldwild was selected manually and the parameters set as default, the epitopes and there binding alleles was selected manually as well.

6. Multi-epitope vaccine construction

The potential epitopes selected will be used to construct the multiepitope vaccine candidate. In order to reinforce the potency of immune response, we will use heparinbinding hemagglutinin (HBHA) of Mycobacterium tuberculosis (P9WIP9 ID number) as an adjuvant according to the litterature [103].the adjuvant will be embedded in N-terminal of the constructed vaccine. As

known, the linkers are indispensable to join the epitopes, fragments and molecules, therfore The linker **'GPGPG'** were used for B-cell epitopes and MHC-I epitopes whereas the linker '**AAY**' were used for MHC-II epitope [104]. To join the HBHA adjuvant and the epitopes we will use EAAAK linker [104].Finally, the antigenicity of the designed vaccine will be evaluated by the VaxiJen server.

7. Assessment of physicochemical features

The multiepitope vaccine condidate devolopped should be analysed in terme of it's sollubility, using Solpro umminoinformatic tool. This latter was selected based on the litterature as it's the best comparing to the other tools [105]. For the physiochemical properties of the designed vaccine construct, including molecular weight, half-life, GRAVY, aliphatic index, instability index and point isoelectric (PI), ExPASy-Protpram tool will be used [106].

8. Secondary and tertiary structure and refinement of vaccine candidate

In order to predict the secondary structure of the developed vaccine candidate, we will paste the amino acid sequence of the vaccine in one of the effective tools [107]. The main coils, helix and plates will be determined by the help of this tool. For the tertiary structure, will be modelled by **I-TASSER** server, (https://zhanglab. ccmb.med.umich.edu/I-TASSER/) using default parameters, The top five predicted models were given by I-TASSER and the best predicted model was selected on the base of C-score.

9. Docking analysis

For the docking analyzes, regarding the MHC-I alleles, receptor alleles specific to each epitope will be selected from the protein database (PDB; http://www.rcsb.org/pdb/). For the selection of MHC-I receptor models, we will use free 3D structures and models of selected epitopes (MHC-I specific) and that will be created with **I-TASSER** (http://zhanglab.ccmb.med.umich.edu/I-TASSER) [108]. Each modeled epitope will be docked with its specific MHC-I allele receptor using **ClusPro** (https://cluspro.bu.edu/home.php) [109] then imaged with **UCSF Chimera** 1.14 tool [110]. For docking analyzes with MHC-II alleles, epitope models will similarly be predicted with I-TASSER, and each epitope modeled (MHC-

II specific) can be found in EpiDock program (EpiDock Server Page (ddg-pharmfac.net) with their own MHC-II docking analysis will be performed by selecting alleles [111].

10. Modeling of the TCR-peptide-MHC-I complex

Peptide-MHC-I (pMHC-I) structure obtained for each epitope as a result of molecular docking TCR (PDB ID number 4WW1) with TCRpMHC models-1.0 program (http://www.cbs.dtu.dk/services/TCRpMHCmodels/) [112] and TCR-pMHC-I complexes will be formed. Modeled structures will be displayed in the UCSF Chimera 1.14 program [113]

11. Immune simulation

For in silico estimation of the immunological response to the designed vaccine, using the C-ImmSim 10.1 server (Rapin et al., 2010) (http://www.cbs.dtu.dk/services/C-ImmSim-10.0/) and selecting the default parameters. simulation will be performed.

RESULTS

1. Antigenicity:

On the 15 surface and secretory proteins studied, 10 of them are predicted as probable antigen and the rest as non-antigen. Antigenicity value varied from 0,5152 to 0,7875. The highest antigenicity predicted on **adhesin protein AP33-1,** while the lowest on **AP65-2 adhesin protein.** (Table 2)

2. Estimation of physicochemical parameters and secondary structures:

The number of amino acids varied from 117 to 827. The largest one was **LP07818P1 family protein** with ~ 94 kDa, while the smallest was **hypothetical protein TVAG_2v0540870** protein with ~ 14 kDa. The theoretical PI value was between 4,55 and 10,16. Among the 15 proteins, seven of them were predicted negatively charged and the rest was positively charged except, **succinyl-CoA synthetase beta-subunit** and **AP65-1 adhesin** that were neutral. The estimated half life was 30h for all proteins. Regarding the instability, only putative TBP-associated protein, LP07818P1 family, calponin-homology domain, CH-domain family and myeloid leukemia factor family were unstable, the other 11 proteins were stable. The aliphatic index among all proteins showed significant variation value between 56,92 and 102,4. The grand average of hydropathicity was estimated negative in the majority of the proteins except 4 of them (Table 3). The parameters of secondary structures is noted in table 4

3. Signal peptide prediction:

According to the prediction of signal peptide based on the four different parameters, sensitivity, specificity, Balanced prediction and Cleavage site. Only putative TBP-associated protein estimated to have a signal peptide in term of sensitivity. Regarding the cleavage site all of the proteins have a signal peptide except, **adhesin protein AP33-1**, **adhesin protein AP33-3**, **AP65-1 adhesin, LP07818P1 family and myeloid leukemia factor family**. (Table 4)

4. Prediction of BetaWrap motifs

Among all proteins analyzed only three were predicted to contain beta Wrap motifs, those proteins are cytotoxic cysteine proteinase, putative surface protein and surface protein named ‘’LP07818P1 family ‘’.

5. Estimation of subcellular localization and number of transmembrane helices:

The number of transmembrane helices among all proteins varied between 0 and 1. Only **surface immunogen P270 protein** and **putative surface protein** predicted to have one transmembrane helices. The other proteins had no transmembrane helices. When subcellular localization predictions were exanimated. putative TBP-associated protein, surface immunogen P270, putative surface protein is predicted to be in the **nucleus**, Otherwise AP65-1, AP65-2, AP65-3 adhesin proteins predicted to be located in the **Chloroplast. Cytoplasm**. adhesin protein AP33-1 as well as **adhesin protein AP33-3**, **AP51-2, and AP51-3** were located in the **Hydrogenosome**. The only protein predicted to be located on the **Lysosome** was cytotoxic cysteine proteinase. LP07818P1 family and **calponin-homology domain**, **CH-domain family** were located only in the Cytoplasm, However, myeloid leukemia factor family protein, was located in both Cytoplasm and Nucleus. (Table 2)

6. Allergenicity estimation:

All proteins analyzed predicted to be non-allergenic. The protein sequences does not contain experimentally proven IgE epitope. For cytotoxic cysteine proteinase protein, no results showed by the AlgPred program regarding IgE epitopes or MEME/MAST motif.

table 2: Antigenicity, solubility, transmembranes helics and Localisation results by Vaxijen, SolPro, TMHMM and Euk-Mploc respectively

Proteins	Antigenicity (Vaxijen)	Solubility (SolPro)	Transmembrane helices(TMHMM)	Localisation(Euk-Mploc)
adhesin protein AP33-1	0.7875 (Probable ANTIGEN).	SOLUBLE	0	Hydrogenosome.
adhesin protein AP33-3	0.7682 (Probable ANTIGEN).	SOLUBLE	0	Hydrogenosome.
succinyl-CoA synthetase beta-subunit	0.4163 (Probable NON-ANTIGEN).	SOLUBLE with probability 0.884417	0	Hydrogenosome.
succinyl-CoA synthetase beta-subunit	0.4011 (Probable NON-ANTIGEN).	SOLUBLE with probability 0.822566	0	Hydrogenosome.
AP65-1 adhesin	0.4758 (Probable NON-ANTIGEN).	SOLUBLE with probability 0.521599	0	Chloroplast. Cytoplasm
AP65-2 adhesin	0.5152 (Probable ANTIGEN).	INSOLUBLE with probability 0.653523	0	Chloroplast. Cytoplasm.
AP65-3 adhesin	0.4865 (Probable NON-ANTIGEN).	INSOLUBLE with probability 0.645290	0	Chloroplast. Cytoplasm.
putative TBP-associated protein	0.4078 (Probable NON-ANTIGEN).	INSOLUBLE with probability 0.720484	0	Nucleus.
cytotoxic cysteine proteinase	0.6074 (Probable ANTIGEN).	INSOLUBLE with probability 0.828211	0	Lysosome.
surface immunogen P270	0.5810 (Probable ANTIGEN).	INSOLUBLE with probability 0.514314	1	Nucleus.
putative surface protein	0.6586 (Probable ANTIGEN)	SOLUBLE with probability 0.872084	1	Nucleus.
LP07818P1 family	0.5668 (Probable ANTIGEN).	SOLUBLE with probability 0.971611	0	Cytoplasm.
calponin-homology domain, CH-domain family	0.5378 (Probable ANTIGEN).	SOLUBLE with probability 0.761095	0	Cytoplasm.
hypothetical protein TVAG_2v0540870	0.7196 (Probable ANTIGEN).	INSOLUBLE with probability 0.537861	0	Nucleus.
myeloid leukemia factor family	0.5689 (Probable ANTIGEN).	INSOLUBLE with probability 0.525027	0	Cytoplasm. Nucleus.

