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# Chapter 1

## THE ROLE OF PERCEIVED NEED WITHIN THE THEORY OF PLANNED BEHAVIOUR: A COMPARISON OF EXERCISE AND HEALTHY EATING

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Nowadays people are much aware of the importance of healthy eating. As Norman and Smith (1995) mentioned that the issue of exercise and healthy eating behaviours has received a lot of attention in recent years. So many evidences showed that the role of blood cholesterol levels as a major risk factor for coronary hearth disease (Law et al. 1994). For instance, Stampler et al. (1986) study showed that followed over 350.000 adults for six years and found a linear relationship between blood cholesterol level and the incidence of CHD and stroke. Healthy eating is also linked to cancer; Austoker (1994) for example suggested that up to 25 percent of cancer related deaths are related to dietary factors, including high fat, saturated fat, preserved food and salt. Additionally physically active people throughout their life live longer than those who are sedentary. Paffenbarger et al (1986), for example, monitored leisure-time activity in a cohort of Harvard graduates for a period of 16 years those who expended more than 2000 kcal of energy in active leisure activities per week lived. On average, two and half years longer than those classified as inactive. Blair et al. (1986) found that exercise is protective against both CHD and some cancers. Finally exercise and healthy eating are both widely regarded as an important factor in improving our physical and psychological well being. However, as with much health-related behaviour adherence to health promotion advice may be determined by many different factors. After all this article will critically review a study conducted by Payne N., Jones F., and Harris P.R in which the effect of perceived need on exercise and healthy eating using the theory of planned behaviour (TPB) with investigated the determinants of perceived need.

The Theory of Planned Behaviour (TPB), developed by Ajzen in 1991, is a social cognition model that has been used in predicting exercise behaviour. It is helps to understand why people exercise and is also useful for discriminating among various stages the individuals may go through when changing their exercise behaviour or eating. As Ajzen and Madden (1986) mentioned as follows Behavioural intention is defined as the outcome of combination of several beliefs that are conceptually independent variables. They consist of attitude, subjective norm and perceived behavioural control. Attitude towards behaviour is composed of both positive and negative evaluation of performing the behaviour and beliefs about the outcome behaviour. Subjective norm is reflected in the perception of social norms and pressure to perform behaviour. Perceived behavioural control is understood as a belief that individual can perform a behaviour in question after considering internal control factors (e.g. skills, abilities) and external control factors( e.g. opportunities, obstacles), both of which relate to past behaviour.

Paisley and Sparks (1998) examined perceived body weight and

estimated fat intake and found them to be significant predictors of perceived need. For instance, Maslow's (1943, cited Payne et al, 2004) hierarchy of needs suggests that needs, cognitive needs, aesthetic needs and self-actualization. Therefore need may be an important addition to the TPB. Paisley and Sparks (1998) note the possibility that need will be reflected in cognitive attitude, but suggest that it is distinct from cognitive attitude, therefore further emphasizing the potential importance of perceived need within the TPB. Also they argue that perceived need is not the same as attitude and should be measured as a separate predictor.

Payne et al. (2004) study extends the existing research by investigating the role of perceived need in relation to behaviour other than healthy eating. They had two aims for their study. The first aim of their study was to examine and compare the predictive ability of perceived need as an addition to the TPB when applied to healthy eating and physical exercise. For that they used the past research. Therefore these two behaviours were chosen in their study. On the other hand second aim was to examine the determinants of perceived need in order to examine the differences across the two behaviours and to further understanding of concept of perceived need. Paisley and Sparks1998) examined perceived body weight and estimated fat intake and found them to be significant predictors of perceived need. Rhodes et al. (2003) study investigating multiple components of attitude, subjective norm and perceived control: examination of the theory of planned behaviour in the exercise domain. Another study which belong to Payne at al (2005) invested of exercise and healthy eating with the impact of the job strain on the predictive validity of the theory of planned behaviour

Payne et al. (2004) participants were UK employees of a large company (about 3000 employees) involved in the design, marketing and sale of computer hardware and software to other companies. However it is very important that they did not mention that how the sample for the study selected. Volunteer for the research was recruited via an internal global e-mail containing the questionnaire as an attachment. One week after each questionnaire had been received; each employee was sent the follow up questionnaire measuring behaviour again by e-mail. 286 employees' responded to this questionnaire. % 70 were male. Seven percent were aged 16-41, 21% were aged 25-34, and 32% were aged 35-44. 33% were aged 45-55 and 7% were aged 55-64. The random of ages were very good.

Also there are not enough participants in Payne et al (2004) study. That might had effect on result and it might affect reliability of study result. Schifter & Ajzen (1985) in their study had participant problem as well. In contrast in Cournaya et al (2001) study there was 683 adults and that was more reliable than other study which has less or not enough participant.

Additionally Cournaya et al (2001) study had a randomly selected population based sample but Payne et al. (2004) was not population based study which the participant sample based on company employees which didn't represent the populations. Especially when consider on some factors like socio-economic, ethnic gender etc. because at the end employees who work in that kind of company more likely have good socio-economic status which has a big effect on health behaviours such as exercise and healthy eating even on intention to do these two health behaviors.

On the other hand gender another problem in Payne et al.(2004) stud, for example %70 of participants were male and this more likely has big effect on research results because it is very important that health behaviors are different from men to women. For example Sallis and Hovell (1990) found that those who engage in exercise are more likely to be young male and well educated adults, members of higher socio-economic groups and those who have exercised in the past. Additionally low participation for women are frequently ascribed to family responsibilities and joint work home responsibilities and social norms which support such behaviours choices (Green et al, 1986) Also men are more likely to eat less healthily than women but they exercise more than women (Reddy et al., 1992)

Also in Payne et al. (2004) study for first questionnaire three hundred thirty one employees responded to that, one week after each questionnaire had been received, each participant was sent the follow-up questionnaire measuring behaviour again via-email. However 286 employees responded to this questionnaire. That result seems like there is effect of intention to treat analysis. Participant who didn't respond to send back second questionnaire (45 participants) might have big impact on result of this study.

Payne et al. (2004) study is a cross-sectional study. However they would get more benefit if they use longitudinal design rather than cross-sectional one, because 1 week is not enough time for this study which give participant before they complete second questionnaire. For example Courneya et al (2001) study was longitudinal design that gives more credit to their study and study result.

Moreover in Payne et al. (2004) definitions for exercise and healthy eating are very clear and useful which is very important for Theory of Planned Behaviour. Definition of behaviour is most important part for the theory. Also objective of the research is clearly stated. Participant of study can understand very easily definitions of the exercise and healthy eating. The methods for measuring result clearly explained in the study.

Payne et al. (2004) study using multiple scales for attitude and perceived behaviour control give big advantage to study. Also using multiple scale items give reflected in the highest internal consistency for

this variable. Courneya et al (2001) had similar result by using multiple measurements. Also in Courneya at al (2001) PBC was assessed by two items on so was subjective norm, which is even of more credit to their study and attitude was measured by three items and this advantage of using multiple scale items was reflected in the highest internal consistency for this variable as well.

On the other hand the one item scales weakened the reliability and validity of the measures. For instance in Payne et al. (2004) study, intention to eat healthy was assessed by a single item that taken from Paisley & Sparks (1998). But besides of that there was one advantages of using open-ended item for perceived need and its determinants.

Also Subjective norm, the normative component was the last addition to the TRA (Fishbein & Ajzen, 1975) and several authors have argued that it is the weakest component. The measurement explanations of the weak predictive power of subjective norms relates to the fact that norms are typically measured by a single item, despite the potentially low reliability of such measures (Armitage & Conner 2001). However in Payne et al. (2004) study they used single item measure for subjective norm. But according to Armitage & Conner multiple item measures of subjective norm and normative beliefs have significantly stronger correlations with intention than any of the other measures. In Payne et al. (2004) study for exercise, attitude and subjective norm explained 14% of the variance in intention. For healthy eating, attitude and subjective norm explained 27% of the variance in intention. However for both behaviours subjective norm and cognitive attitude were not significant contributors to intention. Finally according to Armitage and Conner (2001) subjective norm shows a reasonably strong relationship with intention when approximately measured with multiple-item scales.

Based on their findings Payne et al. (2004) propose a revised the role of perceived need in relation to a behaviour. They found that all the TPB variables were correlated with intention for both exercise and healthy eating. For exercise, PBC was most highly correlated with intention ($r=.43$, $p<.001$) and for healthy eating, affective attitude was most highly correlated with intention ($r=.47$, $p<.001$). This shows the importance of perceptions of control over exercise and the importance of enjoying healthy eating.

Briefly, in Payne et al. (2004) study for healthy eating, the most important predictor of behaviour was intention and the most important factor in the formation of this intention was that people enjoy the foods, followed by perceived need and finally PBC. Therefore perceived need contributed slightly more the explanation of intention to eat healthy than did PBC. For exercise the most important predictor of behaviour was

intention and the most important factor in the formation of this intention was perception of control over whether or not exercises, followed by affective attitude. However perceived need did not add to the explanation of intention to exercise, suggesting that need is not an important factor in decisions to engage in exercise.

In Armitage&Conner (1997) study they tried to find about the predicting intention to eat a low fat diet, However in this research Perceived Control of Behaviour doesn't predict eating low fat food, in other words it just predict intention to eat low fat food. But in Payne et. al (2004) study, Perceived Behavioural Control predict both Intention to eat healthily (.17), and healthy eating (.20). But still intention to eat healthy (.36) is stronger predictor than perceived behavioural control. In other words intention is main predictor. For example in Schiften & Ajzen(1985) study which was about intention, perceived control and weight loss, intention didn't predict losing weight but PBC and self knowledge predicted weight loss.

Payne et al. (2004) found that for healthy eating, the most important predictor of behaviour was intention and the most important factor in the formation of the intention was that people enjoy the foods followed by perceived needs and PBC. It is very important that perceived behavioural control is not main prediction but intention to eat healthy main predictor. However although PBC is not main predictor is in Payne et al. (2004) study it still has significant correlation with healthy eating. In contrast Armatege&Corner (1999) studied predicting intention to eat a low fat diet and they found that Perceived Control Behaviour is not predict to eating low fat food, it is only predict intention to eat low food.

However according to Armitage and Conner (2001) in the TPB, the PBC construct should be top perception of the factors that may facilitate or inhibit performance of behaviour. Specifically, the relationship between PBC and behaviour should be stronger when intention measures are used, because intention measures do not consider on facilitating/inhibiting factors

With regards to the determinant of perceived need to engage in both behaviours were physical health and weight control which the most frequently reported reasons. However Payne et al. (2004) found that perceived need to eat healthy was more frequently reported to be due to physical health. And perceived need did not add to explanations of intention to exercise, suggesting that need is not an important factors in decisions to engage in exercise.

Interestingly, Payne et al. (2004) speculate that the large proportion of participants who did not perceive a need to exercise but still intended to, may in part be responsible for the absence of a significant correlation

between need and intention to exercise.

As a result Payne et al. (2004) recognise that there are some limitations for their study especially definitions and measurments problems. According to that, firstly the measure of intention to exercise used in this study was rather unusual. Because previous operationaltizatons of the TPB tend to measure stength of intentions rather than frequency.Second, intention, perceived need and beahviour were all measured using single items, which may undermine their reliability. Finally, the definition and measurement of healthy eating behaviour is particularly complex. Although people may have different ideas as to what constitues exercise, people have even more differing interpretations of healthy and unhealthy eating.

According to Payne et. al (2004) the findings of this study suggest that people fall into one four groups based on their level of perceived need and behavioural intention. First, there are people who do perceive need and intend to exercise/eat healthily . These people have got the health promotion message. Second, there are people who do percive need but do not intend to exercise/eat healthily. These people have got the message but other things such as negative attitudes or low PBC may be getting in the way. Third, there are people who do not perceive need and do not intend to exercise/eat healthily . This group of people are discusssed by Paisley and Sparks (1998) and are likley to be responsible for the fact that perceived need is not always reflected in cognitive attitude. Moreover, there are some people who do not perceive need but do exercise/eat healthily . These people gave contradictory reasons for perceived need

To conclude, although it is very diffucult examinig two diffirent health behaviour such as healthy eating and exercise at the same time, Payne et al.(2004) study represents an important stepforward in the research on the role of perceived need within the theory of planned behaviour by comporasion of exercise and healhy eating Additionaly It is very important to point that perceived need may not be a particularly important or useful predictor of all health behaviours. However, the concept of perceived need may be rather ambiguous, particularly in the case of exercise. Therefore further reserach is needed to evaluate the role of perceived need in relation to a variety of health behaviours. However research must be carefully conceptualize and operationalize perceived need using multiple-item measure, .it may be useful to consider on motivation theory in the further research. For instance, there may be different levels of need to engage in health behaviours such as esteem and safety.

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# Chapter 2

## WEB-BASED AND VIDEO CONFERENCING COUNSELING FOR INDIVIDUALS WITH SCHIZOPHRENIA AND THEIR FAMILIES

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Schizophrenia is a disorder which has a high tendency to become chronic and can decrease functions of thinking, perception, emotions, language, sense of self, and behaviour, because of leading deficiency of individual it affects family members of individual with schizophrenia as individual itself (WHO, 2016). The most common assumption which clarifies the schizophrenia is the Diathesis-Stress Model.

According to this model, during the disorder, interaction of biological tendency and socio-environmental stressors is efficient (NIMH, 2015; Sota et al., 2008; Nicholson & Neufeld, 1992). Psychotic symptoms of this disorder can be stressful for both individuals themselves and their families (Lobban et al., 2013). Because it is defined that most of individuals with schizophrenia live with their families, families are important part of social network for individuals with mental health problems (Lieberman & Fenton, 2000).

It is explained that individuals with schizophrenia and their families do not have sufficient knowledge about schizophrenia and they are insufficient to cope with responsibilities in the role of caregiver effectively (Haley et al., 2011). Individuals and their families can be assisted with the help of counseling and psychoeducation programs related this topic. Individual and family intervention programs can be given individually or in group and they comprise cognitive therapy techniques and psychoeducation (Birchwood et al., 1992; Caqueo-Urízar et al., 2011; Nirmala et al., 2011).