Table 3: Physico-chemical parameter resultes by ExPasyProtRaram

Protein	Number of amino acids	Molecular Weight	Theoretical P	Total Number of Negatively Charged Residues (Asp + Glu)	Total Number of Positively Charged Residues (Arg + Lys)	The estimated half-life (hour)	The instability index (II)	Aliphatic Index	GRAVY
adhesin protein AP33-1	309	32467.91	9.48	25	36	30	25.06/stable	90.36	0.026
adhesin protein AP33-3	309	32528.9	9.5	24	35	30	26.3/stable	89.71	0.009
adhesin protein AP51-2	409	43781.56	7.05	47	47	30	27.87/stable	98.75	0.043
adhesin protein AP51-3	409	43791.54	7.63	47	48	30	26.88/stable	97.56	0.022
AP65-1 adhesin	567	63281.92	7.22	67	67	30	31.16/stable	88.1	-0.202
AP65-2 adhesin	567	63087.79	8.35	64	68	30	33.24/stable	86.7	-0.204
AP65-3 adhesin	567	63133.88	8.15	67	70	30	30.88/stable	86.9	-0.211
putative TBP-associated protein	438	49945.1	6.03	50	45	30	40.89/unstable	102.4	-0.148
cytotoxic cysteine proteinase	305	33662.71	6.31	32	30	30	24.39/stable	64.33	-0.446
surface immunogen P270	526	58554.51	4.61	78	48	30	44.09/stable	83.37	-0.417
putative surface protein	625	67021.51	4.55	66	39	30	28.07/stable	75.65	-0.323
surface protein	827	93883.93	5.54	103	91	30	50.32/unstable	84.78	-0.404
surface protein	777	86035.66	9.52	114	147	30	62.85/unstable	56.92	-1.094
surface protein	117	13346.62	10.16	17	34	30	36.22/stable	57.69	-1.397
surface protein	344	40851.66	4.57	90	53	30	70.26/unstable	58.28	-1.224

Table 4 Signal peptide results by Signal BLAST

Proteins	Sensitivity	Specificity	Balanced prediction	Cleavage site
adhesin protein AP33-1	NO	NO	NO	NO
adhesin protein AP33-3	NO	NO	NO	NO
adhesin protein AP51-2	NO	NO	NO	YES
adhesin protein AP51-3	NO	NO	NO	YES
AP65-1 adhesin	NO	NO	NO	NO
AP65-2 adhesin	NO	NO	NO	YES
AP65-3 adhesin	NO	NO	NO	YES
putative TBP-associated protein	YES	NO	NO	YES
cytotoxic cysteine proteinase	NO	NO	NO	YES
surface immunogen P270	NO	NO	NO	YES
putative surface protein	NO	NO	NO	YES
surface protein	NO	NO	NO	NO
surface protein	NO	NO	NO	YES
surface protein	NO	NO	NO	YES
surface protein	NO	NO	NO	NO

Table 5: Secondary structure results by GOR IV

Proteins	Alpha helix (%)	Extended strand (%)	Random coil (%)
adhesin protein AP33-1	26.54%	25.24%	48.22%
adhesin protein AP33-3	28.16%	22.98%	48.87%
adhesin protein AP51-2	45.72%	15.40%	38.88%
adhesin protein AP51-3	41.32%	20.29%	38.39%
AP65-1 adhesin	32.28%	18.87%	48.85%
AP65-2 adhesin	29.45%	20.46%	50.09%
AP65-3 adhesin	29.98%	21.87%	48.15%
putative TBP-associated protein	36.53%	16.89%	46.58%
cytotoxic cysteine proteinase	28.85%	24.26%	46.89%
surface immunogen P270	17.68%	21.29%	61.03%
putative surface protein	6.72%	26.24%	67.04%
surface protein	34.70%	13.30%	52.00%
surface protein	24.97%	5.28%	69.76%
surface protein	58.97%	4.27%	36.75%
surface protein	34.30%	10.47%	55.23%

Table 6: Allergenicity, BetaWrap motifs results predicted by AlgPred, BetaWrap, respectively.

Protein	Allerginicity (AlgPred)		Beta wrap motif
	IgE epitope	MEME/MAST motif	
adhesin protein AP33-1	_	NON ALLERGEN	no
adhesin protein AP33-3	_	NON ALLERGEN	no
adhesin protein AP51-2	_	NON ALLERGEN	no
adhesin protein AP51-3	_	NON ALLERGEN	no
AP65-1 adhesin	_	NON ALLERGEN	no
AP65-2 adhesin	_	NON ALLERGEN	no
AP65-3 adhesin	_	NON ALLERGEN	no
putative TBP-associated protein	_	NON ALLERGEN	no
cytotoxic cysteine proteinase			P-value: 0.049
surface immunogen P270	_	NON ALLERGEN	no
putative surface protein	_	NON ALLERGEN	P-value: 0.00036
surface protein	_	NON ALLERGEN	P-value: 0.085
surface protein	_	NON ALLERGEN	no
surface protein	_	NON ALLERGEN	no
surface protein	_	NON ALLERGEN	no

7. B cell epitopes:

Among the fifteen-protein studied, only **adhesin protein AP33-1**, **adhesin protein AP33-3, LP07818P1 family, and calponin-homology domain CH-domain family protein**, were selected for the detection of B cell epitopes using IEDB program. Those proteins predicted to be antigenic, non-allergenic and soluble based on previous predictions.

six linear B cell epitopes were predicted for **adhesin protein AP33-1**, all of them were antigenic, and the value of antigenicity varied from 0.5696 to 1.7650. Regarding adhesin protein AP33-3 protein, Eight Linear B cell predicted, there were all antigenic excluding, NQGQFH and APPGKRMG with a value of -0.3170 and 0.2300 respectively.

The value of antigenicity for the other epitopes varied between 0.5696 and 1.8806. For the third protein, we predicted ten linear B cell epitopes, two of them were non-antigenic EDMKAFQA and STVKKIQGTSVLDAMKTSKVLDAIKTTK, with 0.1588 and -0.0776 respectively, the rest were predicted antigenic with a value varied between 0.6045 and 0.9655.

Regarding the fourth surface protein, we predicted one linear B cell epitopes , with an antigenicity valued 0.5284.

Epitopes predicted using IEDB were presented in table 7. The results obtained of the epitopes regarding the antigenicity showed that all epitopes had more antigenicity value than most of their own proteins.

8. Prediction of MHC-I and MHC-II epitopes

Not a lot of MHC-I epitopes were predicted antigenic (Table 8). Antigenicity values belonging to epitopes were generally predicted higher than those of their own proteins. For the First protein, **GTIIAGLPIF** epitope was predicted with the highest value of antigenicity (1.3258). regarding adhesin protein AP33-3 protein, we have predicted **GSIIAGLPIF** epitope with the highest antigenicity with a value of 1.2982. For LP07818P1 family protein, **MMKWVLAIYI** epitope had the highest value of antigenicity, 1.9328. Finaly, the last surface protein (calponin-homology domain, CH-

domain family) had **RTRRNTVFVK** epitope predicted as a highest antigenic epitope with a value of 1.1834. Only IQAIGPFLFF epitope showed a low IC50 value less than 10 and a Percentile rank of 0,03 indicating a strong binding among epitope and MHC-I alleles.
On the other hand, most of the MHC-II epitopes were predicted antigen (Table 9). Also, nearly all epitopes had higher antigenicity values than those of their own proteins. **PLLFIDKDTKVVIQG** and **QSFIINPEANIMSDI** epitopes showed the highest antigenicity with a value of 1.5237 and 1.3988 respectively.

9. Population Coverage Analysis

In order to find out the interaction of epitopes and their coverage to the world, separately with MHC-I alleles and as well with MHC-II alleles, we carried out 5 alleles of the most antigenic epitopes constructing the vaccine developped. For MHC-I and MHC-II separately. IEDB population coverage analysis tool from Immune epitope database was utilized. In order to design an efficae and opperative vaccine, the population coverage of the vaccine should be taking into consideration because the MHC HLA allele distribution may change in different regions worldwide and it can affect the efficacy of the vaccine. The maximum value of population exposure is observed in Finland (88.75%), followed with Germany and England (79.45%, 78.22% respectively). For turkey the coverage calculated 54.49%. However, the lowest value of population exposure was calculated 1.99% in Costa Rica. Three epitopes in MHC-I were found to be responsible for most of the interaction (GTIIAGLPIF, MMKWVLAIYI and RTRRNTVFVK), they caracterize a significant coverage. İn the other hand, only PLLFIDKDTKVVIQG was present in the interaction with MHC class II alleles.

Table 7: B cell epitopes predicted by both IEDB and antigenicity value predicted by Vaxijen v2.0.