As well as coping with patients' devastating symptoms, families have to cope with unstable family routines, decadent social relationships and impaired economic status. In this coping process, families can experience feelings like shame, guilt, anxiety, anger, and sadness. These difficulties enforce coping and compliance skills of family members intensively and this enforcement generally ends with anxiety, depression, and guilt (Schene, 1990; Baronet, 1999; Kuipers et al., 2010). Taking care of a patient with schizophrenia creates an important burden for their families and in consequence of this, negative feelings can be developed. There are lots of interventions and their effectiveness is proved in dealing feelings of individuals and their families.

Among of them, there are web-based interventions and videoconferencing interventions.These methods are new and up to date and they demand to be studied deeply. According to literature, with these methods, psychoeducation, counseling and monitoring can be achievedmostly (Kuipers et al., 2010; Caqueo-Urízar et al., 2011; Haley et al., 2011; Nirmala et al., 2011).

It is stated that essential topics in the psychoeducation are education about disease and its prognosis, problem solving and coping skills

education, development of communication, and decrement of stress (APA, 2000).Considering mental state of individuals and their families, psychoeducation has important role in methods of coping with patient, symptoms and their reasons of disease, enabling psychological and social support for individuals and family members (Birchwood et al., 1992; Goto, 1991).Psychoeducation oriented patients and their families can be planned like individually, in group or distant.

When examining results of the studies related this topic, there is evidence that web-based and video conferencing interventions can be used in family environment but the number of researches that analyzes this situation is limited. Web-based and video conferencinginterventions enable modern psychiatry services for geographically isolated, restricted, or jailed people who cannot have sufficientpsychiatry service.

The individual with schizophrenia and his/her family benefit increasing productivity and patients also benefits developed caring services.The patient can take mental health service in the comfort of home and in own determined time.The aim of this study is defining effects of web-based and video conferencinginterventions for individuals with schizophrenia and their families and collecting results of studies in this topic and analyzing them.

**Subjects and Methods**

**Search strategies**

This research is accomplished for reviewing results of tele psychiatric interventions which is given to individuals with schizophrenia and their caregivers. In the scope of this research, all relatives are considered family members.

Studies were includedin Ulakbim Medicine Database, Web of Science, Science Direct, and Pubmeddatabases in December 2016. For keywords, terminology of Cochrane Schizophrenia Group and Central Register of Controlled Trials are used.In this study, combining terms of *schizophrenia* and/or *psychosis* and *psychoeducation/ psychoeducational interventions* and *family/caregiver* and *telemedicine/telehealth/telecare/website/online/ videoconferencing*, reviews were carried out.

Respectively, these criterions were used for incorporating specified research: 1) written in English language, full text articles; 2) individuals who got diagnosis of schizophrenia and their care takers; and 3) using interventions with web or video conference from tele psychiatry attempts.

**Study selection**

Examinations with the help of determined keywords related this topic were performed in four databases. Related titles and summaries were

viewed (n=722). Among researchers, researchers which are proper to the aim of the research were determined. Among these researchers, repeated ones were determined and deleted. Full texts of remained studies were obtained and according to inclusion criteria, appropriate articles were chosen. Six studies which examined web-based intervention oriented individuals with schizophrenia; three studies which examine web-based interventionfor caregivers of individuals with schizophreniaand two studies which examine video-based intervention for caregivers of individuals with schizophrenia (Table 1).

**Results**

**Results of Web-based Tele Psychiatric Interventions for Individuals with Schizophrenia**

In the research of Maijalaa et al. (2015) they used a website named Mieli.net to evaluate usage of web-based question and answer corner among individuals with schizophrenia. According to result of analysis, total 85 questions and comments are sent to the"question answer corner". These questions are related usage of question answer corner (29%), medicine treatment (31%), disease and examinations (25%), daily life and coping (4%), treatment centers (2%), other questions and comments (9%). In the result of this study, services related question answer corner via web can be part of health care of individuals with schizophrenia and enhancing responsibility toward care, information level can be developed.

In Anttilaa et al. (2012)'s research, which was performed as part of Mieli.net's study, they examined usage of web based patient education in individuals who take inpatient treatment. Respectively, titles of patient education interviews are mental distortions, treatment, wellbeing condition, patient rights, web appliance which sends questions to psychiatric nurses (eSupport) and daily life with support of relatives. It is reported that 93 patients took total 508 patient education sessions during their treatment in hospital. All patient education sessions were participated to 73 of patients and three fourths of sessions were performed successfully. It is claimed that taking web-based patient education during treatment in hospital and investing for patients who have serious mental disorders to utilize taking this education are important.

Steinwachs et al. (2011) in their research, evaluate web-based intervention which is developed for enabling individuals with schizophrenia communicate with clinicians about evidence based treatment. Interactive web-based intervention consists of six areas as medicine treatment, side effects, guidance, family support, appointment and life quality respectively. It is found that patients in intervention group compared to patients in control group are more active in communication; ask more questions about

their treatment; inform about life style further; control available knowledge frequently; their tone of voice are more dominant and respectful. In the result of this study, with the help of web based intervention, individuals with schizophrenia get strong with patient focused communication about their treatment.

In the research of Välimäki et al. (2008) who defined design and development process of web-based patient support program in the four episode, the process of web based program is defined analysis of users' necessities, development knowledge area, development of model, and user evaluation. Based on the need analysis result of users, five general informative areas in the titles of disease, treatment, wellbeing, daily activities and patient rights are defined. In terms of the content, form, visual image and availability of model, evaluation of the first version are made by 76 participants (20 nursing students, 35 nurses, and 21 patients) and according to the result of analysis, web based support program is practicable and reliable. According to the result of evaluation related web-based system oriented nurses (n=38), system consists of basic, interesting, well defined knowledge.

Farrell et al. (2004) defined web based intervention oriented personnel and patients in community mental health clinics, in their research. The sample of this study consists of nine patients and with the help of standard user test method data is collected. The home page of the website comprises of ten categories as mental health treatment; crisis, stress and coping skills; health and dental care; accommodation; income support and help; peer support; family and society support; job and rehabilitation centers; protection and advocacy and information about mental health. According to the results of study, this website is functional in respects to education, communication, and support.

In the research of Kaplan et al. (2014) they examine effectiveness of web-based family education and social support intervention oriented mother with serious mental disorder. Experimental group (n=31) takes intervention with web based family education course and e-mail sent from mental health professional and family with mental health problems and control group (n=29) takes intervention with web based healthy life course. Web-based family education course consists of episodes like introduction, child development, decrement in stress, mental health and parenting, positive parenting, and results. Data is collected with questionnaires in the beginning of the study and after three monthsfrom the beginning. It is evaluated that satisfaction and effectiveness of parenting, parenting skills and coping skills, social support and parent stress. In the result of web based intervention, it is found that parenting skills and coping skills are developed and parent stress is decreased. There is no support for

development of parent effectiveness. Consequently, mothers with serious mental problems are interested with web based family education and can do it.

**Results of web-based tele psychiatric interventions for caregivers of individuals with schizophrenia**

In their study, which aims to evaluate validity of telehealth psychoeducation interview, Rotondi et al. (2005) managed 51 participants. According to the results, individuals with schizophrenia reach 17292 total number of pages in the website, caregivers reached 2,527. Therapy group is most frequently used item of the website and individuals with schizophrenia visit this website mostly. Comparing with control group, perceived stress from individuals with schizophrenia in experimental group is significantly lower and their social support perception shows bigger tendency. Caregivers in experimental group report low or moderateloneliness feeling and low or moderate stress level. According to results of the study, web based psychosocial intervention oriented individuals with schizophrenia and their families is effective and applicable.

In the research of Glynn et al. (2010), they evaluate validity of web-based multiple family group program oriented families of schizophrenic people who can reach from their homes. In internet, Family Support Program consists of personal-group home page; discussion board; source links for knowledge and activities; short video training presentations; real time conversation items. According to evaluation results, 84.6 percent of families uses discussion board as a platformwhere anxiety, questions, experiences, and knowledge about relationship between patient relatives are shared. In the result of treatment, web-based intervention is found like positive.

In the research of Rotondi, et al. (2010), they evaluated usage of dedicatedly designed home computers and web site for giving multiple family psychoeducation service for individuals with schizophrenia and their families. In the web based intervention for telehealth group participants (n=29), a web site named as SOAR (Schizophrenia Online Access to Resource) is designed for enabling main themes of family psychoeducation. These main themes consist of development of empathetic responsibilities of participants, education about disease and treatment, support network and coping strategies. Individuals with schizophrenia have significantly development in the knowledge about schizophrenia; the caregivers show significant development in the knowledge about process of disease. During intervention, individuals with schizophrenia show significantly lower in positive symptoms.

**Results of video conference interventions for caregivers of individuals with schizophrenia**

The aim of the study of Haley et al. (2011) is comparing effectiveness of tele psychiatry education and face to face education. Experimental group takes intervention with video conference (n=35), control group take intervention with slide show via projector (n=21). In the study, information questionnaire is used for measuring information level of participants about schizophrenia and knowledge level of caregivers about schizophrenia is used as evaluation criteria. In the beginning, there is no difference between participants in the experimental group and control group in terms of knowledge of schizophrenia. After taking education, care takers from two groups show development significantly and knowledge increment between groups becomes equal in six weeks.

In the study of Stengard (2003) two different methods which are used in education for families of people with schizophrenia are compared. In this research, main aims of the intervention are improving knowledge level about disease and decreasing feeling expression, objective burden and psychological stress level.

Experimental group takes intervention with video training(n=128) and control group takes intervention with traditional report method (n=69). For collecting data, measurement tools contain personal information form, objective burden, General Health Questionnaire (GHQ), family questionnaire and group evaluation form in order. The education significantly causes information increment and development of psychological wellbeing of participants. After intervention, there is no significant change in feeling expression or family burden. Participants in video training group find lessons more beneficial.

**Discussion**

This systematic review aims to evaluate effectiveness of web-based and video conference interventions' results for individuals with schizophrenia and their families and to define methodological limitations to guide future researches enabling development of property of data in this area. According to the literature, a number of researches, which benefited technology using internet or video conference based interventions is limited.Web-basedinterventions showed not only improving a number of outcomes including positive psychotic symptoms, hospital admissions, socialization, depression and medication adherence but also improved family outcomes they may need to specifically address carers' needs, distress, and burden.

If interventions including web based and video conference are available for coping with interferences in starting and sustaining treatment, all mental health team's access of duty devoted to mental health care increases (Smith & Pell, 2003).When enabling widespread and social care to individuals with serious mental health distortions and their families, some obstacles can be encountered. These obstacles can be high prices of educated personnel and evidence based programs, difficulty of appropriate monitoring, logistic differences between people and fewness of patients who take this service from internet. Services including web-based and video conference provide an opportunity for learning specific abilities, participating interaction groups, taking active role in seeking support for patients.

Thus, the internet method aids developing patients' life quality and reaching their purposes (Rotondi et al., 2010). This systematic review uses web based method in psychiatric intervention (Rotondi et al., 2005; Glynn et al., 2010; Rotondi et al., 2010). In the result of these researches, it is found that web-based interventions for individuals with schizophrenia and their families are applicable and effective.

Video conference and psychotherapy are known as one of the areas of tele psychiatry which enables patients and their families reach mental health professionals (Mair & Whitten, 2000). In the review study of related video conference psychotherapy (VCP) shows that video conference psychotherapy's availability and usability in various therapeutic formats and different populations (Backhaus et al., 2012).According to the research evaluates routine tele psychiatric services in Canada, most of psychiatrists claim that counseling via video conference is more effective than face to face counseling (Simpson et al., 2001). In this systematic review, in the result of psychoeducation is given with the method of video conference,web-based and video conferencing interventions for families of people with schizophrenia is effective (Haley et al., 2011; Stengard, 2003).

According to a systematic review study of web-based and video conferencing interventions is applicable in the treatment and evaluation of patients with schizophrenia (Kasckow et al., 2013). In this systematic review, in the result of the articles which were evaluated, interventions including web-based and video conference have specific advantages. They involved increment in the level of disease information (Haley et al., 2011; Rotondi et al., 2010), decrement in positive symptoms of disease (Rotondi et al., 2010), decrement in stress perception and development in social support perception (Rotondi et al., 2005; Glynn et al., 2010). According to results of articles which are examined in the scope of research, psychoeducation which is given with web based and video conferencing interventions is beneficial for individuals with schizophrenia and their families.

## Conclusion

Web-based interventions and video conferencing interventions provide convenience for individuals, who have difficulty accessing health care for various reasons, such as living off the people, as well as, economic and cultural barriers. In the field of mental health, it also provides opportunities for education and communication and social support to both the patient and the caregiver in a new and different alternative way.