		Peptide	**Antigenity value of epitope**
adhesin protein AP33-1	0.7875 Probable ANTIGEN	GDFSRNLHKPLLFID	1.3750 (Probable ANTIGEN).
		KKAGTIIAG	1.7650 (Probable ANTIGEN).
		IPQHDMIK	0.5696 (Probable ANTIGEN).
		TSEEDAAE	0.9578 (Probable ANTIGEN).
		APPGKRM	0.6605 (Probable ANTIGEN)
		VSGGKGTAEG	1.4547 (Probable ANTIGEN).
adhesin protein AP33-3	0.7682 Probable ANTIGEN	SFERNLHQPLLFI	0.9823 (Probable ANTIGEN)
		NQGQFH	-0.3170 (Probable NON-ANTIGEN
		KAGSIIA	1.0683 (Probable ANTIGEN)
		IPQHDMIK	0.5696 (Probable ANTIGEN)
		GTSEEDAAE	1.1737 (Probable ANTIGEN).
		KTKLTQ	1.8806 (Probable ANTIGEN
		APPGKRMG	0.2300 (Probable NON-ANTIGEN
		VSGGKGTAEG	1.4547 (Probable ANTIGEN)

LP07818P1 family	**0.5668 Probable ANTIGEN**	**TNGLNKKSKGAE**	**0.6830 (Probable ANTIGEN)**
		EDMKAFQA	0.1588 (Probable NON-ANTIGEN
		NKLFAETIQFYEKLTMYSFQLSEQIEEIF GPKEIKPVMAILQIVSKSHHFQALRLD ISELKPFTEKLTNLTLVYPESKHIGKTTPA HSFVITDLNHMKTPHTYLCKSIPDMMK WVLAIYITANAPESSLIPPAQRLENMLN QNAPAQPAVVKSVPKITVQTTQTQTKK PVPQETPKSQEPPKQPEPAKPAVAPPKP PEEVPKPAEPEKPKSEVQENKSEQKTEKT EPTQQEQPKTEETPQPAPQPAPPKPIFQ ITIETQPPPPPKKEQPTPIKKPLIITVKEAVS PTKSSVKLRKLKKEGEQNKQEENADNEQK VNLPPEVIAANYQSKPLDVQLNGYLETYSKI KKKSQLQLRINTIDKSLLPNSFKYDEKFIETEL EKICENFDKIEPNYDLSIPMDTVNPDQLVEST LTKLSGNPTINLSSIFDWNKFGIQYDIYFSEA SPKCPITTQLSICSQNFYASGNQIQSDTPDAFR FVFLV	0.6045 (Probable ANTIGEN)
		IFLNGLIEDSLVEPIKELALQTPELTSLVPKLFNISD	0.8441 (Probable ANTIGEN
		WSEQYYKKTAMMRNETSL	0.9557 (Probable ANTIGEN)
		EANIMSDIPACDKTRFIDTPI	0.8907 (Probable ANTIGEN
		FDEYKTNPSKMAESILTNGILQKKNFLQQYYAQ	0.7237 (Probable ANTIGEN).
		LQDNEITNS	0.9655 (Probable ANTIGEN).
		STVKKIQGTSVLDAMKTSKVLDAIKTTK	-0.0776 (Probable NON-ANTIGEN).
		SNWYEEEAIL	0.8291 (Probable ANTIGEN)
calponin-homology domain, CH-domain family	0.5378 Probable ANTIGEN	QKATKWLQQFLAIKGGSFTHIGTAFQDV QNLNIVIQLLTDEKDANYETINDALEAILN HKLMPKWDRLQETDINQSNMQKIVQFV NRLAGQYTIDQVKKDNANAKPSAKPAQK LPSPKSSAAAAPAPAHKPKPEKTSSVETTH LAKPPSPTSNSKPSPAAQSKPAEEDATISR SDFVSRPMMNKHRSNTIAPKRAAESILAA QAAIAGKPITPKAADTNSRPISRGSPREIS PKSSDSDKPNSFKARLAFAQSNLAHQNA LNPSAKISCNPPGHRNTSPTKPQPTFAEE PPKEEEKPVTRPALRPVPKEEPKKEVEEEK LVIKPALRPVPKEEPKKKVEPEKPISKPALK PVPKEEPKPEPKPVPKPEPKKEEPKKEEEEK PISRPPLRPVPKKEEEEKPVIKPALKPVPKEE PKPEPKKEEEEKPKEVEEEKPKPAFKPEPKK EEKPRASFNLPKKEEEEKKETPEPTKEEEEN KEAPKPTENEVLPPPKPVLTTDEVCVNSDM LNFPTAFRRTRRNTVFVKRPSLKDVSPKEPER PKPAESPVMRPAAMAGIPDQFSSPVAGGGG RARRPPMPGMGPGMPMPGLGAGMPKLRK VNQPGVPVYTKNEAGTPRPGKEEKKKEEKKE KKEKDPKLNEVPKPSGGGFFKKLFGKDKGKE EKTKEKSPKSSGNDINGWLSLMGVEVSNIAT DFKDGTNLIKLIKSMTGDEIEVKPVKPALLKFQ NPNLDEAIKYFDSKNLTAGKNHISKDDFMNGNEN	0.5284 (Probable ANTIGEN)

Table 8: Epitopes specifc to selected MHC-I alleles.

	Allele	start	end	lenght	Peptide	IC50	Penrcentile rank	Antigenecity
adhesin protein AP33-1	HLA-B*40:01	30	39	10	TYEAAYATTL	18.16	0.05	0.4349 (Probable NON-ANTIGEN
	HLA-A*03:01	36	45	10	SRLMRQYGTK	25.38	0.08	-0.0760 (Probable NON-ANTIGEN)
	HLA-B*15:01	56	65	10	**GTIIAGLPIF**	26.58	0.13	1.3258 (Probable ANTIGEN)
	HLA-B*15:01	4	13	10	RTDANASLIF	32.21	**0.16**	0.7814 (Probable ANTIGEN
	HLA-B*40:01	24	33	10	IEAAEAGMGL	33.14	0.08	0.2047 (Probable NON-ANTIGEN
	HLA-B*15:01	29	38	10	LTQEKPVVAF	37.78	0.2	0.4886 (Probable NON-ANTIGEN)
	HLA-B*15:01	22	31	10	IVSRSGTLTY	40.88	0.22	-0.1700 (Probable NON-ANTIGEN
	HLA-A*03:01	57	66	10	TIIAGLPIFK	43.96	**0.16**	0.5798 (Probable ANTIGEN
adhesin protein AP33-3	HLA-B*40:01	30	39	10	TYEAAYATTL	18.16	0.05	0.4349 (Probable NON-ANTIGEN
	HLA-B*15:01	56	65	10	**GSIIAGLPIF**	19.09	0.09	1.2982 (Probable ANTIGEN
	HLA-A*03:01	36	45	10	SRLMRQYGTK	25.38	0.08	-0.0760 (Probable NON-ANTIGEN
	HLA-B*15:01	4	13	10	RTDANASLIF	32.21	**0.16**	0.7814 (Probable ANTIGEN
	HLA-A*03:01	57	66	10	SIIAGLPIFK	32.6	0.11	0.4600 (Probable NON-ANTIGEN
	HLA-B*15:01	29	38	10	LTQEKPVVAF	37.78	0.2	0.4886 (Probable NON-ANTIGEN
	HLA-B*40:01	24	33	10	IEAAQAGMGL	38.95	0.1	0.3195 (Probable NON-ANTIGEN
	HLA-B*15:01	22	31	10	IVSRSGTLTY	40.88	0.22	-0.1700 (Probable NON-ANTIGEN