Most individuals with mental illness are more likely to experience difficulties with the use of the website due to their difficulties in learning and remembering. In this context, it is thought that it is important to create web sites designed for web-based interventions according to the people with mental health problems and their families.Future online interventions should be designed for safety protocols and reported on adverseevents and the perceived safety of online interventions

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*Table 1 Characteristics of included studies*

| Study | Study aim | Study sample size | interventions | Measurement | Outcome interventions |
|---|---|---|---|---|---|
| Maijala et al. 2015 Finland | To describe the use of an Internet delivered question and answer column among patients with schizophrenia | Intervention group= 100 Control group= 211 | Web-based intervention | Patient characteristics using questionnaires Question and answer column about Mieli. Net | Services related question answer corner via web can be part of health care of individuals with schizophrenia and enhancing responsibility toward care, information level can be developed. |
| Anttila et al. 2012 Finland | To evaluate the use of web-based patient education sessions in the psychiatric inpatient care | 93 patients | Web-based intervention | Evaluation report developed for the study A pilot test | Patient education sessions can be used with patients with serious mental disorders. |
| Steinwachs et al. 2011 USA | To evaluate web-based intervention which is developed for enabling individuals with schizophrenia communicate with clinicians about evidence based treatment | Intervention group= 24 Control group= 26 | Web-based intervention | Roter Interaction Analysis System (RIAS) | With the help of web based intervention, individuals with schizophrenia get strong with patient focused communication about their treatment. |
| Välimäki et al. 2008 Finland | To design and development process of web based patient support program (Mieli.Net portal) | 76 participants Nursing students= 20 Nurses= 35 Patients= 21 | Web-based intervention | Satisfaction survey Interviews with patients and relatives A literature review was conducted | Based on need analysis result of users, five general informative areas in the titles of disease, treatment, wellbeing, daily activities and patient rights are defined. Web based support program is practicable and reliable. According to the result of evaluation related web based system oriented nurses, system consists of basic, interesting, well defined knowledge. |
| Farrell et al. 2004 USA | To define web based intervention oriented personnel and patients in community mental health clinics | 9 patients | Web-based intervention | Standard user test method | This web site is functional in respect to education, communication and support. |
| Kaplan et al. 2014 USA | To examine effectiveness of web based family education and social support intervention oriented mother with serious mental disorder. | Intervention group= 31 Control group= 29 | Web-based intervention | Online survey | Parenting skills and coping skills are developed and parent stress is decreased. |

| | | | | | |
|---|---|---|---|---|---|
| Rotondi et al. 2005 USA | To evaluate the applicability of a telehealth psychoeducation intervention | Intervention group= 27 Control group= 24 | Web-based intervention | Stress and perceived social support<br>Distress related disease and perceived social support<br>Website Evaluation Tool | Web-based psychosocial intervention oriented individuals with schizophrenia and their families is effective and applicable. |
| Glynn et al. 2010 USA | To evaluate validity of web based multiple family group program oriented families of schizophrenic people who can reach from their homes | Intervention group= 26 Control group= 16 | Web-based intervention | Use of website<br>Brief Psychiatric Rating Scale<br>Brief Symptom Inventory<br>Family Attitude Scale<br>Multidimensional Scale of Perceived Social Support | Participants in the program uses discussion board where anxiety, questions, experiences and knowledge about relationship between patient relatives are shared. In the result of treatment, web based intervention is found like positive. |
| Rotondi et al. 2010 USA | To evaluate usage of dedicatedly designed home computers and web site for giving multiple family psychoeducation service for individuals with schizophrenia and their families | Intervention group= 29 Control group= 26 | Web-based intervention | Scale for the Assessment of Positive Symptoms<br>Knowledge About Schizophrenia Instrument<br>Usage of the Website | Individuals with schizophrenia have significantly development in the knowledge about schizophrenia; caretakers show significant development in the knowledge about process of disease. During intervention, individuals with schizophrenia show significantly lower in positive symptoms. |
| Haley et al. 2011 Ireland | To compare effectiveness of tele psychiatry education and face to face education | Intervention group= 35 Control group= 21 | Video conference intervention | Knowledge Questionnaire | Care takers from two groups show development significantly and knowledge increment between groups becomes equal. |
| Stengard et al. 2003 Finland | To compare two different methods which are used in education for families of people with schizophrenia | Intervention group= 128 Control group= 69 | Video conference intervention | The Knowledge Test<br>The objective burden<br>General Health Questionnaire<br>The Family Questionnaire<br>The group evaluation form | Education significantly causes information increment and development of psychological wellbeing of participants. After intervention, there is no significant change in feeling expression or family burden. Participants in video group find lessons more beneficial. |

# Chapter 3

## GENERAL CHARACTERISTICS OF HUMAN PAPILLOMA VIRUS (HPV) AND IT'S INTERACTION WITH THE CELL CYCLE

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Human Papilloma Viruses (HPV) are a small, non-enveloped DNA viruses that infects basal keratinocyte of the stratified mucosal or cutaneous epithelium. These viruses contain a viral capsid with icosahedral symmetry consisting of 72 pentameric capsomeres. **(Figure 1).** Each viral capsid composes of two structural proteins. One of these proteins is the major capsid protein L1, which has a molecular weight of about 55 kiloDaltons (kD) and makes up about 80% of the viral genome. The minor capsid protein L2 is 70 kD in weight. The virus is 52-55nm in length and contains 8000 base pairs of episomal (circular) DNA. The viral proteins especially early genes interact with cell cycle and checkpoints during infection and carcinogenesis and also inhibit the functioning of tumor suppressor genes p53 and retinoblastoma gene (Rb) **(Safi, 2004, Geith, 2019)** In this chapter, the general characteristics of HPV and the relationships between the viral proteins and cell cycle were evaluated.

The cell cycle includes a series of events that a cell goes through as it grows and divides. This process is controlled by cyclins (Cyc A-D), cyclin-dependent kinases (CDK 1-11), and cyclin-dependent kinase inhibitors (CKI). The cell cycle consists of two parts, interphase and mitosis (M). A cell spends most of its time in interphase. In this phase, cell grows, its chromosomes replicates and the cell prepares for division. Interphase is divided into three phases, G1 (first gap), S (synthesis), G2 (second gap). In G1 phase, ATP, RNA and protein are synthesized, the number of organelles increased and the growth factors activated. If a cell does not have enough growth factor, it enters the Go phase. When the cell receives a signal they enter the interphase. S phase is proceeding after G1 (Miller et al., 2017). In S phase, RNA synthesis continued, protein synthesis is highest level up. DNA replicated and centrosome begins to replicate itself. DNA replication is highly orchestrated process. In G2 phase, RNA and protein synthesis continued, centrosome duplication is completed. The cell then leaves interphase, undergoes mitosis. Mitosis consists of two consecutive processes; karyokinesis and cytokinesis. Prophase, metaphase, anaphase and telophase are major sections of karyokinesis.

The control of cell cycle is very crucial in preventing tumor genesis. There are three checkpoints on cell cycle process; one of them at the end of the G1 stage, the other end of the G2 stage, and the last one on metaphase stage (spindle checkpoint) to regulate the cell cycle. Cell cycle checkpoints are responsible for transition between phases. The checkpoints can delay cell cycle progression or induce cell cycle exit or cell death in response if DNA damage cannot be repaired. These are DNA surveillance mechanisms and prevent the accumulation of genetic errors during cell division. At the G1/S checkpoint, sufficient time is allowed for DNA repair in G1. (Hussain, et.al., 2021; Matthews et .al., 2022) At these control points, it is decided

whether the cell will continue the cycle or not. At the G1 checkpoint, if the cell has grown to a sufficient size and the DNA is not damaged, division occurs. In G1 phase, pRB is phosphorylated by cyclin-dependent kinases (CDKs). The activity of E2F is regulated by pRb. Rb binds and also inhibits the transcription factor, E2F. E2F, controls expression of genes for DNA synthesis and effects to transition to S phase. (Miller et al., 2017). While the size of the cell and DNA damage are controlled at the G2 checkpoint, the attachment of the chromosomes to the spindle fibers is controlled at the M checkpoint. Cyclins are synthesized at certain stages of the cycle, while CDKs are synthesized at every stage of the cell cycle. Each CDK forms a complex with a different cyclin. Inactive forms of CDKs are phosphorylated and activated by binding to proteins called cyclins. While cyclin D increases at the onset of the G1 phase; cyclin E is in late G2 and S phase; Cyclin A takes place from the beginning of the S phase to the end of the G2 phase and cyclin B takes part in the G2-M phase. CDK4 or CDK6, which complexes with cyclin D, and CDK2, which combines with cyclin E, controls the progression of the G1 phase (Canpolat, 2016). Cyclin A and B are expressed during G2/M and Cyclin E is upregulated during G1/S progressions. (Vermeulen, et.al., 2003) **(Figure 1).**

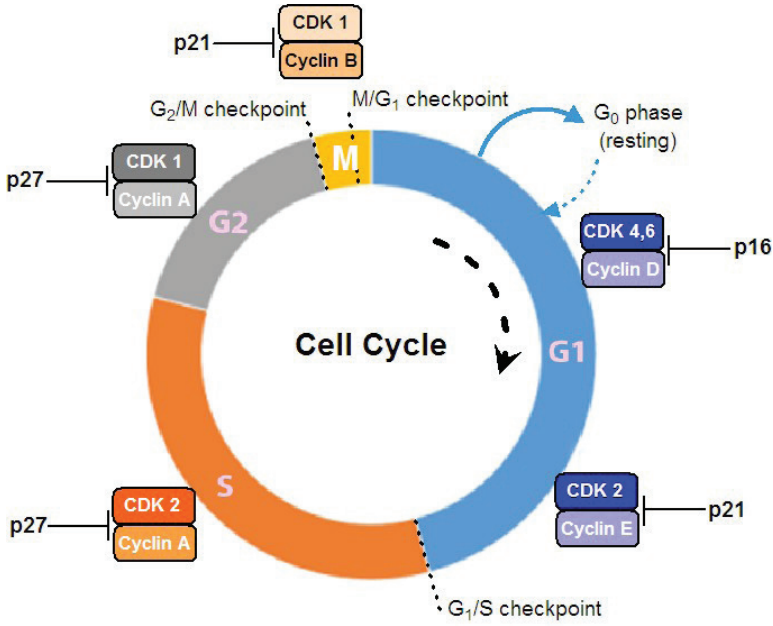



**Figure 1.** *Cell cycle regulation by checkpoints*

Many ds DNA viruses affect the cell cycle of infected host cells. HPV is non-enveloped icosahedral viruses with a circular, approximately 8.0 kbp, covalently closed double stranded DNA virus (Figure 1). HPV manipulates the cell cycle of the host cell to provide conditions for viral

genome replication (Reinson, et.al., 2015). This virus causes benign and malignant lesions in the skin, oral cavity and anogenital region in men and women. It causes papilloma on the penis, scrotum and anogenital region in men, and on the vulva, perianal region and cervix and vaginal walls in women. **(**Boda, et.al., 2018). HPV infections are often asymptomatic (Mattoscio, et.al.,2021 ).

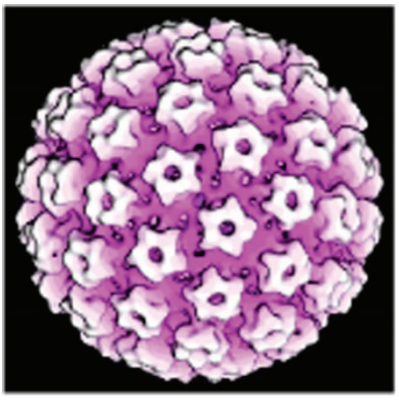


**Figure 2.** *Electron microscopic view of Human Papilloma Virus (Howley, 1996; Safi, 2004)*

To date more than 200 HPV types have been identified and divided into five major genera: alpha, beta, gamma, mu, and nu (α, β, γ, μ, ν) according to their nucleotide sequence (www.hpvcenter.se, on 2019-01-30). The alpha papillomaviruses (α HPVs) was referred to as high-risk (HR) HPV types and infect mostly mucosal and genital epithelia. α HPVs (n=65) were classified into carcinogenic (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) and probably carcinogenic (HPV 26, 53, 66, 67, 68, 70, 73, and 82) group. HR-HPVs are also involved in the formation of a number of other anogenital malignancies. The small number of the α HPV and β, γ, μ, ν types show tropism for cutaneous epithelia. The beta and gamma genera are cutaneous HPV types **(**Boda, et.al., 2018; Gheit, 2019; Malla and Kamal, 2021; Mattoscio, et.al.,2021). The genus gamma includes 98 types. The genera mu and nu include only 3 and 1 types Gheit, 2019).

Papillomaviruses have host specificity and cause benign and malignant lesions in their natural hosts. HPVs exist in two different forms in precancerous lesions and invasive cancer. The first of these is the plasmid form. Here, the virus DNA is not integrated into the host cell DNA. The second type of DNA replication is vegetative DNA replication and occurs in differentiated epithelial cells. In invasive cancer, the virus DNA is integrated into the host cell DNA **(Figure 3).**

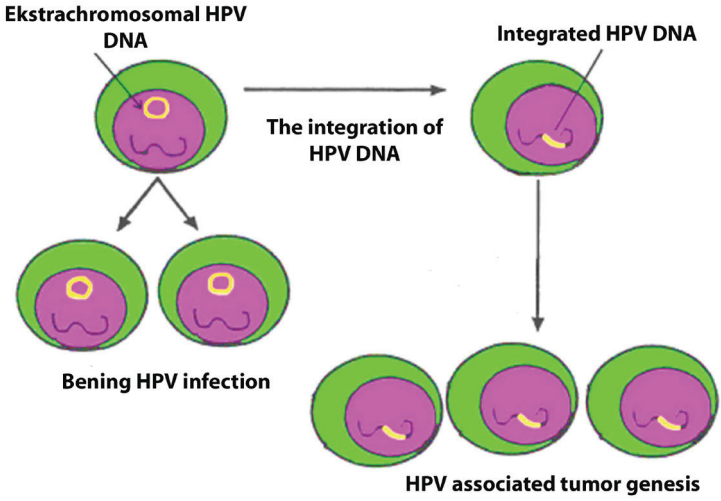



**Figure 3.** *Benign and malignant transformation of HPV (Alberts et.al., 1994; Safi, 2004)*

Different stages of HPV-induced disease can be characterized by virus mRNA expression profiles (Graham, 2010). The double stranded DNA genome is transcribed in one direction from one DNA strand. The viral DNA consists of nine open reading frames (ORF), including the early genes (E1, E2, E4, E1^E4, E5, E6, E7, E8 and E8E2) the late genes (L1, L2) and long control region (LCR). LCR is the non-coding region where viral transcription and replication are controlled. Replication originates from this region. LCR contains binding sites for various transcription factors and the viral helicase E1 and E2 (Safi, 2012; Boda et.al., 2018; Miller et al., 2017; Mac and Moody, 2020). HPV early region may be actively engaged in transcription of the genome and controlled in response to epithelial differentiation (Graham, 2010). The viral E1 and E2 proteins initiate viral DNA replication and maintain and partition viral genomes. E5, E6 and E7 proteins play a role in providing the necessary environment for viral DNA replication and avoiding the host immune response (Della Fera, et.al., 2021). E6 and E7 oncoproteins promote immortalization of host cells through the degradation of tumor suppressor proteins, p53 and pRb.

(Díaz-Tejeda, et.al., 2021; Gheit, 2019; Graham, 2017; Scheurer, et.al., 2007) Increased E6 and E7 activity can stimulate cell growth, interrupted cell differentiation, and induce chromosomal instability (Graham, 2017). E7 can also induce chromatin condensation and abnormal centrosome duplication (Scheurer, et.al., 2007).

L1 and L2 proteins are synthesized in the part of the viral genome called the late region. These proteins are the structural proteins that make up the viral capsid (Safi Z., 2004; Safi Oz, 2012). The LCR comprises roughly 800–900 bp and contains the early promoter driving expression of E6 and E7 oncogenes (Díaz-Tejeda, et.al., 2021). Most researchers consider that heparan sulfate proteoglycans play a critical role in the process of attachment of pathogens to epithelial cells (Schiller et. al., 2010). HPV viral proteins play a crucial role in the it's life cycle and replication. Structural proteins of HPV and their relationship with the cell cycle are presented under the following headings; E1, E2, E4, E1^E4, E5, E6, E7, E8 and E8E2, L1 and L2 proteins. These HPV proteins and their properties are summarized in **Table 1 and 2.**

**Table 1.** *Early genes of HPV*

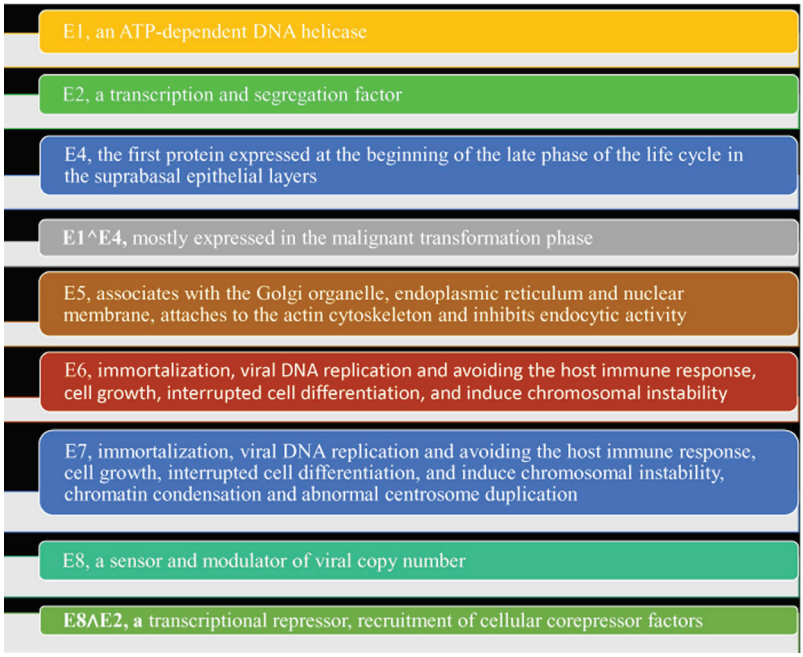
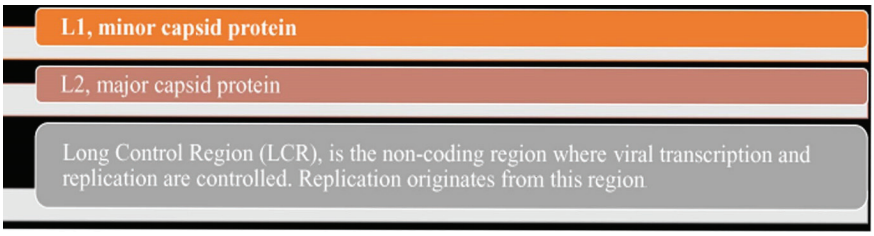


**Table 2.** *LCR and late genes of HPV*




**E1 protein**

The E1 region of HPVs is the largest gene region in papillomaviruses. The papillomavirus E1 is a arguably most complex protein with a molecular weight of approximately 73 kDa. This is encoded by papillomaviruses is an ATP-dependent DNA helicase, which uses energy from ATP hydrolysis to unwind dsDNA during replication. HPV replicates under the control of virus E1 and E2 proteins (Drews, et.al., 2020). HPV E1 plays a central role in the viral life cycle and interact with DNA replication factor and also orchestrates of the viral DNA replication. E1 unwinds DNA at the origin using a spiral escalator mechanism and ahead of the replication fork and also interacts with cellular DNA replication factors. This protein engages in multiple nucleic acid- protein and protein- protein interactions. E1 also connects with the p180 subunit of the cellular polymerase α-primase to initiate DNA replication. Nuclear accumulation of E1 arrests cells in S-phase and triggers a DNA damage response. Although the driving force of E6 and E7 in carcinogenesis is known, the potential contributions of E1 and E2 in the early stages of disease development should not be forgotten (Bergvall, et.al., 2013).

**E2 protein**

The papillomavirus E2 is a protein with a molecular weight of approximately 48kD and the main transcriptional regulators of the papillomaviruses. This protein plays a crucial role in the viral life cycle, vegetative DNA and viral genome replication, RNA transcription, post-transcriptional processes and possibly packaging (McBride, 2013; Ren et.al.,2020). E2 is a transcription and segregation factor. E2 also increases E1's binding specificity to the origin of viral replication (Reinson, et.al., 2015). The E2 protein contains three regions. These; 1- Aminoterminal trans acting region (N), 2- Central binding (hinge) region (H), 3- Carboxyl terminal (C) protein dimerization and DNA binding region. Studies with papillomaviruses that cause lesions in the genital tract show that the expression of early gene in the viral genome is under the control of E2 proteins. There are four regions in the LCR region to which E2 can bind. Binding of E2 to its specific region in the LCR results in modulation of viral promoter activity. In summary, the E2 protein plays a role in the

regulation of transcription. It also helps the E1 protein, which initiates viral replication and assemble into a double hexamer of viral (McBride, 2013; Ren et.al.,2020). HPV E5, E6, and E7 proteins are expressed under the transcriptional control of cellular transcription factors together with the E1 and E2 proteins and also contribute to the formation of the papilloma (Drews, et. al., 2020).

**E4 protein**

The papillomavirus E4 acts as a biomarker of active virus infection. E4 proteins are expressed before L2 and L1. This protein is located centrally within the E2 gene, with the primary E4 gene-product (E1^E4) being translated from a spliced mRNA that includes the E1 initiation codon and adjacent sequences (Doorbar, 2013). Although the HPV E4 proteins are expressed in the early region of the ORF, they also play a role in the formation of infection as if they are the product of late genes. E4 is the first protein expressed at the beginning of the late phase of the life cycle in the suprabasal epithelial cells with the L1 and L2 capsid proteins being expressed last of all in the upper granular layer (Graham, 2010). This result suggested that E4 gene products may have a role in differentiation-dependent virus replication. The E4 proteins of the beta, gamma and mu HPV types assemble into distinctive inclusion granules (Doorbar, 2013). Different forms of E4 proteins that 10/11 kD, 16/17 kD, 21/23 kD and 32/34 kD were found in different warts formed by the virus in epithelial tissue. The 10/11kD form is mostly seen in the superficial cells of the epithelial tissue. While the 17kD form is detected in parabasal cells, other forms appear as epithelial cells differentiate (Doorbar, 2013; Safi., 2004; Safi Oz, 2012). E4 protein causes the formation of koilocytosis by disrupting the cytoskeleton of the squamous cell (Krause et.al., 2021).

It has been determined that HPV18 E4 protein inhibits cell growth and ensures that the host cell is arrest in the G2-M phase of the cell cycle. In addition to all these features, this protein destroys the host cytoskeleton and regulates the division of the host cell according to the virus life cycle. The kinases and proteases regulate E4. HPV E4 also affects other viral proteins simultaneously, and include cyclin-dependent kinase, protein kinase A, protein kinase C and members of the MAP Kinase family (Doorbar, 2013). HPV E4 also induce G2/M arrest and facilitate E6/E7 viral amplification. This viral protein play a role in maintenance of MAPK activation and may interact and stabilize E2 and viral genome amplification (Ren et.al., 2020).

**E1^E4 protein**

The E1^E4 protein of HPV16 is mostly expressed in the malignant transformation phase. It is a protein that is abundantly synthesized in the uppermost layer of the epithelial tissue during the proliferation period of

the infection. The first 5 amino acids of this protein are synthesized from the ORF of E1, while the remaining amino acids are synthesized from the ORF of E4. This protein leads the way in synthesizing the structural proteins of the virus and in vegetative viral DNA replication. Although the E1^E4 protein is associated with viral DNA replication, it also has the ability to be distributed filamentous in the cytoplasm of epithelial cells. It is stated that the filamentous distribution of the E1^E4 protein depends on the relationship between them and the cytoskeleton in HPV 16. The inability to culture cells of HPV can be explained by the inability to form a keratin filament network in the culture medium, and therefore the lack of connection between the HPV 16 E1^E4 protein and this keratin filament. It is also known that this protein changes the ATPase activity of RNA helicase Davy, et. al., 2002; Safi Z., 2004; Safi, 2012)

**E5 protein**

E5 is a highly hydrophobic membrane protein consisting of 83 amino acids. Unlike other viral proteins, this protein associates with the Golgi organelle, endoplasmic reticulum and nuclear membrane, attaches to the actin cytoskeleton and inhibits endocytic activity. E5 protein immortalizes these cells by providing an uncontrolled increase in human keratinocytes and reduces cell-cell communication through gap junctions. The high amount of mRNAs belonging to the E5 protein in cervical intraepithelial neoplasia (CIN 1) cases suggested that this protein may play a role in the first step of the transformation into malignancy. HPV 16 E5 is the most synthesized protein of the virus in it's episomal form after infecting epithelial cells (Kabsch and Alanso., 2002; Safi Z., 2004; Safi Oz, 2012). Disruption of genetically regulated apoptosis causes uncontrolled cell proliferation and malignancy. The HPV 16 E5 protein prevents apoptosis in the infected cells, just like in other viruses. While the Fas receptor, which plays an important role in apoptosis in normal tissues such as ovaries, endometrium and cervix, is synthesized in a controlled manner, abnormalities in the synthesis of this receptor are observed in gynecological cancers. The amount of Fas decreases and tumor cells gain resistance against Fas L-mediated apoptosis. In addition, virus E5 protein increases the number of host cells uncontrollably, giving the virus time to multiply. In summary, E5 reduces apoptosis mediated by TRAIL and Fas L receptors and ligands involved in apoptosis in human keratinocytes. In fact, it has been shown in cell culture studies with human keratinocytes that this protein protects cells against apoptosis mediated by TRAIL and Fas L. (Kabsch and Alonso, 2002). HPV16 E5 also cooperate with E7 in cell transformation (Ren et.al., 2020). Krawczyk et al. They suggest that the HPV proteins E5 and E6 work together to facilitate the formation of the perinuclear cavity. (Krawczyk et al.,2008).

**E6 onkoprotein**

The E6 protein of HPV consists of approximately 150 amino acids. In oncogenic HPV types, the E6 and E7 proteins are often associated functionally. These proteins provide the cellular conditions necessary for viral DNA synthesis. In the initial phase of viral DNA replication, some factors such as the host cell DNA polymerase α are also important in addition to the E1 and E2 proteins. The common feature of all E6 proteins is that they contain Cys-X-X-Cys motifs. These motifs are found in the zinc finger regions where proteins bind to DNA. It has not been fully elucidated whether the Cys-X-X-Cys motifs of the chain fingers are required for the binding of E6 to DNA. However, these motifs are stated to be important in the transcription activation of E6, transformation, immortalization, and association with cellular proteins (Safi, 2004; Safi Oz, 2012).

All viral promoters are active at different stages of their life cycle. E6 ORF drives the expression of early viral genes in undifferentiated cells. The late promoter is located within the E7 ORF and is activated upon epithelial differentiation to induce expression of late viral genes, including the L1 and L2 capsid genes (Mac and Moody, 2020).

HPV E6 and E7 are effect in important biological events such as tumorigenesis, control of transcription and apoptosis. E6 inhibits the functioning of cell cycle checkpoints and inhibits the course of apoptosis. p53 and pRb are two of the most universally tumor-suppressor genes. Viral E6 and E7 mediate degradation of the cell cycle regulators p53 and Rb in carcinogenesis (Fan and Chen., 2004; Hussain, et.al., 2021;) Normally low levels of p53 are elevated in conditions such as viral infection, DNA damage or cellular stress. When p53 is high, it keeps the cell in G1 phase and controls the cell cycle. Thus, uncontrolled cell division does not occur. p53 creates a defense mechanism against uncontrolled divisions by leading the cell to apoptosis or keeping the cell cycle at one point. The situation is different in cells where the HPV E6 protein is synthesized, and this defense mediated by p53 mechanism is broken. HPV E6 connects with p53 and prevents p53 from attaching to target sites in DNA. In addition, E6 degrades the p53 with the E3 ubiquitin-protein ligase via proteasome (Mac and Moody, 2020; Safi, 2004; Safi Oz, 2012). Epigenetic silencing of p53 by DNA methylation in CpG island of gene promoters is often observed in HR-HPV associated infection (Mac and Moody, 2020; Safi., 2004; Safi Oz, 2012).

Interestingly, not all the p53 in the cell is degraded and it is possible that E6/E6AP only targets cellular pools of p53 destined to activate the transcription of downstream pro-apoptotic factors, such as Bax, Fas, PUMAβ, Apaf-1 and PIG (Vats et.al., 2021).

The p53 tumor suppressor gene induces cell cycle arrest. p53 -repressed genes required for the G2 phase. This arrested process is also important for all checkpoints up to the completion of cell division. This p53-dependent arrested mechanism is controlled by the p53–p21–DREAM–E2F/CHR pathway (p53–DREAM pathway). p53–p21–DREAM–E2F/CHR pathway controls of cell cycle genes. DREAM (The dimerization partner, RB-like, E2F and multi-vulval class B) is a transcriptional repressor that binds to the E2F or CHR promoter regions. In humans, the key proteins in the complex are muv B core complex, E2F4-5/ DP, and p130 or p107 proteins, which are related to the retinoblastoma tumor suppressor pRB, both of which are homologs of p105 and bind repressive E2F transcription factors (Fischer et.al., 2016a; Fischer et.al., 2016b; Engeland, 2018)

The p53-DREAM pathway is controlled by more than 250 cell cycle-related genes. This pathway participates in the control of all points from DNA synthesis to cytokinesis, including the G1/S, G2/M, and spindle assembly checkpoints. When DREAM binding to E2F and CHR elements, it regulates a larger set of target genes leading to regulatory functions distinct from pRB/E2F (Engeland, 2018).

The defects of p53–DREAM pathway contribute to a general loss of checkpoint control. Furthermore, deregulation of DREAM target genes promotes chromosomal instability and aneuploidy of cancer cells (Fischer et.al., 2016a; Fischer et.al., 2016b; Engeland, 2018).