LP07818P1 family	**HLA-B*15:01**	**43**	**52**	**10**	**LQIVSKSHHF**	**4.19**	**0.02**	**0.4266 (Probable NON-ANTIGEN**
	HLA-B*15:01	12	21	10	LQQYYAQDPF	5.7	0.02	0.3558 (Probable NON-ANTIGEN
	HLA-B*58:01	38	47	10	KSIPDMMKWV	7.01	0.03	0.0562 (Probable NON-ANTIGEN
	HLA-B*58:01	37	46	10	CKSIPDMMKW	7.21	0.03	0.0162 (Probable NON-ANTIGEN)
	HLA-B*15:01	6	15	10	LQKKNFLQQY	8.56	0.03	0.2743 (Probable NON-ANTIGEN
	HLA-B*15:01	3	12	10	IQAIGPFLFF	8.84	0.03	0.9420 (Probable ANTIGEN
	HLA-B*15:01	31	40	10	LQDNEITNSF	10.66	0.04	0.4532 (Probable NON-ANTIGEN
	HLA-A*03:01	25	34	10	KLFNISDVIK	12.88	0.03	-0.9883 (Probable NON-ANTIGEN
	HLA-B*15:01	50	59	10	KVFMPISIVF	14.95	0.08	0.8726 (Probable ANTIGEN
	HLA-B*15:01	36	45	10	ILYDFFRAKF	16.96	0.09	1.5284 (Probable ANTIGEN
	HLA-B*15:01	59	68	10	FQSFEDMKAF	17.38	0.09	-0.3421 (Probable NON-ANTIGEN
	HLA-A*03:01	35	44	10	AILYDFFRAK	18.41	0.05	0.8639 (Probable ANTIGEN
	HLA-A*03:01	38	47	10	TMAAILINDK	23.26	0.07	1.0051 (Probable ANTIGEN
	HLA-B*15:01	56	65	10	RLDISELKPF	24.72	0.12	-0.2763 (Probable NON-ANTIGEN
	HLA-A*02:01	12	21	10	QMYKWLCILV	25.14	0.22	0.8623 (Probable ANTIGEN
	HLA-A*03:01	4	13	10	KLFAETIQFY	26.03	0.08	0.6531 (Probable ANTIGEN
	HLA-B*15:01	53	62	10	MPISIVFQSF	26.63	0.13	1.3520 (Probable ANTIGEN
	HLA-A*02:01	43	52	10	**MMKWVLAIYI**	27.34	0.24	1.9328 (Probable ANTIGEN
	HLA-B*15:01	4	13	10	KLFAETIQFY	27.67	0.14	0.6531 (Probable ANTIGEN
	HLA-B*15:01	47	56	10	IQGTSVLDAM	28.52	0.14	0.0517 (Probable NON-ANTIGEN
	HLA-B*40:01	7	16	10	AETIQFYEKL	30.42	0.07	0.2490 (Probable NON-ANTIGEN
	HLA-A*02:01	21	30	10	FQLSEQIEEI	31.25	0.28	-0.2401 (Probable NON-ANTIGEN
	HLA-B*15:01	55	64	10	VQLNGYLETY	31.37	0.16	1.6798 (Probable ANTIGEN)
	HLA-B*15:01	10	19	10	IQFYEKLTMY	31.86	0.16	0.4614 (Probable NON-ANTIGEN
	HLA-B*40:01	34	43	10	KEIKPVMAIL	32.26	0.08	0.6937 (Probable ANTIGEN
	HLA-B*15:01	43	52	10	ITIDSLGKVF	32.51	0.17	-0.3363 (Probable NON-ANTIGEN
	HLA-B*15:01	16	25	10	IDSIVNSQSF	33.41	0.17	0.5665 (Probable ANTIGEN
	HLA-B*58:01	21	30	10	VANHSIISNW	34.46	0.21	0.1979 (Probable NON-ANTIGEN
	HLA-A*02:01	6	15	10	SIFDWNKFGI	34.47	0.31	0.3068 (Probable NON-ANTIGEN
	HLA-A*02:01	45	54	10	FIDTPIFSYL	35.06	**0.31**	1.2743 (Probable ANTIGEN
	HLA-A*24:02	15	24	10	YYAQDPFDCI	39	0.07	0.3652 (Probable NON-ANTIGEN
	HLA-B*15:01	11	20	10	MQMYKWLCIL	39.23	0.2	0.8277 (Probable ANTIGEN
	HLA-A*03:01	36	45	10	ITNSFISTVK	41	0.14	0.3864 (Probable NON-ANTIGEN

calponin-homology domain, CH-domain family	**HLA-B*07:02**	**18**	**27**	**10**	**MPGMGPGMPM**	**3.28**	**0.01**	**0.1821 (Probable NON-ANTIGEN)**
	HLA-B*07:02	25	34	10	MPMPGLGAGM	4.63	0.02	-0.5050 (Probable NON-ANTIGEN
	HLA-B*07:02	51	60	10	RPKPAESPVM	10.88	0.04	0.3129 (Probable NON-ANTIGEN
	HLA-A*03:01	59	68	10	AILNHKLMPK	12.27	0.03	0.6248 (Probable ANTIGEN
	HLA-A*03:01	33	42	10	TVFVKRPSLK	17.91	0.05	0.8159 (Probable ANTIGEN
	HLA-B*07:02	55	64	10	APKRAAESIL	23.44	0.09	0.0673 (Probable NON-ANTIGEN
	HLA-B*15:01	39	48	10	KVNQPGVPVY	24.5	0.12	0.9022 (Probable ANTIGEN
	HLA-B*15:01	20	29	10	GSFTHIGTAF	24.57	0.12	0.5174 (Probable ANTIGEN
	HLA-B*58:01	40	49	10	KSSGNDINGW	25.31	**0.15**	0.7807 (Probable ANTIGEN)
	HLA-B*08:01	45	54	10	MMNKHRSNTI	25.86	0.07	0.6679 (Probable ANTIGEN
	HLA-A*03:01	28	37	10	**RTRRNTVFVK**	26	0.08	1.1834 (Probable ANTIGEN
	HLA-B*07:02	16	25	10	PPMPGMGPGM	26	0.11	-0.4054 (Probable NON-ANTIGEN
	HLA-B*07:02	12	21	10	RARRPPMPGM	26.18	0.11	0.0871 (Probable NON-ANTIGEN
	HLA-B*07:02	57	66	10	SPVMRPAAMA	36.03	0.15	0.5675 (Probable ANTIGEN
	HLA-A*02:01	51	60	10	SLMGVEVSNI	43.31	0.39	0.0163 (Probable NON-ANTIGEN)
	HLA-A*03:01	19	28	10	MLNFPTAFRR	46.51	0.17	-0.7803 (Probable NON-ANTIGEN

Table 9: Epitopes specifc to selected MHC-II alleles

	Allele	start	end	lenght	Peptide	IC50	Penrcentile rank	Antigenecity
adhesin protein AP33-1	HLA-DRB3*01:01	11	25	15	HKPLLFIDKDTKVVI	18.86	0.19	0.9966 (Probable ANTIGEN
	HLA-DRB3*01:01	12	26	15	KPLLFIDKDTKVVIQ	18.92	0.19	1.1104 (Probable ANTIGEN
	HLA-DRB3*01:01	10	24	15	LHKPLLFIDKDTKVV	24.62	0.36	0.8238 (Probable ANTIGEN)
	HLA-DRB1*03:01	12	26	15	KPLLFIDKDTKVVIQ	25.75	0.17	1.1104 (Probable ANTIGEN
	HLA-DRB5*01:01	55	69	15	AGTIIAGLPIFKNMK	25.88	1.4	1.1170 (Probable ANTIGEN
	HLA-DRB1*03:01	13	27	15	**PLLFIDKDTKVVIQG**	28.07	0.23	1.5237 (Probable ANTIGEN
	HLA-DRB1*03:01	14	28	15	LLFIDKDTKVVIQGI	29.58	0.27	1.0903 (Probable ANTIGEN
	HLA-DRB3*01:01	13	27	15	**PLLFIDKDTKVVIQG**	29.84	0.54	1.5237 (Probable ANTIGEN
	HLA-DRB5*01:01	56	70	15	GTIIAGLPIFKNMKE	34.1	2.2	1.0522 (Probable ANTIGEN
	HLA-DRB1*03:01	11	25	15	HKPLLFIDKDTKVVI	37.13	0.41	0.9966 (Probable ANTIGEN
	HLA-DRB5*01:01	54	68	15	KAGTIIAGLPIFKNM	37.83	2.5	0.9359 (Probable ANTIGEN
	HLA-DRB1*03:01	15	29	15	LFIDKDTKVVIQGIG	41.11	0.52	0.9863 (Probable ANTIGEN
	HLA-DRB1*15:01	36	50	15	SRLMRQYGTKVVGAV	43.04	1.8	0.7326 (Probable ANTIGEN
	HLA-DRB5*01:01	53	67	15	KKAGTIIAGLPIFKN	43.24	3	0.9438 (Probable ANTIGEN
	HLA-DRB5*01:01	10	24	15	SLIFVPAPGAAAACI	43.45	3	0.2085 (Probable NON-ANTIGEN).
	HLA-DRB3*01:01	9	23	15	NLHKPLLFIDKDTKV	43.91	0.94	0.8569 (Probable ANTIGEN)
	HLA-DRB5*01:01	8	22	15	NASLIFVPAPGAAAA	46.18	3.3	0.0448 (Probable NON-ANTIGEN
	HLA-DRB3*01:01	55	69	15	FAGQLHTDVVKRFAA	48.05	1	0.6050 (Probable ANTIGEN)
	HLA-DRB1*15:01	35	49	15	HSRLMRQYGTKVVGA	48.43	2.2	0.8001 (Probable ANTIGEN
adhesin protein AP33-3	HLA-DRB3*01:01	12	26	15	QPLLFIDKDTKVVIQ	20.59	0.26	1.0758 (Probable ANTIGEN
	HLA-DRB3*01:01	11	25	15	HQPLLFIDKDTKVVI	20.68	0.27	0.9974 (Probable ANTIGEN
	HLA-DRB5*01:01	55	69	15	AGSIIAGLPIFKNMK	26.37	1.4	1.0999 (Probable ANTIGEN
	HLA-DRB1*03:01	12	26	15	QPLLFIDKDTKVVIQ	26.68	0.2	1.0758 (Probable ANTIGEN
	HLA-DRB3*01:01	10	24	15	LHQPLLFIDKDTKVV	27.57	0.47	0.9080 (Probable ANTIGEN
	HLA-DRB1*03:01	13	27	15	**PLLFIDKDTKVVIQG**	28.07	0.23	1.5237 (Probable ANTIGEN
	HLA-DRB1*03:01	14	28	15	LLFIDKDTKVVIQGI	29.58	0.27	1.0903 (Probable ANTIGEN)
	HLA-DRB3*01:01	13	27	15	**PLLFIDKDTKVVIQG**	29.84	0.54	1.5237 (Probable ANTIGEN).
	HLA-DRB5*01:01	56	70	15	GSIIAGLPIFKNMKE	34.88	2.3	1.0364 (Probable ANTIGEN
	HLA-DRB5*01:01	54	68	15	KAGSIIAGLPIFKNM	38.89	2.6	0.8694 (Probable ANTIGEN
	HLA-DRB1*03:01	11	25	15	HQPLLFIDKDTKVVI	39.78	0.44	0.9974 (Probable ANTIGEN
	HLA-DRB1*03:01	15	29	15	LFIDKDTKVVIQGIG	41.11	0.52	0.9863 (Probable ANTIGEN
	HLA-DRB1*15:01	36	50	15	SRLMRQYGTKVVGAV	43.04	1.8	0.7326 (Probable ANTIGEN
	HLA-DRB5*01:01	10	24	15	SLIFVPAPGAAAACI	43.45	3	0.2085 (Probable NON-ANTIGEN
	HLA-DRB5*01:01	53	67	15	KKAGSIIAGLPIFKN	45.53	3.2	0.8479 (Probable ANTIGEN
	HLA-DRB5*01:01	8	22	15	NASLIFVPAPGAAAA	46.18	3.3	0.0448 (Probable NON-ANTIGEN
	HLA-DRB3*01:01	55	69	15	FAGQLHTDVVKRFAA	48.05	1	0.6050 (Probable ANTIGEN
	HLA-DRB1*15:01	35	49	15	HSRLMRQYGTKVVGA	48.43	2.2	0.8001 (Probable ANTIGEN