In vitro studies have shown that the E6 protein also connects with the ERC-55 (E6-BP) and paxillin. ERC-55 is a calcium-binding protein in the endoplasmic reticulum. Paxillin is a protein associated with focal adhesion proteins (eg vinculin) and is involved in the regulation of the cytoskeleton (Safi , 2004; Safi Oz, 2012). HPV E6 protein also interacts with telomerase Wise-Draper and Wells, 2008). Telomerase activation is critical for the immortalization of primary human keratinocytes by the high-risk HPV E6 and E7 oncoproteins (Liu et.al., 2009).

While the virus replicates in epithelial cells, it makes some changes both in the cytoplasm and in the nucleus. The most important changes in the epithelial cell cytoplasm caused by HPV is the formation of hollow called koilos. (Safi Oz, 2012) Koilocytosis is a descriptive term derived from the Greek adjective *koilos*, meaning hollow. (Krause et.al., 2021). In Papanicolaou stained smears of HPV positive patients, koilocytotic cells consist of the condensed pink, blue, or both cytoplasm and hyperchromatic nucleus. The nucleus with an irregular border and enlarged to at least three times the size of an intermediate cell nucleus. According to the first view regarding, HPV E6 protein and the E6-AP cellular proteins can effective in the formation of koilocytosis. In the mechanism mediated by E6 and

E6-AP, the E6 degrades p53 by connecting E6-AP. The fact that E6-AP is localized around the nucleus and that p53 destruction occurs in this region suggests that this event is effective in the koilocytosis. The second view regarding the koilocytosis is related to the E6 protein and paxillin, which is one of the cellular proteins with which E6 is linked. Paxillin is an adhesion protein and other adhesion proteins in the cell. It takes part in the regulation of the cytoskeleton by connecting with (vinculin). The virus's E6 protein binds to paxillin, disrupting actin filament formation and cytoskeletal regulation. In the light of this information, it is thought that the formation of actin filament and thus the disruption of the cytoskeleton are effective in the formation of the hollow around the nucleus. The third view concerns the E1^E4 protein. In recent studies, the existence of another protein that can disrupt the epithelial cytoskeleton of HPV type 16 has been suggested and this protein has been named the E1^E4 protein. It has been reported that this protein destroys the cytoskeleton by connecting with proteins in the epithelial cell. Based on this information, it was thought that the koilocytosis may be due to the destruction of the cytoskeleton (Davy et al., 2002; Nakahara et al., 2002).

**E7 onkoprotein**

The E6 and E7 genes in HPV 16 and HPV 18 are expressed by the major promoters P97 and P105 in the LCR region. (Bergvall, et.al., 2013). The E7 oncoprotein is a small protein consisting of about 100 amino acids and bind to pRB. In addition to pRB, the E7 protein also associates with p107 and p130 proteins. Phosphorylation of pRB is important in the E7-pRB relationship. The phosphorylated pRB can only be detected in the Go/G1 phase. This structure is the active state of pRB and plays a suppressive role in the cell cycle. In the transition to the S phase, pRB is inactivated by phosphorylating by cyclin-dependent kinases (cdk). Thus, its suppressive role in the cell cycle is eliminated. When the cellular transcription factor E2F-1 binds to the phosphorylated structure of pRB, transcription is suppressed. Phosphorylation of pRB or complexation with E7 releases E2F-1 and activates transcription. With the release of E2F-1, events such as cellular DNA synthesis and progression of the cell to the S phase begin to occur. In biochemical and genetic studies conducted to examine the relationship between the E7 protein and pRB, it has been shown that the binding affinity between this protein and pRB decreases when some polymorphic changes (T663G/C24W) occur in the structure of the E7 protein (Howley, 1996; Safi, 2004; Safi Oz, 2012). Other cellular targets of HPV E7 protein are cyclin-dependent kinase inhibitors and chromatin modifying factors (Wise-Draper and Wells, 2008).

HPV E6 and E7 oncoproteins play the pivotal role in driving the cells toward oncogenesis (Pal and Kundu., 2020). These proteins respectively

inactivate p53 and members of the pRb family, interfering with the cellular control mechanisms of the cell cycle and cellular networks such as the PIK3CA-AKT-mTOR pathway (Hoppe-Seyler et.al., 2018; Ren et.al.,2020). HPV E6 and E7 proteins also alter the expression of Disc Large 1 polarity protein, a scaffolding protein involved in the control of cell polarity and proliferation in epithelial cells (Dizanzo et.al., 2020) Other effects of E6 and E7 proteins are on the centrosome. In the study of Duensing et al. in keratinocyte cell culture, HPV 16 E6 and E7 oncoproteins are associated with abnormalities in the centrosome and genomic arrangement. E6 oncoprotein was found to provoke centrosome accumulation, HPV-16 E7 expression disrupts normal centriole (Fukasawa et.al., 1996; Duensing et.al., 2001; Duensing et.al., 2009)

HPV 16 E7 retinoblastoma protein impairs its tumor suppressor function by binding pRB. It has been reported that DREAM in E7 is disrupted by binding to the pRB-associated protein p130. HPV E6 degrades p53 via ubiquitin. In addition, it has been stated that HPV E7 protein may impair the function of p53 by binding to DREAM components p107 and p130 (Fischer et.al., 2016a; Fischer et.al., 2016b; Engeland, 2018).

The p53–DREAM pathway play a role in the spindle assembly checkpoint, chromosomal instability, aneuploidy in cancer cells and mitotic catastrophe.The p53-DREAM pathway is part of the DNA damage response. The suppression of this pathway causes disruptions in the mitotic process. If the damage cannot be repaired, cells can be arrested in mitosis or driven to programmed cell death. Also, DREAM regulation is abrogated by the human papilloma virus HPV E7 protein linking the p53–DREAM pathway to carcinogenesis by HPV (Fischer et.al., 2016a; Fischer et.al., 2016b; Engeland, 2018).

**E8 protein**

The E8 promoter is both positively and negatively regulated by cellular and viral proteins and thus most likely acts as a sensor and modulator of viral copy number. The E8 promoter is responsible for the expression of E8^E2C. This protein is tightly regulated to allow limiting replication but preventing over replication in undifferentiated cells (Straub, et.al., 2015).

**E8 ∧ E2 protein (E8^E2C)**

The Papillomavirus E8∧E2 protein, part of the E8 gene, transcriptional repressor, suppresses DNA replication from extrachromosomal origins (Zobel et.al., 2003). It binds to the C terminal of the E2 gene and also involves the recruitment of cellular corepressor factors. These proteins are potent repressors of transcription and the E1/ E2-dependent replication. E8∧E2 modulates the levels of replication in the maintenance phase of

replication in HPV16, 18 and 31 but only appears to be essential for episomal maintenance in HPV 31 (Mc Bride, 2013; Straub, et.al., 2015; Zobel et al., 2003).

Structural proteins of HPV and their relationship with the cell cycle process are summarized in **Figure 4.**

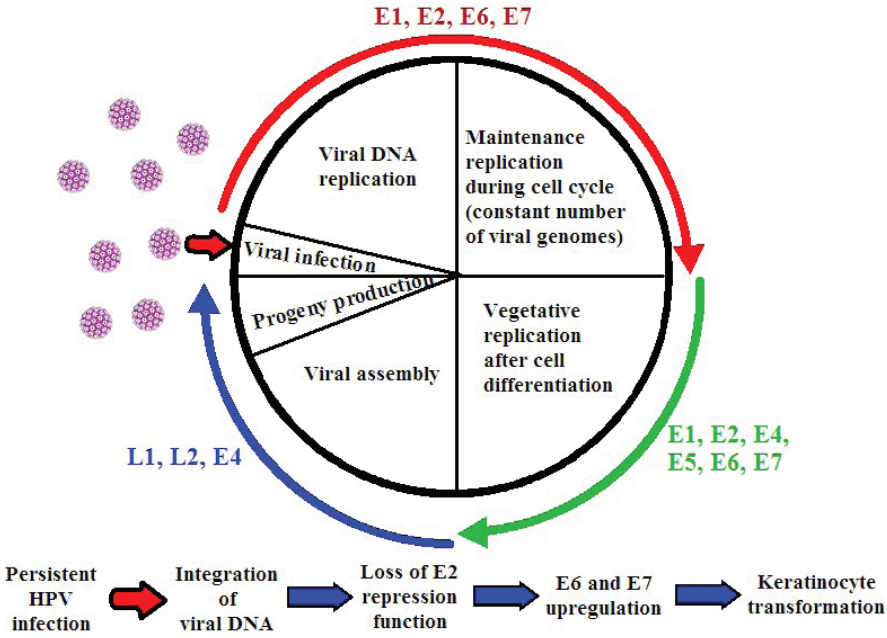



**Figure 4:** *Structural proteins of HPV and the interaction of cell cycle (Gheit, 2019)*

HPV life cycle, replication and epigenetic regulation are presented in a separate topic below.

**Overview of the HPV Life Cycle and Epigenetic Regulation**

Micro-wounds in the epidermis also facilitates the entry of the virus into the epithelial cell. In HPV life cycle requires a three dimensional epithelium. HPV infects the actively proliferating, undifferentiated basal cell of the stratified squamous epithelium. The HPV life cycle is tightly associated with epithelial differentiation of host keratinocytes. Epithelial differentiation induces the productive phase of the viral life cycle. It is also support to express of late viral genes (E4, E5, L1, L2), as well as high levels of E1 and E2 (Mac and Moody, 2020). When the virus integrated its DNA into the genome, it replicates in the maturing squamous cells (Graham, 2010; McBride, 2013; Krause et.al., 2021).

The process from uptake of the virus by the host cell is shown in Figure 4. (Mac and Moody, 2020). HPV infects undifferentiated basal

keratinocytes (1). It is known that the attachment of the virus to the cell is the most important stage in viral replication. The major capsid protein L1 plays a role in the attachment of HPV to the host epithelial cell. In addition to this information, it is stated that the receptor protein called α 6-integrin plays a role in the adhesion of HPV 6 to the epithelial cell, while the cell surface heparin sulfate and proteoglycan also play a role in this adhesion (Hussain, et.al., 2021; Mattoscio, et.al.,2021; Safi , 2004; Safi Oz, 2012) . After attachment to the host cell, the virus is taken in by endocytosis (2). While being transported to the nucleus, it also uncoat capsid proteins (3) Low pH serves as an important role of uncoating process (Kilcher and Mercer, 2015). Following infection of the basal cells of the virus, the early promoter becomes active and activates the expression of the E1 viral helicase (4). Thus, viral episomes are rapidly amplified (5). As the infected basal cells divide, the viral DNA divides into daughter cells that begin terminal differentiation (6,7). In HPVs, the proteins synthesized in the cytoplasm are transported to the nucleus, so that the entire virus particle is formed in the nucleus. It is thought that during the formation of the virus particle, first empty capsids are formed, and the viral nucleic acid is packaged into the empty capsid. The mature virus particle breaks the nuclear membrane and passes into the cytoplasm (8,9). Events such as vegetative viral DNA replication, formation of L1 and L2 capsid proteins, disruption of the nuclear membrane and release of virus are observed in differentiated keratinocytes (10) (Mac and Moody, 2020; Safi, 2004; Safi Oz, 2012). Normally, naked viruses leave the cell by lysing the host cell, while it has been reported that HPV is released into keratinized cells with differentiation in the basal cell (11). While most of the infections are cleared, some of them remain in the host and induce disease progression. **(Figure 5).** Virus replication cycle is completed in the upper epithelial layers from where newly synthesised virions are released (Graham, 2010).

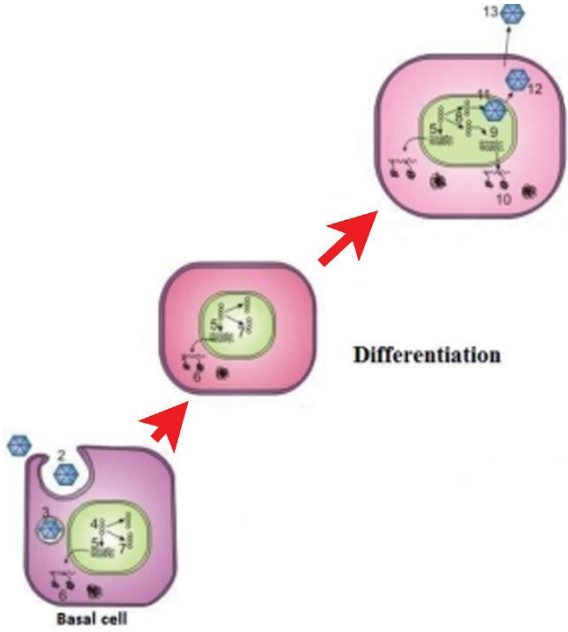



**Figure 5.** *HPV life cycle. Attachment to basal cell (1), virus by endocytosis (2), transport to the nucleus and uncoating of viral capsid (3) transcription of early genes (4), translation of early proteins (5), viral DNA replication (6), all the stages described occur in basal and parabasal cells (7), transcription of late genes (8), formation of capsid proteins L1 and L2 (9), formation of virus particle (10) disruption of the nuclear membrane (11) release of the virus (12,13) (Safı , 2004)*

The HPV life cycle and it's replication also regulated epigenetically. Epigenetic modifications, a phenomenon that changes gene expression without altering DNA sequence, of HPV chromatin is also regulate viral gene expression throughout the viral life cycle. HPV genomes are epigenetically regulated by DNA methylation and post-translational histone modifications, including methylation, phosphorylation and acetylation. That the HPV E6 and E7 proteins modulate the expression of histone acetyltransferase (HATs) and histone deacetylases (HDACs), creates the appropriate environment for replication in infected cells. HPV infection can also induce DNA and histone methylation (Burley et.al., 2020; Mac and Moody, 2020)

## CONCLUSION

In this chapter viral HPV proteins and it's interaction of cell cycle process were discussed. It is thought that detailing the effect of HPV on the cell cycle will contribute to the elucidation of the HPV-induced tumor formation mechanism. One of the structural proteins, HPV E1 arrests cells in S phase and triggers a DNA damage response. Although the important

power of E6 and E7 in carcinogenesis is known, the potential contribution of E1 and E2 in the early stages of disease development is an important issue that should not be forgotten. E2 plays a crucial role in the viral life cycle, vegetative DNA and viral genome replication, RNA transcription and packaging. E2 is a transcription and separation factor. It shows that in HPV-specific genital lesions, early gene expression in the viral genome is under the control of E2 proteins. HPV18 E4 protein inhibits cell growth and causes the cell cycle to stop in the G2-M phase and regulates the division of the host cell according to the virus life cycle by disrupting the host cytoskeleton. HPV E4 also facilitates amplification of E2, E6 and E7. HPV 16 E5 protein prevents apoptosis in infected cells as in other viruses. While the Fas receptor, which plays an important role in apoptosis, is synthesized in a controlled manner in normal tissues such as ovaries, endometrium and cervix, abnormalities in the synthesis of this receptor are observed in gynecological cancers. The role of the E5 protein in this process needs to be fully elucidated. HPV E6 and E7 are effective in many important biological processes such as tumorigenesis, control of transcription and apoptosis. Viral E6 and E7 mediate the disruption of cell cycle regulators p53 and pRb, which are universally the most common tumor suppressor genes in carcinogenesis. The p53–p21–DREAM–E2F/CHR pathway controls a large number of cell cycle genes, helping to arrest the cell cycle in case of error and/damage. Therefore, activation of this pathway will be important in the treatment of cancer.

Detailed knowledge of HPV on cell cycle may help in understanding the HPV mechanism during the process of HPV infection and carcinogenesis. Elaborating the HPV cell cycle relationship may help to understand the HPV mechanism in carcinogenesis. It is thought that the information obtained in this process may help develop biomarkers for cell cycle arrest and specific drug targeting in HPV-associated carcinogenesis.

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# Chapter 4

## ROLE OF BIOMARKERS IN ACNE VULGARIS

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## 1. Introduction

Acne vulgaris is a prevalent skin ailment that can affect people of all ages and it can be seen as a side effect in many clinical cases. The pathophysiology of acne is still uncertain, and no specific cause has been found. Acne vulgaris is a sebaceous follicular chronic inflammatory condition caused by abnormal follicular differentiation, androgens, sebum, and the anaerobic bacteria Propionibacterium acnes (P acnes) ( Figure 1). Acne typically appears during puberty, when adrenal androgens generated by the growing adrenal gland cause a rise in sebum production, the lipid-rich secretion of the sebaceous glands. Dietary influences, for example, have been suggested but not verified (2). Among the 11 and 30 years of age, 80 percent of people suffer from acne vulgaris (1). The prevalence of acne beyond puberty declines with age, yet the disease burden in younger adults remains substantial (3). Although there is no perfect cure for acne, most individuals can find a regimen that helps them reduce their lesions (2).

Acne is commonly seen on the face, chest, and back of a person, also its detection all over the body changes the reason of occurrence and treatment approach. The high prevalence of acne biomarkers plays an essential role in differential diagnosis and treatment selection. Acne therapy differs depending on whether it is mild, moderate, or severe.

Adipokines (irisin, adiponectin, resistin, neuropeptide Y, desnutrin, visfatin, and ghrelin), cytokines (IL-1β, IL-6, IL-8, IL-17and IL-19), and antioxidant enzymes (glutathione peroxidase, superoxide dismutase, and catalase) were found to be a biomarker of acne and have a relationship with disease severity.

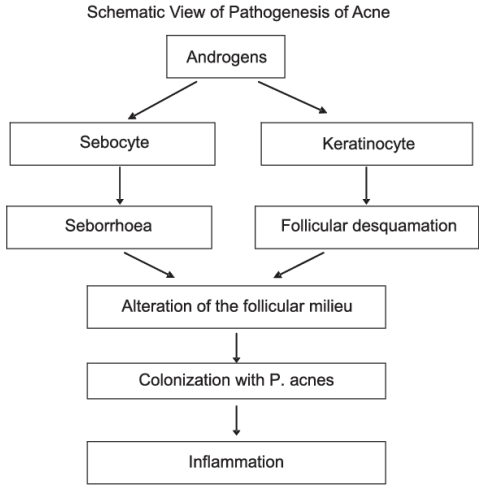


*Figure 1*

*Androgen influence proliferation or differentiation of sebocyte which develop seborrhoea (excessive oil on skin) and keratinocyte cause follicular desquamation. Both ways alter the follicular milieu. Unbalanced milieu become vulnerable to colonization of propionibacterium acne. P. acne led to inflammation on skin and acne development occur.*

## 2. Adipokines

The storage of triglycerides under situations of excess calories and their release during fasting periods, thermoregulation, and mechanical organ protection are the principal activities of adipose tissue. Adipose tissue is now classified as an endocrine organ. Adipokines (proteins/ protein factors) are molecules that are generated in adipocyte (Figure 2). Appetite and satiety, fat distribution, insulin secretion and sensitivity, energy expenditure, endothelial function, inflammation, blood pressure, and hemostasis are all regulated by adipokines (4). Therefore, adipokines can be used as biomarkers.

Extensive studies have begun in dermatological research to discover the likely targets and cutaneous sources of these proteins by identifying changes in their serum levels in dermatological illnesses such as acne vulgaris (5). According to Kovács et al. (2020), importantly, additional research revealed that the skin is not only a target but also a source of adipokine signaling. Sebocytes, in addition to being critical regulators of skin homeostasis, are an intriguing cell type that combines lipid metabolism with inflammation at the cellular level, a key trait shared by adipocytes (6).

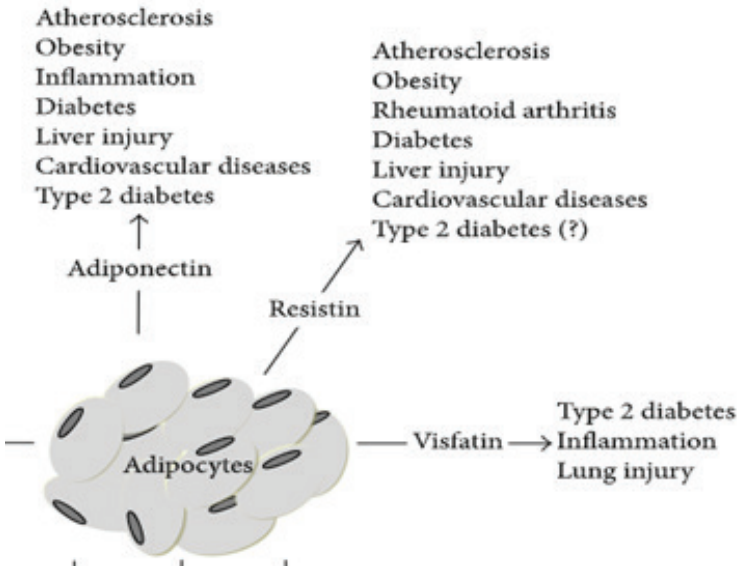


*Figure 2*

*Adipokins such as visfatin, resistin, and adiponectin cause some disease including diabetes, cardiovascular diseases, liver injury, autoimmune disorders, obesity, inflammation, and atherosclerosis.*

### 2.1 Irisin

Adipose tissue and muscles after cleavage of fibronectin type III domain-containing protein 5 (FNDC5) in response to stimuli such as cold exposure and physical activity, earning it the nickname "exercise hormone" secrete new adimyokine "irisin" (Boström et al., 2012). The same authors state that irisin increases thermogenesis and energy consumption in subcutaneous adipose tissue, which protects against obesity and insulin resistance (7). Mustafa AI& El-Shimi aimed to show the connection between irisin and acne vulgaris, also together with insulin resistance (calculated by Homeostasis Model Assessment of Insulin Resistance (HOMA-IR index)).

The result of the study indicates that with the severity of the lesions, serum irisin decreased while the HOMA-IR index increased considerably in acne vulgaris patients. There is a remarkable opposite interaction among irisin and HOMA-IR index. Serum irisin levels were also observed to have a negative relationship with the severity of acne lesions (8). Irisin may be alerting us to the onset of insulin resistance. Early detection of insulin resistance may aid in improved care of acne sufferers. But insulin resistance may not be a significant factor in the development of postadolescent acne (18).

### 2.2 Adiponectin

Adiponectin is a collagen-like plasma protein secreted mainly by adipocytes (9). Furthermore, in hepatocytes (10), myotubes (11), and skeletal muscle (12) adiponectin gene expression can be stimulated. Adiponectin levels in the blood decrease with obesity and are positively linked to insulin sensitivity, as well as being raised in response to insulin (13). The remarkable effect of weight loss is the increased plasma levels of adiponectin (14). Increased adiponectin serum levels improve insulin sensitivity and so regulate energy balance in muscle and liver tissues (15).

According to Çerman et al. (2016), in contrast to healthy control subjects, acne vulgaris patients show quite low serum adiponectin concentration. Adiponectin levels that are low may decrease anti-inflammatory cytokines while activating pro-inflammatory cytokines (16). Due to adiponectin's anti-inflammatory and anti-apoptotic activity, decreased level of adiponectin may impair the protection of the vascular, heart, lung, and colon (17). Another research made by Ozuguz et al. (2017) between healthy group and nonobese with moderate to severe acne vulgaris patients indicates that adiponectin levels do not differ significantly. Isotretinoin (the most effective anti-acne drug) boosted adiponectin levels, which was surprising (18).

### 2.3 Resistin

Due to its resistance to insulin, an international committee named the adipokine "Resistin" (15). In rodents and humans, resistin is a small secretory protein that plays a pleiotropic role (19). The level of resistin in the body increases as the fat mass grows (15). The relationship between fat, inflammation, and atherosclerosis in humans may be explained by resistin (20). Furthermore, mounting data suggest that resistin serves as a host defense peptide of innate immunity, with broad antimicrobial action, immune modulation, and microbial product-induced inflammatory limitation (21).

Resistin gene polymorphisms (RETN -420 C/G) may have a positive relationship in acne vulgaris development and have a link to serum resistin levels and disease severity (22). Younis et al. (2016) found a link between RETN genotypes and serum lipid levels in acne sufferers. Moreover, they discovered acne vulgaris and the severity of acne symptoms strongly linked to polymorphisms in the RETN gene (23). Elevated serum lipids are linked to RETN variant genotypes, which might also modify the sebum composition in sebaceous glands, leads to inflammation during acne development. Along with its combined impacts on lipid metabolism and inflammation, those data support the idea that RETN may play a key role in acne etiology (24). Resistin can be a promising therapeutic target (23).

### 2.4 Neuropeptide Y

Neuropeptide Y (NPY) is an amino acid peptide that is used to manage and cure a variety of disorders including the central nervous system, cardiovascular system, respiratory system, gastro-intestinal system, and endocrine system (25). NPY influences dietary intake through a variety of mechanisms, including signaling the CNS for an energy requirement in the hypothalamus, influencing appetite, and having anorexigenic effect. NPY suppresses keratinocyte proliferation while preventing the buildup of cAMP that occurs when forskolin is induced in the cells. Similarly, NPY prevents pathogenic microorganisms from reproducing in the human skin in an irreversible manner (15).

According to new research on the physiology of the sebaceous gland, the gland has receptors for a variety of neuropeptides. By modifying the function of the pilosebaceous unit, emotional stress linked to the synthesis of hormones, neuropeptides, and inflammatory cytokines promotes the chronic course and worsening of acne (26). If stress is the reason for acne, an interdisciplinary therapeutic approach should be used in eligible patients, comprising not only dermatologists but also psychologists and psychiatrists.

### 2.5 Desnutrin

Desnutrin is an adipokine that controls fat burning in the adipose tissue (15). By enhancing fatty acid oxidation and re-esterification within adipocytes, increasing lipolysis and overexpression of desnutrin in adipose tissue reduces adipocyte triglyceride content and attenuates diet-induced obesity, at least in part. Desnutrin overexpression in adipocytes also improves insulin response by increasing peripheral and hepatic insulin sensitivity (27).

Demir et al. (2014) found that a negative connection between serum glucose and desnutrin levels was seen in the entire cohort (patients and controls) ($r = -0.31$, $p<0.05$). Insulin and desnutrin levels were found to have a positive connection ($r = 0.42$, $p<0.001$). Desnutrin activity was decreased in acne vulgaris patients as a result of higher blood glucose and insulin levels, possibly contributing to insulin resistance (28). Serum desnutrin levels were considerably lower in acne vulgaris patients, but they were unrelated to acne severity and were unaffected by sex (29).

### 2.6 Visfatin

The adipokine visfatin/nicotinamide phosphoribosyltransferase (NAMPT) is primarily synthesized by visceral fat tissues. Vascular remodeling, vascular inflammation, and atherosclerosis have all been linked to high circulating levels of visfatin/NAMPT, all of which raise

the risk of cardiovascular diseases (30). Concerning the glucose and lipid homeostasis, this hormone has insulin-like actions. Visfatin levels will rise in lockstep with body mass (15).

Visfatin expression on sebocytes was identified by Kovács et al. in acne patients. Acne sufferers had significantly greater tissue and serum visfatin levels than the healthy group in the study (Samir et al., 2021). Visfatin may play a role in acne etiology by binding to insulin receptors, causing insulin resistance, which increases duct hyperkeratinization and stimulation of proinflammatory cytokines. These researches are complementary and reveal a link between visfatin and acne.

**2.7 Ghrelin**

Ghrelin, also known as appetite hormone, is distinguished by the fact that, although having a peptide structure, it lacks amino acids and is thus unique to ghrelin in comparison to other hormones. Ghrelin also has anti-inflammatory, anti-microbial, antioxidant properties and releases the growth hormone in addition to being responsible for the regulation of appetite (15).

Ghrelin immunoreactivity is prominent in hair follicles and sebaceous glands in human skin sections, but remarkably low in acne vulgaris patients compared to controls, according to immunohistochemistry studies (32). Inflammation in acne cannot be reduced, and reproduction of bacteria that play major roles in the etiology of the disease cannot be avoided, according to Kanat et al. (2019) who noticed a decrease in levels of these ghrelins with antimicrobial properties. As a result, restoring normal quantities of this hormone may aid in the treatment of acne.