LP07818P1 family	**HLA-DRB3*02:02**	**17**	**31**	**15**	**LCILVANHSIISNWY**	**12.71**	**0.22**	**0.5054 (Probable ANTIGEN**
	HLA-DRB3*02:02	16	30	15	WLCILVANHSIISNW	13.96	0.26	0.6414 (Probable ANTIGEN
	HLA-DRB1*07:01	23	37	15	HIVFVPTSTNGLNKK	14.09	0.5	-0.4298 (Probable NON-ANTIGEN
	HLA-DRB1*07:01	22	36	15	VHIVFVPTSTNGLNK	14.39	0.55	-0.4980 (Probable NON-ANTIGEN
	HLA-DRB3*02:02	18	32	15	CILVANHSIISNWYE	14.43	0.28	0.6260 (Probable ANTIGEN
	HLA-DRB1*15:01	7	21	15	GPFLFFYQNNDLKDM	15.15	0.21	0.5902 (Probable ANTIGEN
	HLA-DRB1*07:01	21	35	15	MVHIVFVPTSTNGLN	15.22	0.69	-0.1275 (Probable NON-ANTIGEN)
	HLA-DRB1*07:01	24	38	15	IVFVPTSTNGLNKKS	15.25	0.69	-0.2556 (Probable NON-ANTIGEN
	HLA-DRB3*02:02	19	33	15	ILVANHSIISNWYEE	15.75	0.33	0.8585 (Probable ANTIGEN
	HLA-DRB3*02:02	15	29	15	KWLCILVANHSIISN	15.98	0.34	0.7063 (Probable ANTIGEN)
	HLA-DRB1*15:01	5	19	15	AIGPFLFFYQNNDLK	16.2	0.28	0.8400 (Probable ANTIGEN
	HLA-DRB1*07:01	20	34	15	DMVHIVFVPTSTNGL	16.29	0.74	-0.2346 (Probable NON-ANTIGEN
	HLA-DRB1*15:01	8	22	15	PFLFFYQNNDLKDMV	16.68	0.35	0.9166 (Probable ANTIGEN
	HLA-DRB1*15:01	6	20	15	IGPFLFFYQNNDLKD	17.48	0.4	0.7439 (Probable ANTIGEN
	HLA-DRB3*02:02	21	35	15	NSQSFIINPEANIMS	17.54	0.42	1.0512 (Probable ANTIGEN
	HLA-DRB1*07:01	14	28	15	MLIDSIVNSQSFIIN	20.29	1.2	0.9370 (Probable ANTIGEN
	HLA-DRB1*07:01	15	29	15	LIDSIVNSQSFIINP	20.74	1.2	0.8401 (Probable ANTIGEN
	HLA-DRB3*02:02	20	34	15	VNSQSFIINPEANIM	21.27	0.58	1.0335 (Probable ANTIGEN
	HLA-DRB3*02:02	22	36	15	SQSFIINPEANIMSD	23.22	0.66	1.2282 (Probable ANTIGEN)
	HLA-DRB1*07:01	41	55	15	FRAKFVISQIYQFEK	23.63	1.4	0.3233 (Probable NON-ANTIGEN
	HLA-DRB1*03:01	52	66	15	FQALRLDISELKPFT	24.28	0.15	0.3802 (Probable NON-ANTIGEN
	HLA-DRB1*03:01	51	65	15	HFQALRLDISELKPF	25.22	0.15	0.1005 (Probable NON-ANTIGEN
	HLA-DRB1*07:01	16	30	15	IDSIVNSQSFIINPE	25.26	1.5	0.7169 (Probable ANTIGEN
	HLA-DRB1*07:01	13	27	15	LMLIDSIVNSQSFII	25.91	1.7	0.6927 (Probable ANTIGEN
	HLA-DRB3*02:02	56	70	15	FDEYKTNPSKMAESI	26.08	0.72	0.5492 (Probable ANTIGEN
	HLA-DRB1*15:01	9	23	15	FLFFYQNNDLKDMVH	26.1	0.7	0.7625 (Probable ANTIGEN
	HLA-DRB1*07:01	25	39	15	VFVPTSTNGLNKKSK	26.13	1.7	0.0335 (Probable NON-ANTIGEN
	HLA-DRB3*01:01	45	59	15	GNQIQSDTPDAFRFV	26.15	0.41	0.9318 (Probable ANTIGEN
	HLA-DRB3*02:02	23	37	15	**QSFIINPEANIMSDI**	26.39	0.72	1.3988 (Probable ANTIGEN
	HLA-DRB4*01:01	1	15	15	SQLQLRINTIDKSLL	27.11	0.12	0.4592 (Probable NON-ANTIGEN
	HLA-DRB4*01:01	2	16	15	QLQLRINTIDKSLLP	27.56	0.12	0.4804 (Probable NON-ANTIGEN
	HLA-DRB3*02:02	48	62	15	LAIYITANAPESSLI	27.58	0.75	1.1620 (Probable ANTIGEN
	HLA-DRB1*07:01	42	56	15	RAKFVISQIYQFEKG	28.09	1.8	-0.2153 (Probable NON-ANTIGEN
	HLA-DRB1*07:01	40	54	15	FFRAKFVISQIYQFE	28.74	1.9	0.5632 (Probable ANTIGEN
	HLA-DRB3*01:01	46	60	15	NQIQSDTPDAFRFVF	29.91	0.54	0.6691 (Probable ANTIGEN
	HLA-DRB1*03:01	50	64	15	HHFQALRLDISELKP	31.81	0.31	0.2535 (Probable NON-ANTIGEN
	HLA-DRB1*03:01	53	67	15	QALRLDISELKPFTE	31.94	0.31	0.4514 (Probable NON-ANTIGEN