**3. Cytokins**

Cytokines are tiny proteins that serve as signals in an intercellular communication language. Interleukins, chemokines, interferons, lymphokines, and tumor necrosis factors are examples of cytokines. The physiologic role is to coordinate the developmentally planned, constitutive, and unscheduled modeling and remodeling of tissues. Because cytokines can be found in the matrix and cell binding to matrix proteins can both stimulate and complement with cytokines, it's probable that apparent cell differentiation induction by matrix proteins alone is sometimes due to the simultaneous effect of matrix proteins and cytokines going to act in an autocrine or paracrine function (34). To regulate the human immune response, cytokines work in tandem with cytokine inhibitors and soluble cytokine receptors.

Fluctuations in cytokine concentrations are to be expected in acne patients. Acne vulgaris pathogenesis is influenced by a variety of cytokines.

Inflammatory cytokines (pro-inflammatory or anti-inflammatory) have been studied in relation to the condition. With the release of cytokines, inflammation plays a key role in the creation of both inflammatory and noninflammatory acne lesions (35).

### 3.1 Interleukin-1 beta

Acne lesions include increased inflammation and Propionibacterium acnes invasion of the pilosebaceous unit. In monocytic cells, Propionibacterium acnes causes pro-inflammatory IL-1β production (36). IL-1β is produced not just by immune cells, but also by adipose tissue (3). ElAttar et al. (2022) compared IL-1β levels in acne patients, post-acne scar patients, and healthy people. When comparing acne vulgaris to post-acne scars and controls, there was a statistically significant increase in IL-1β expression in both ($p<0.001$). Both the clinical severity of acne vulgaris ($p=0.022$) and the degree of histological inflammation ($p=0.011$) were significantly positively linked with IL-1β expression (37). Overall, IL-1β may be a crucial role cytokine in acne causation and severity.

### 3.2 Interleukin-6

Inflammatory diseases have also been investigated extensively with IL-6, a pleiotropic cytokine. This cytokine modulates immunological responses, hematopoiesis, and inflammation and plays an important role in host defense systems (38). Ji et al. (2018) discovered that the levels of IL-6 expression in acne skin lesion tissues were substantially greater than in normal skin tissues (39). Two more investigations found a positive link between acne and the IL-6 gene promoter. In individuals with acne, there was a substantially larger relationship of IL-6 572 variant genotypes compared to the healthy control group ($P<0.001$), with a higher incidence of the IL-6 572 CC polymorphism in acne patients. However, no significant correlations were seen between the IL-6 572 variant genotypes and patient sex or AV severity (40-41).

### 3.3 Interleukin-8

IL-8, also known as neutrophil-activating peptide-1, is a chemokine expressed by macrophages that induce neutrophils to move to the infection site. P. acnes-induced IL-8 production by acne patients' peripheral blood mononuclear cells was substantially higher than that of normal donor peripheral blood mononuclear cells (42). To suppress IL-8, targeting the production of it may help in minimizing the adverse effects of IL-8-mediated inflammatory response and angiogenesis in inflammatory acne vulgaris compared to normal skin. (43).

### 3.4 Interleukin 17

IL-17 has been related to adaptive immunity due to its interaction with T helper 17. When compared to the levels of IL-17 and 25-hydroxycholecalciferol expression in healthy skin of the controls, active acne lesions have higher IL-17 and lower 25-hydroxycholecalciferol expression (44). This inverse sequence could play a function in active acne vulgaris and the severity of the condition (45). Serum IL-17 levels were considerably greater in acne vulgaris patients than in the control group, suggesting that it could be used as a biomarker for disease pathogenesis as well as a potential prognostic predictor for acne vulgaris severity and scarring (46). Because of the important involvement of IL-17 in the etiology of acne, novel treatment methods may be developed.

### 3.5 Interleukin 19

In both immunological and non-immune cells, IL-19 promotes an anti-inflammatory response. The considerable difference in IL-19 serum levels between individuals with moderate acne vulgaris and patients with severe acne vulgaris could indicate that inflammation plays a key role in acne vulgaris severity (47-48). Due to its distinct characteristics, IL-19 may be linked to acne vulgaris inflammation and severity, which could lead to further investigation.

## 4. Antioxidant Enzymes

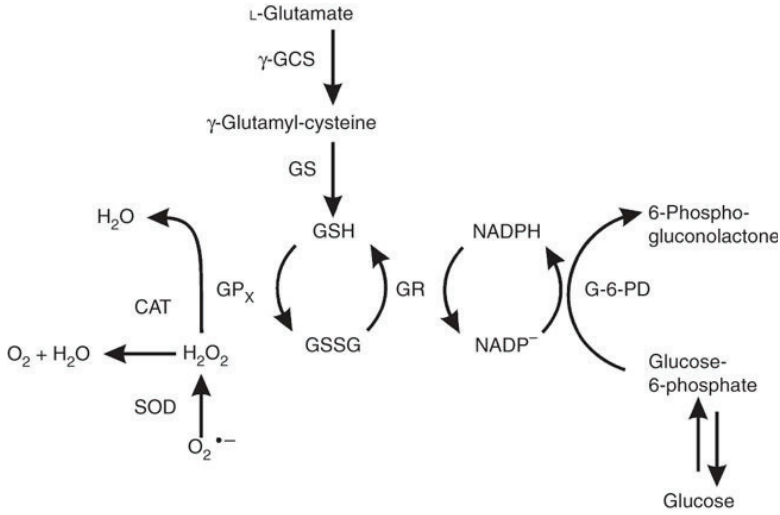


*Figure 3 (Glutathione peroxidase ($GP_x$) and catalase (CAT) converts hydrogen peroxide to water, superoxide dismutase (SOD) catalyze conversion of superoxide ion ($O_2^-$) to hydrogen peroxide ($H_2O_2$). $GP_x$ also have role in gluthatione (GSH) conversion to gluthatione dissulfide (GSSG). If reduction of GSSG to GSH by gluthatione reductase (GR) is inhibited, the ROS enzymes will have negative impact. Inadequate glucose uptake will also affect the ROS enzymes negatively due to relationship with GR.)*

Highly reactive, short-lived, compounds produced from the molecule oxygen are known as reactive oxygen species (ROS). Increased levels of ROS are linked to the aging process and a variety of human diseases, including cancer, ischemia, and immune and endocrine dysfunction. Several non-enzymatic and enzymatic antioxidant actions exist to protect against the buildup of ROS (49). Oxidative stress occurs when oxidants (free radicals) outnumber antioxidants, favoring free radicals (50). When oxidative stress occurs because of a pathologic event, the defense system encourages the regulation and expression of these enzymes. The antioxidant defense system and ROS scavenging are important in maintaining normal cellular physiology, fighting disease, and fostering immunity (49).

The early steps in the pathogenic processes of acne formation include oxidative stress-induced inflammation and its maintenance inside the pilosebaceous unit. The favorable environment for Propionibacterium acnes growth provided by sebum oxidation is also a contributing component in these processes. This process suggests that oxidative stress could be used as a therapeutic target for acne vulgaris (50).

### 4.1 Glutathione Peroxidase

The enzyme glutathione peroxidase converts hydrogen peroxide to water, and its main biological function is to protect the organism from oxidative damage (Figure 3). The importance of oxidative stress in the reduction of glutathione peroxidase levels is attributed to the significant negative association between glutathione peroxidase levels and their negative correlation with disease severity (51). The levels of glutathione peroxidase were not substantially different between control and groups in mild and moderate acne, but there was a significant difference between control and groups of patients with severe acne (52).

### 4.2 Superoxide Dismutase

Conversion of the superoxide radical into an oxygen molecule and hydrogen peroxide is catalyzed by the superoxide dismutase enzyme (Figure 3). In contrast to patients with moderate and severe acne, and controls, patients with mild acne had statistically significantly higher superoxide dismutase activity. Moreover, when compared to other patient subgroups and controls, severe acne exhibited statistically significantly lower superoxide dismutase activity (53). The higher quantities of superoxide anion radicals in the epidermis could be due to the reduced activity of superoxide dismutase in polymorphonuclear leukocytes (54). New drugs for acne treatment can stimulate lymphocytes with the anti-oxidative property.

### 4.3 Catalase

Catalase is an enzyme that converts hydrogen peroxide to water and oxygen and is found in almost every living organism (Figure 3). Al-Shobaili et al in 2014 discovered that the sick group had considerably reduced catalase activity than the control group. However, there was no statistically significant variation in catalase activity levels amongst patients with varying degrees of disease severity (55). According to research, high hydrogen peroxide levels but low catalase levels were determined. Acne vulgaris can be worsened by reduced catalase plasma levels (56).

## 5. Conclusion

Since acne is a multifactorial disease, determining an accurate diagnosis and prognosis can be difficult. Hence, understanding the pathophysiology of acne is critical. It is important to select the appropriate biomarkers in order to comprehend the disease's etiology. Acne vulgaris biomarkers, fortunately, have characteristics that can be employed in both diagnosis and treatment. They allow us to better understand the severity, triggering factors, and natural history of the disease. Targeting the correct biomarker will aid the rebalance of the natural microbiome.

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# Chapter 5

## BIOLOGICAL PROPERTIES OF VITAMIN D AND ITS EFFECT ON THE PATHOGENESIS OF PERIODONTAL DISEASE

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## 1.Introduction

Vitamins are essential for the human organism, and they are organic molecules that take part in various enzymatic reactions that cannot be produced in the body, and in which specific diseases occur in their deficiencies (Jameson & AP, 2004). One of the most important vitamins is vitamin D and its importance is increasing day by day. Its main effect is on the regulation of calcium and phosphorus metabolism and bone mineralization (ATAŞ, ÇAKMAK, & SORAN, 2008; Jameson & AP, 2004). In addition, cell differentiation and proliferation, cardiovascular functions, cellular humoral immunity play a role in biological mechanisms. On the other hand, vitamin D has been associated with many systemic conditions and diseases such as neurological diseases, cancers, heart diseases, metabolic syndrome, infectious, rheumatic and autoimmune diseases, COVID-19, etc. (Holick, 2008; Hypponen, Boucher, Berry, & Power, 2008; Pludowski et al., 2013).

Vitamin D is among the fat-soluble vitamins. Unlike other vitamins, it can be synthesized endogenously, so it is considered a steroid hormone rather than a vitamin (Holick, 2004). Vitamin D is similar to a steroid hormone, especially cholesterol, because it is actively synthesized in the organism, acts on tissues through receptors, and is regulated by feedback mechanisms (Vieth, 2004).

### Vitamin D Structure and Biosynthesis

There are 5 forms of vitamin D: D1 (ergocalciferol with lumisterol) , D2 (ergosterol with ergocalciferol), D3 (cholecalciferol), D4 (22 dihydrocalciferol) and D5 (cytocalciferol). Among the compounds that have the effect of vitamin D, the most important biochemically are vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol) (Ketharanathan, Torgersen, Petrovski, & Preus, 2019). Although both forms are chemically similar, their biological origins are different; While cholecalciferol is synthesized endogenously in the skin under the influence of sunlight, ergocalciferol is of vegetable origin (Yücel, 2018) . The D2 form differs from the D3 form in that it has a 24-methyl group and double bonds at the 22nd and 23rd carbons, causing it to be more biologically active. In addition, the D3 form can be produced synthetically. D1 and D2 forms of vitamin D are shown in Figure 1. (Armas, Hollis, & Heaney, 2004).

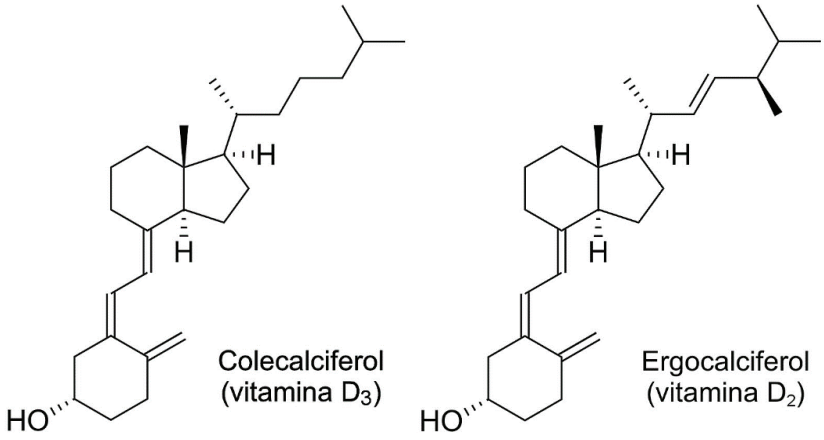


**Figure 1.** *Cholecalciferol and ergocalciferol (Armas, Hollis, & Heaney, 2004) .*

95% of the need for vitamin D is produced in the skin by the effect of sunlight. A small portion is met with food (Bouillon, Norman, & Lips, 2007). Vitamin D2 (Calciferol, Ergocalciferol): Plant-derived ergosterol, which is a provitamin, is taken in foods and stored in the skin. With the effect of UVB, it is converted to ergocalciferol in the stratum basale and stratum spinosum layer of the skin. This substance enters the hydroxylation reaction in the liver and kidneys. Vitamin D3 (Cholecalciferol): It is partially taken with animal foods and synthesized in the body. It is a hormone analog precursor, not a true vitamin. After a two-step bioactivation, cholecalciferol is converted to calcitriol, the most active form of vitamin D, 1,25-dihydroxycholecalciferol.