	HLA-DRB1*07:01	**39**	**53**	**15**	**DFFRAKFVISQIYQF**	**31.94**	**2.1**	**0.4196 (Probable NON-ANTIGEN**
	HLA-DRB4*01:01	3	17	15	LQLRINTIDKSLLPN	32.39	0.19	0.4659 (Probable NON-ANTIGEN)
	HLA-DRB1*15:01	45	59	15	KWVLAIYITANAPES	33.26	1.2	1.3829 (Probable ANTIGEN
	HLA-DRB1*07:01	17	31	15	DSIVNSQSFIINPEA	34.26	2.3	0.7036 (Probable ANTIGEN
	HLA-DRB4*01:01	9	23	15	IKELALQTPELTSLV	34.29	0.22	0.8230 (Probable ANTIGEN
	HLA-DRB3*02:02	49	63	15	AIYITANAPESSLIP	34.32	0.92	1.0446 (Probable ANTIGEN
	HLA-DRB4*01:01	49	63	15	SHHFQALRLDISELK	35.31	0.23	0.1801 (Probable NON-ANTIGEN
	HLA-DRB1*07:01	18	32	15	SIVNSQSFIINPEAN	35.53	2.4	0.6820 (Probable ANTIGEN
	HLA-DRB4*01:01	50	64	15	HHFQALRLDISELKP	36.46	0.24	0.2535 (Probable NON-ANTIGEN
	HLA-DRB3*01:01	44	58	15	SGNQIQSDTPDAFRF	36.67	0.69	0.8646 (Probable ANTIGEN
	HLA-DRB5*01:01	56	70	15	QLNGYLETYSKIKKK	37.69	2.5	0.6975 (Probable ANTIGEN).
	HLA-DRB1*15:01	44	58	15	MKWVLAIYITANAPE	38.27	1.5	1.2680 (Probable ANTIGEN
	HLA-DRB4*01:01	48	62	15	KSHHFQALRLDISEL	39.77	0.29	0.2096 (Probable NON-ANTIGEN
	HLA-DRB4*01:01	8	22	15	PIKELALQTPELTSL	39.79	0.29	0.6462 (Probable ANTIGEN
	HLA-DRB1*07:01	43	57	15	LQIVSKSHHFQALRL	40.83	2.9	0.4905 (Probable NON-ANTIGEN
	HLA-DRB1*15:01	4	18	15	QAIGPFLFFYQNNDL	40.91	1.7	0.4253 (Probable NON-ANTIGEN)
	HLA-DRB3*02:02	55	69	15	DFDEYKTNPSKMAES	41.99	1.1	0.4729 (Probable NON-ANTIGEN
	HLA-DRB3*02:02	24	38	15	SFIINPEANIMSDIP	42.24	1.1	1.3700 (Probable ANTIGEN
	HLA-DRB1*15:01	43	57	15	MMKWVLAIYITANAP	42.99	1.8	1.3035 (Probable ANTIGEN
	HLA-DRB3*02:02	1	15	15	SQLQLRINTIDKSLL	43	1.1	0.4592 (Probable NON-ANTIGEN)
	HLA-DRB3*02:02	14	28	15	YKWLCILVANHSIIS	45.12	1.2	0.4297 (Probable NON-ANTIGEN
	HLA-DRB3*02:02	2	16	15	QLQLRINTIDKSLLP	45.2	1.2	0.4804 (Probable NON-ANTIGEN
	HLA-DRB4*01:01	10	24	15	KELALQTPELTSLVP	45.64	0.39	0.9934 (Probable ANTIGEN
	HLA-DRB5*01:01	16	30	15	QYDIYFSEASPKCPI	46.81	3.3	0.8393 (Probable ANTIGEN)
	HLA-DRB1*07:01	35	49	15	AILYDFFRAKFVISQ	46.86	3.6	1.0071 (Probable ANTIGEN
	HLA-DRB4*01:01	51	65	15	HFQALRLDISELKPF	46.96	0.4	0.1005 (Probable NON-ANTIGEN
	HLA-DRB1*07:01	45	59	15	IVSKSHHFQALRLDI	47.61	3.6	0.6563 (Probable ANTIGEN
	HLA-DRB3*01:01	47	61	15	QIQSDTPDAFRFVFL	47.72	1	1.0363 (Probable ANTIGEN
	HLA-DRB3*02:02	54	68	15	LDFDEYKTNPSKMAE	48.42	1.3	0.5242 (Probable ANTIGEN
	HLA-DRB1*07:01	43	57	15	AKFVISQIYQFEKGD	49.01	3.8	-0.4980 (Probable NON-ANTIGEN).

calponin-homology domain, CH-domain family	**HLA-DRB3*02:02**	**17**	**31**	**15**	**LCILVANHSIISNWY**	**12.71**	**0.22**	**0.5054 (Probable ANTIGEN)**
	HLA-DRB3*02:02	16	30	15	WLCILVANHSIISNW	13.96	0.26	0.6414 (Probable ANTIGEN
	HLA-DRB1*07:01	23	37	15	HIVFVPTSTNGLNKK	14.09	0.5	-0.4298 (Probable NON-ANTIGEN
	HLA-DRB1*07:01	22	36	15	VHIVFVPTSTNGLNK	14.39	0.55	-0.4980 (Probable NON-ANTIGEN)
	HLA-DRB3*02:02	18	32	15	CILVANHSIISNWYE	14.43	0.28	0.6260 (Probable ANTIGEN)
	HLA-DRB1*15:01	7	21	15	GPFLFFYQNNDLKDM	15.15	0.21	0.5902 (Probable ANTIGEN
	HLA-DRB1*07:01	21	35	15	MVHIVFVPTSTNGLN	15.22	0.69	-0.1275 (Probable NON-ANTIGEN
	HLA-DRB1*07:01	24	38	15	IVFVPTSTNGLNKKS	15.25	0.69	-0.2556 (Probable NON-ANTIGEN
	HLA-DRB3*02:02	19	33	15	ILVANHSIISNWYEE	15.75	0.33	0.8585 (Probable ANTIGEN
	HLA-DRB3*02:02	15	29	15	KWLCILVANHSIISN	15.98	0.34	0.7063 (Probable ANTIGEN
	HLA-DRB1*15:01	5	19	15	AIGPFLFFYQNNDLK	16.2	0.28	0.8400 (Probable ANTIGEN)
	HLA-DRB1*07:01	20	34	15	DMVHIVFVPTSTNGL	16.29	0.74	-0.2346 (Probable NON-ANTIGEN
	HLA-DRB1*15:01	8	22	15	PFLFFYQNNDLKDMV	16.68	0.35	0.9166 (Probable ANTIGEN
	HLA-DRB1*15:01	6	20	15	IGPFLFFYQNNDLKD	17.48	0.4	0.8988 (Probable ANTIGEN
	HLA-DRB3*02:02	21	35	15	NSQSFIINPEANIMS	17.54	0.42	1.0512 (Probable ANTIGEN)
	HLA-DRB1*07:01	14	28	15	MLIDSIVNSQSFIIN	20.29	1.2	0.9370 (Probable ANTIGEN
	HLA-DRB1*07:01	15	29	15	LIDSIVNSQSFIINP	20.74	1.2	0.8401 (Probable ANTIGEN
	HLA-DRB3*02:02	20	34	15	VNSQSFIINPEANIM	21.27	0.58	1.0335 (Probable ANTIGEN
	HLA-DRB3*02:02	22	36	15	SQSFIINPEANIMSD	23.22	0.66	1.2282 (Probable ANTIGEN
	HLA-DRB1*07:01	41	55	15	FRAKFVISQIYQFEK	23.63	1.4	0.3233 (Probable NON-ANTIGEN
	HLA-DRB1*03:01	52	66	15	FQALRLDISELKPFT	24.28	0.15	0.3802 (Probable NON-ANTIGEN)
	HLA-DRB1*03:01	51	65	15	HFQALRLDISELKPF	25.22	0.15	0.1005 (Probable NON-ANTIGEN
	HLA-DRB1*07:01	16	30	15	IDSIVNSQSFIINPE	25.26	1.5	0.7169 (Probable ANTIGEN
	HLA-DRB1*07:01	13	27	15	LMLIDSIVNSQSFII	25.91	1.7	0.6927 (Probable ANTIGEN
	HLA-DRB3*02:02	56	70	15	FDEYKTNPSKMAESI	26.08	0.72	0.5492 (Probable ANTIGEN
	HLA-DRB1*15:01	9	23	15	FLFFYQNNDLKDMVH	26.1	0.7	0.7625 (Probable ANTIGEN
	HLA-DRB1*07:01	25	39	15	VFVPTSTNGLNKKSK	26.13	1.7	0.0335 (Probable NON-ANTIGEN
	HLA-DRB3*01:01	45	59	15	GNQIQSDTPDAFRFV	26.15	0.41	0.9318 (Probable ANTIGEN
	HLA-DRB3*02:02	23	37	15	**QSFIINPEANIMSDI**	26.39	0.72	1.3988 (Probable ANTIGEN
	HLA-DRB4*01:01	1	15	15	SQLQLRINTIDKSLL	27.11	0.12	0.4592 (Probable NON-ANTIGEN
	HLA-DRB4*01:01	2	16	15	QLQLRINTIDKSLLP	27.56	0.12	0.4804 (Probable NON-ANTIGEN
	HLA-DRB3*02:02	48	62	15	LAIYITANAPESSLI	27.58	0.75	1.1620 (Probable ANTIGEN
	HLA-DRB1*07:01	42	56	15	RAKFVISQIYQFEKG	28.09	1.8	-0.2153 (Probable NON-ANTIGEN
	HLA-DRB1*07:01	40	54	15	FFRAKFVISQIYQFE	28.74	1.9	0.5632 (Probable ANTIGEN
	HLA-DRB3*01:01	46	60	15	NQIQSDTPDAFRFVF	29.91	0.54	0.6691 (Probable ANTIGEN
	HLA-DRB1*03:01	50	64	15	HHFQALRLDISELKP	31.81	0.31	0.2535 (Probable NON-ANTIGEN)
	HLA-DRB1*03:01	53	67	15	QALRLDISELKPFTE	31.94	0.31	0.4514 (Probable NON-ANTIGEN
	HLA-DRB1*07:01	39	53	15	DFFRAKFVISQIYQF	31.94	2.1	0.4196 (Probable NON-ANTIGEN
	HLA-DRB4*01:01	3	17	15	LQLRINTIDKSLLPN	32.39	0.19	0.4659 (Probable NON-ANTIGEN
	HLA-DRB1*15:01	45	59	15	KWVLAIYITANAPES	33.26	1.2	1.3829 (Probable ANTIGEN
	HLA-DRB1*07:01	17	31	15	DSIVNSQSFIINPEA	34.26	2.3	0.7036 (Probable ANTIGEN

	HLA-DRB4*01:01	**9**	**23**	**15**	**IKELALQTPELTSLV**	**34.29**	**0.22**	**0.8230 (Probable ANTIGEN**
	HLA-DRB3*02:02	49	63	15	AIYITANAPESSLIP	34.32	0.92	1.0446 (Probable ANTIGEN
	HLA-DRB4*01:01	49	63	15	SHHFQALRLDISELK	35.31	0.23	0.1801 (Probable NON-ANTIGEN
	HLA-DRB1*07:01	18	32	15	SIVNSQSFIINPEAN	35.53	2.4	0.6820 (Probable ANTIGEN
	HLA-DRB4*01:01	50	64	15	HHFQALRLDISELKP	36.46	0.24	0.2535 (Probable NON-ANTIGEN
	HLA-DRB3*01:01	44	58	15	SGNQIQSDTPDAFRF	36.67	0.69	0.8646 (Probable ANTIGEN
	HLA-DRB5*01:01	56	70	15	QLNGYLETYSKIKKK	37.69	2.5	0.6975 (Probable ANTIGEN
	HLA-DRB1*15:01	44	58	15	MKWVLAIYITANAPE	38.27	1.5	1.2680 (Probable ANTIGEN
	HLA-DRB4*01:01	48	62	15	KSHHFQALRLDISEL	39.77	0.29	0.2096 (Probable NON-ANTIGEN
	HLA-DRB4*01:01	8	22	15	PIKELALQTPELTSL	39.79	0.29	0.6462 (Probable ANTIGEN
	HLA-DRB1*07:01	43	57	15	LQIVSKSHHFQALRL	40.83	2.9	0.4905 (Probable NON-ANTIGEN
	HLA-DRB1*15:01	4	18	15	QAIGPFLFFYQNNDL	40.91	1.7	0.4253 (Probable NON-ANTIGEN
	HLA-DRB3*02:02	55	69	15	DFDEYKTNPSKMAES	41.99	1.1	0.4729 (Probable NON-ANTIGEN
	HLA-DRB3*02:02	24	38	15	SFIINPEANIMSDIP	42.24	1.1	1.3700 (Probable ANTIGEN)
	HLA-DRB1*15:01	43	57	15	MMKWVLAIYITANAP	42.99	1.8	1.3035 (Probable ANTIGEN
	HLA-DRB3*02:02	1	15	15	SQLQLRINTIDKSLL	43	1.1	0.4592 (Probable NON-ANTIGEN
	HLA-DRB3*02:02	14	28	15	YKWLCILVANHSIIS	45.12	1.2	0.4297 (Probable NON-ANTIGEN)
	HLA-DRB3*02:02	2	16	15	QLQLRINTIDKSLLP	45.2	1.2	0.4804 (Probable NON-ANTIGEN
	HLA-DRB4*01:01	10	24	15	KELALQTPELTSLVP	45.64	0.39	0.9934 (Probable ANTIGEN)
	HLA-DRB5*01:01	16	30	15	QYDIYFSEASPKCPI	46.81	3.3	0.8393 (Probable ANTIGEN
	HLA-DRB1*07:01	35	49	15	AILYDFFRAKFVISQ	46.86	3.6	1.0071 (Probable ANTIGEN
	HLA-DRB4*01:01	51	65	15	HFQALRLDISELKPF	46.96	0.4	0.1005 (Probable NON-ANTIGEN
	HLA-DRB1*07:01	45	59	15	IVSKSHHFQALRLDI	47.61	3.6	0.6563 (Probable ANTIGEN)
	HLA-DRB3*01:01	47	61	15	QIQSDTPDAFRFVFL	47.72	1	1.0363 (Probable ANTIGEN
	HLA-DRB3*02:02	54	68	15	LDFDEYKTNPSKMAE	48.42	1.3	0.5242 (Probable ANTIGEN
	HLA-DRB1*07:01	43	57	15	AKFVISQIYQFEKGD	49.01	3.8	-0.4980 (Probable NON-ANTIGEN

10. Vaccine Construction and Sequence Assembly

Finally, 4 B-Cell epitopes, 3 MHC-I and 2 MHC-II epitopes were be combained to construct a multiepitope vaccine. An addition of an adjuvant is necessary to the N terminus of the peptide sequence, the heparin-binding hemagglutinin (HBHA) of Mycobacterium tuberculosis (199 aa) was the adjuvant added to increase the immune response. İt was attached to the first B-Cell epitope through an **EAAAK linker** in order to prompt a specific immune response. Moreover the binding of B-Cell epitopes and MHC-I epitopes was done via **CPGPG linkers** whereas for connecting the MHC-II epitopes, **AAY linkers** were utilized. The final vaccine construct was consisted of 1081 amino acids and the calculated molecular weight was 117754.82 kDA. Diagrammatic explanation of vaccine construct showen in Figure 3

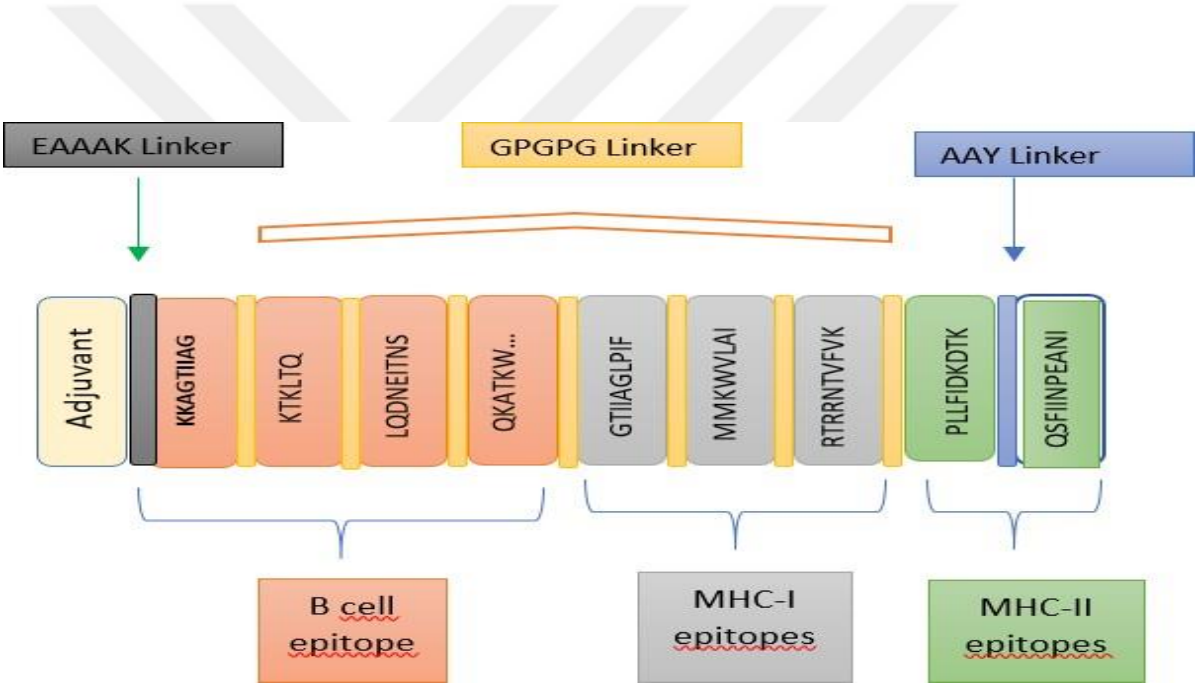

Figure 3. Schematic representation of the final multiepitope vaccine construct containing an adjuvant protein, B, and MHC-I and II epitopes linked with linkers. Colors indicated as follows; Light yellow: adjuvant, Dark grey: EAAAK linker, orange: B cell epitopes, dark yellow: GPGPG linkers, light grey: MHC-I epitopes, green: MHC-II epitopes, and blue: AAY linkers

11. Antigenicity or Allergenicity Assessment of Vaccine Protein

Using vaxigen 2.0 tool, we determined the antigenicity of the constructed vaccine sequence, attached to the adjuvant, found to be antigen with a value of 0.5677 . By eliminating the adjuvant, the value of antigenicity predicted to be 0.5571, so it was an antigen according to the predictions.

The results determined that the sequence have an antigenic nature, attached or not to the adjuvant. For the allergenicity, we used AlgPred 2.0 tool, the sequence in the absence or the presence adjuvant is non allergenic by nature.

12. Solubility and Physiochemical Properties Analysis

We used ExPASy-ProtParam in order to determine the Physico-chemical parameters of the specific vaccine construct, we found that the Molecular weight 117754.82 Da. The theoretical Isoelectric Point Value (PI) was found to be 9.49. Total number of negatively charged residues (Asp + Glu) predicted to be 152, Total number of positively charged residues (Arg + Lys) found to be 190. The estimated half-life of the developed vaccine is 30 hours (mammalian reticulocytes, in vitro), >20 hours (yeast, in vivo); and >10 hours (Escherichia coli, in vivo).

The instability index (II) for the vaccine sequence was predicted by Protpram as The instability index (II) is computed to be 55.38 .This classifies the protein as unstable..

The estimation of aliphatic index was about 64.72. The prediction of the Grand Average of Hydropathicity (GRAVY) was found to be about -0.891. Further annalysize must be conducted regarding the stability of the vaccine. The SOLpro server was utilized for the solubility rate calculation of the vaccine construct that was found to be soluble with a value of 0.861165.

13. Prediction of Secondary and Tertiary Structure

the prediction of secondary structure was done using GOR IV tool. The fenerated structure confirmed that the protein consists of 34.97% alpha helix, 7.59% extended strand and 57,45% coil. Secondary strucure is given in Figure 4. The tertiary structure prediction chimeric construct of vaccine was done using I-TASSER server. There were total 5 models provided by the server 3D structure is shown in Figure 5.

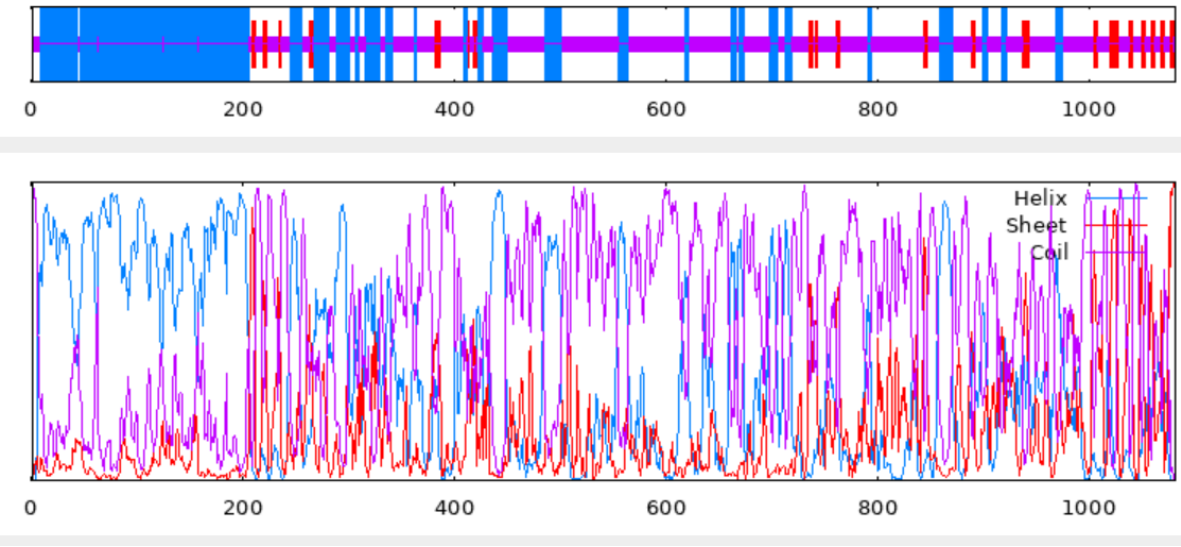

Figure 4: Graphical representations of secondary structure of final multiepitope vaccine candidate. Results of GOR IV online server.

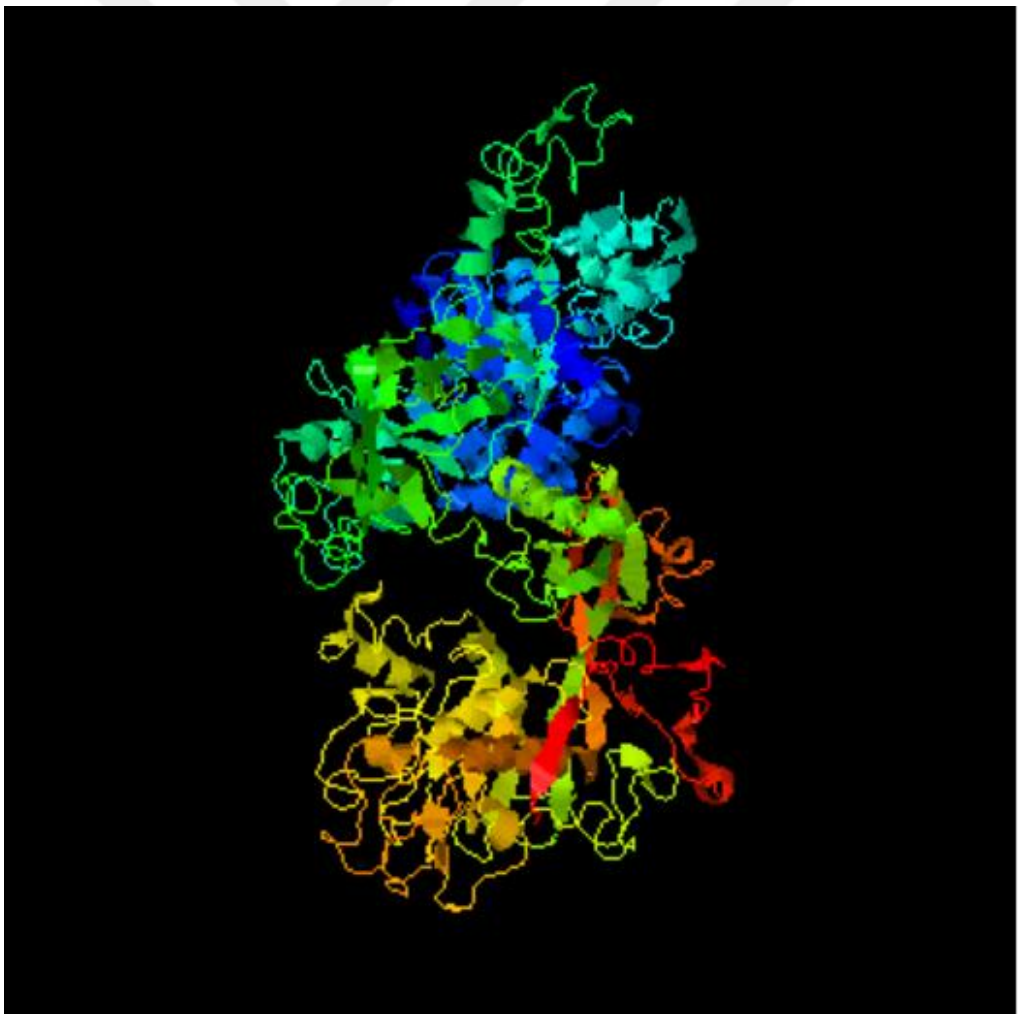

Figure 5: Tertiary Structure of the constructed vaccine

DISCUSSION

Trichomonisis is the most common, sexually transmitted disease, caused by **Trichomonas vaginalis flagelatted protozoan, the amount of symptoms are numerous including , vaginitis** and infections have been associated with preterm delivery, low birth weight and increased infant mortality, as well as predisposing to HIV/AIDS and cervical cancer in men and women. In order to enhance the pathogenicity of this disease, the 5- nitroimidazole drugs, are the most prescribed and approaved, effective drugs to treat trichomoniasis. However, Some treatment failures demonstrate that T. vaginalis provide mechanism resistance against metronidazole [114]. Therefore it is necessary to find a new cure to fight against this viral disease.

In this computationally advanced era of computer aided drug design and immunoinformatics based technology, bioinformatics and immunoinformatics tools provide a novel approach for the designing of multiepitope peptide-based vaccine.

According to the in silico analyses results ,the physiochemical properties revealed the high antigenicity with or without the adjuvant, non allergenicity and non- toxic, which make a good vaccine candidate. The analyses showed a negative GRAVY value indicating that the vaccine is hydrophilic and has a better interaction with surrounding water molecules. Also, it had soluble characteristics which are important parameters for biophysical studies on epitope-based vaccine design. However , The parameter of stability is the only inconvinient for the choice of this vaccine, further studies and analyses must be done regarding this parameter.

Overall, this study was a main focus on the designing of multiepitope subunit vaccine by the use of advanced technological and bioinformatics approaches to increase the humoral and cell mediated immune response. For the design of this vaccine, different componounds were necessary and must be combined to result in such a developped vaccine, B cell epitopes, MHC-I and II , since T-Cell immunity can provide long-lasting response as any antigen can escape the antibody memorial response because of the antigenic drift [115].

Moreover, population coverage analysis was done using IEDB population coverage analysis tool and the presence of interacting human MHC (HLA) alleles was found in thousand diverse humans [116]. For this reason, both MHC Class-I and Class-II were analyzed and the results

concluded the wide coverage of designed vaccine around the globe. 4 B Cell epitopes, 3 MHC-I and 2 MHC II epitopes selected for the developed vaccine.

CONCLUSION

In this study, a multi-epitope based vaccine in designed against Trichomoniasis disease using in sillico umminoinformatics approaches. Artificially, the vaccine can be a good candidate vaccine, especially it is antigenic, non-allergenic and non-toxic. The stability parameter should be studied further. The vaccine candidate shows also a good population coverage in finland and Germany. In-silico designed vaccine candidate could prove effective if it is tested in-vitro and in-vivo and also good for clinically trials. As it can show good immune response in clinical trials,

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ANTIGENIC PEPTIDE DISCOVERY AND MULTI-EPITOPE VACCINE DESIGN OF TRICHOMONAS VAGINALIS PATHOGEN USING IN-SILICO BIOINFORMATICS.

ORİJİNALLİK RAPORU

%18	%15	%17	%12
BENZERLİK ENDEKSİ	İNTERNET KAYNAKLARI	YAYINLAR	ÖĞRENCİ ÖDEVLERİ

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4	Manojit Bhattacharya, Ashish Ranjan Sharma, Pratik Ghosh, Sang-Soo Lee, Chiranjib Chakraborty. "A Next-Generation Vaccine Candidate Using Alternative Epitopes to Protect against Wuhan and All Significant Mutant Variants of SARS-CoV-2: An Immunoinformatics Approach", Aging and disease, 2021 Yayın	%1