Cholesterol synthesized in the liver is converted to 7-dehydrocholesterol (7DHC) here and then passes into the peripheral blood and reaches the malpighi layer of the skin. UV rays penetrating the epidermis, transform the inactive provitamin D3 (7-dehydrocholesterol) in the skin into previtamin D3 by photolysis (Vieth, 2004). 7-dehydrocholesterol is converted to 25-hydroxyvitamin D3 (25(0H)D) by hydroxylation of the 25th carbon atom in the liver. In order for vitamin D to be converted to its active form, it must be converted to 1.25 dihydroxyvitamin D [1,25(OH)2D] by 1 alpha hydroxylase in the kidneys. The enzyme 1 alpha hydroxylase is the key enzyme in vitamin D synthesis. 25(OH)D is hydroxylated in the kidneys to 1,25(OH)2D3 (calcitriol), the most active form of vitamin D (de Tena, Abejón, & Horcajo, 2011; Holick et al., 2011). (FIG 1) In addition, vitamin d can be synthesized by immune system cells, epithelial cells and osteoblasts that express the CYP27B1 gene. (OH)2D3 is synthesized (DAL & İŞLEKEL, 2019). The 1,25(OH)2D3 metabolite is 100-500 times more active than 25(OH)D and increases intestinal calcium absorption. When

calcitriol reaches a sufficient level, it increases the release of 24 hydroxylase enzyme, and the added part is converted into 24,25-dihydroxyvitamin D3 (24,25 (OH)2D3) in the kidneys and then catabolized (13, 15). Vitamin D metabolism is shown in **Figure 2.**

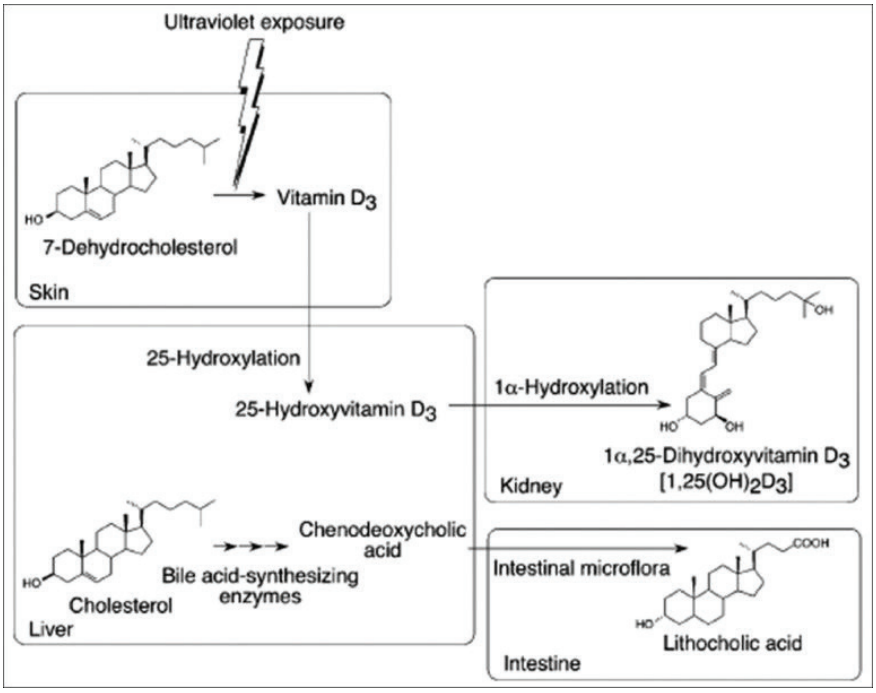


**Figure 2.** *Biosynthesis of vitamin D (Anand, Chandrasekaran, & Rajput, 2013).*

**Vitamin D metabolism**

Vitamin D compounds are transported in the circulation by binding to vitamin D binding protein (DBP) at a rate of 85% and to albumin at a lesser rate (14.6%) (Bikle, 2014). Approximately 0.4-3% of vitamin D is found freely in serum (Holick, 2003; Volmer, Mendes, & Stokes, 2015). 1,25(OH)2D exerts its effect on the vitamin D receptors (VDR) found in the small intestine, kidney and other tissues.

It protects the blood calcium level, which is the general function, by increasing the absorption of Ca from the small intestine and reducing the loss of Ca from the kidneys. In addition, 1,25(OH)2D vitamin has biological effects that inhibit cell proliferation, stimulate terminal differentiation, inhibit angiogenesis, stimulate insulin production and inhibit renin production (Hua-Huy & Dinh-Xuan, 2015; Vickers, 2017). When vitamin D and its metabolites reach a certain level in the blood, they are inactivated by 24 hydroxylase expressed by the CYP24A1 gene found in many tissues. 24,25(OH)2 is converted to D3 and excreted in the urine as calcitroic acid (Thomas et al., 1998; Uitterlinden, Fang, Van Meurs, Pols, & Van Leeuwen, 2004).

Parathyroid hormone (PTH) and Fibroblast Growth Factor-23 (FGF-23) are mainly involved in the regulation of vitamin D. FGF-23 increases the expression of the CYP27B1 gene when the vitamin D level is increased. Thus, 24-α Hydroxylase enzyme is activated and vitamin D is inactivated. PTH works as an antagonist with FGF-23. When the level of active vitamin D decreases, it inhibits the 24-α Hydroxylase enzyme. It also induces vitamin D synthesis (Christakos, Ajibade, Dhawan, Fechner, & Mady, 2012).

**Vitamin D mechanism of action**

Vitamin D is involved in bone homeostasis and regulation of many genes. Vitamin D exerts its effect mainly through the genomic pathway or non-genomic pathway mediated by the vitamin d receptor (VDR) of its active form 1,25(OH)2D3. REF Non-genomic pathway, membrane vitamin D receptor (mVDR) or membrane 1,25 D3 The associated rapid response is activated by steroid-binding protein (1,25 D3-MARRS). Activation of mVDR or 1.25 D3-MARRS provides rapid response to vitamin D. Activation of the non-genomic process activates some transcription factors or opens Ca2+ and Cl- channels (Bouillon et al., 2006; Feldman, Krishnan, & Swami, 2013; Hii & Ferrante, 2016).

Vitamin D exerts its genomic effects through the VDR attached to the nuclear membrane. It is a member of the VDR nuclear receptor family and is involved in gene regulation (Haussler, Jurutka, Mizwicki, & Norman, 2011; Hossein-Nezhad, Spira, & Holick, 2013). VDR is a transcription factor found in almost all cells, although at variable levels. Generally, ligand binding to the receptor initiates biological pathways by activation or inhibition of target genes (Jain & Micinski, 2013). When VDR binds with vitamin D, it forms a heterodimer structure with the retinoic x receptor (RXR) and binds to the vitamin d response element (VDRE). Activation or inhibition of the target gene occurs. The response via the genomic pathway is later (Heikkinen et al., 2011; Ramagopalan et al., 2010).

The VDR protein is directly involved in gene transcription. Therefore, changes in the VDR gene affect many biological functions such as calcium metabolism, immune system, cell differentiation, oxidative stress, etc. (Simon, Munger, & Ascherio, 2012; Uitterlinden et al., 2004).

**Detection of vitamin D level**

1,25OH-D is the most active form, but has a short half-life and its circulating amount varies. Therefore, serum 25(OH)-D is used to evaluate the vitamin D level, since it shows the level of vitamin D that is taken from outside and produced endogenously. , ≥30 ng\ml was accepted as optimal. On the other hand, 25(OH)-D was determined as >150 ng/ml toxic level (Holick, 2006, 2009)

## Anti-Inflammatory and Antioxidant Effect of Vitamin D on the Pathogenesis of Periodontal Disease

### *Periodontal Disease Pathogenesis*

Periodontal disease is an inflammatory disease with a complex pathogenesis with changes in the host immune response caused by microbial dental plaque (Löe & Silness, 1963). Periodontal disease is mainly caused by the disruption of the balance between the microbial dental plaque and the host response. On the other hand, various risk factors such as systemic diseases, genetics, smoking, stress, nutrition are also effective in the progression of the disease (Sahingur & Cohen, 2004).

Dysbiosis occurring at the onset of periodontal disease; It leads to the production of excess cytokines, reactive oxygen products that cause oxidative stress, and matrix metalloproteinases, and host response resulting in periodontal tissue damage. Injury-associated molecular peptides that further enhance the inflammatory response are released and the condition results in chronic inflammation if the subsequent inflammation is not resolved naturally (Bowen, 1976; Guentsch et al., 2011; Liebana & Castillo, 1994).

The host response is initiated by innate immune system cells and moderated by adaptive immune system cells. Vascular changes, vascular damage and increased vascular permeability are seen in the early stage of inflammation. The cells that are active during this period are neutrophils. Neutrophils try to eliminate the agent by synthesizing pro-inflammatory cytokines such as lysosomal enzymes and arachidonic acid metabolites that will cause tissue destruction. In case of insufficient neutrophils, macrophage cells come into play. (38). In chronic inflammatory response; Infiltration of mononuclear cells such as macrophages, lymphocytes and plasma cells, fibroblast proliferation, connective tissue and bone destruction dominate (Page, 1998)

While periodontal disease is initially limited to the gingiva, peripheral attachment loss, bone resorption, periodontal pocket formation, gingival recession, and finally tooth loss are seen in the later stages of the disease (Kantarci, Oyaizu, & Van Dyke, 2003).

### *Vitamin D and Inflammation/Inflammatory Response*

The periodontium is constantly exposed to dental plaque containing pathogenic and non-pathogenic oral microorganisms (Muthukuru, Jotwani, & Cutler, 2005). The gingival barrier is the first line of defense (Kusumoto et al., 2004). 1,25(OH)D3 induces the synthesis of proteins required for intercellular junction of epithelial cells. Thus, the penetration of pathogens and their products into tissues is prevented or slowed down (Hewison,

2008; White, 2008)

Another important step involved in the innate immune response is antimicrobial peptides. Antimicrobial peptides are one of the host defense mechanisms. Antimicrobial peptides, which have cationic and amphipathic molecular structures, protect epithelial surfaces and play a role in the regulation of the host defense mechanism, thanks to their antimicrobial properties (Lemaître et al., 2017). Structurally, they are divided into two subgroups as anionic and cationic according to their amino acid compositions. They are mainly secreted by oral epithelial cells, neutrophils and salivary glands. Antimicrobial proteins have been shown to be effective against gram-negative and gram-positive bacteria, viruses, fungi, and even altered and cancerous cells (Brogden, 2005). Antimicrobial peptides, which have many killing mechanisms against bacteria, are effective against bacterial nucleic acids, as well as by disrupting the structure of the pathogenic cytoplasmic membrane (Izadpanah & Gallo, 2005). Major antimicrobial proteins; cathelicidins, defensins, histatins, granulizins, lactoferrin, and hepcidins (Komatsuzawa et al., 2006; López-García, Lee, Yamasaki, & Gallo, 2005).

Although antimicrobial peptides are effective in killing pathogens, they also have other functions in the innate and adaptive immune response. It is effective in the activation of phagocytosis, the release of pro-inflammatory cytokines and the regulation of the complement system. It has also been reported that they show chemotactic properties and are effective in angiogenesis (White, 2010). Antimicrobial peptides protect the oral epithelium from infection in the early period. It prevents the progress of the current situation. On the other hand, bacterial dental plaque is the primary cause of periodontal diseases. Antimicrobial peptides prevent biofilm formation before dental plaque is formed (Gorr & Abdolhosseini, 2011). Therefore, antimicrobial peptides play a role in the progression of periodontal disease (Gorr & Abdolhosseini, 2011). Vitamin D is considered a potent antimicrobial peptide stimulant. The production of cathelicidin and some defensins (defensins hBD-2) in the human body depends on sufficient circulating 25(OH)D15. 1,25(OH)2D3 induces antimicrobial peptide expression in human keratinocytes, monocytes, and neutrophils (Georgieva et al., 2019; Roider, Ruzicka, & Schauber, 2013). In addition, TLRs, which play an important role in the innate immune response, play a role in vitamin D-mediated antimicrobial peptide production. TLRs are molecules that recognize molecular patterns associated with microbial pathogens. They are involved in the initiation of innate and adaptive immune response (Song et al., 2017). TLRs are found in many cells, including macrophages, dendritic cells, and epithelial cells. In humans, CYP27B1 (1α-hydroxylase) enzyme is activated when TLR2/1 and TLR4

are stimulated with their ligands. Thus, active vitamin D (1,25(OH) 2 D) and production of antimicrobial peptide cathelicidin is induced. Vitamin D supports the innate immune response by activating natural antimicrobial peptides such as defensins (Adams & Hewison, 2008; Medzhitov, 2001). In addition, it has been reported in recent studies that serum 25(OH)D deficiency correlates with decreased hBD-2 and cathelicidin levels in periodontal tissues (Ganz, 2003).

Antimicrobial peptides exert antibacterial action against periodontapathogens. Cathelicidin LL-37, which has the strongest antimicrobial activity, is effective on some oral organisms such as hBD3 and hBD2 Streptococcus salivarius (Ouhara et al., 2005; Schwalfenberg, 2011). In particular, beta defensin 2 kills A. actinomycetemcomitans, a periodontal pathogen associated with AgP (Feucht, DeSanti, & Weinberg, 2003). Thus, Antimicrobial peptides enable the organism to be tolerated by the host on the epithelial surface and actively protect the host from inflammation caused by other pathogens. Therefore, vitamin D deficiency may compromise the host's ability to fight periodontal pathogens (Gombart, Borregaard, & Koeffler, 2005).

Vitamin D shows some of its effects on the immune system through monocytes. Vitamin D reduces the production of proinflammatory factors such as IL-1β, IL-6, TNF-α, RANKL, COX-2 and nitric oxide in macrophages. On the other hand, it increases the production of anti-inflammatory cytokines such as IL-10 (Nurminen, Seuter, & Carlberg, 2019). 1,25-Dihydroxyvitamin D3 activates the secretion of hydrogen peroxide by human monocytes. On the other hand, it increases differentiation into M2 phenotype by decreasing the differentiation of macrophages to M1 phenotype associated with inflammatory processes and autoimmunity (Dankers, Colin, Van Hamburg, & Lubberts, 2017; Zhang, Zhou, Guo, Song, & Liu, 2015). In vitamin D deficiency, dysfunction occurs in macrophages and the immune system is affected because chemotaxis, phagocytosis, and proinflammatory cytokine production cannot be performed (Manolagas, Hustmyer, & Yu, 1990; van Etten & Mathieu, 2005).

Acquired immune response; occurs after the natural immune response. The pathogen-specific host response is elicited by macrophages and dendritic cells, T and B lymphocytes, cytokines and immunoglobulins. Antigen-stimulated T cells differentiate into Th1 and Th2 cells according to their cytokine production status. Th1 cells; They produce pro-inflammatory cytokines, IFN-gamma, IL-2 and TNF-α, and induce a cellular response. Th2 cells, on the other hand, produce anti-inflammatory cytokines, IL-4 and IL-5, and direct the humoral immune response. Disruption of the balance between these two cells indicates the direction of the immune response (Delves & Roitt, 2000). 1,25(OH)D3 is also involved in the regulation

of the specific immune system by affecting macrophages, neutrophils, dendritic cells, B lymphocytes and T lymphocytes (Aranow, 2011). Vitamin D prevents the uncontrolled release of IFN-γ and TNF-α by inhibiting the overactivation of CD8+ cytotoxic T cells. 1,25(OH)D3 is also involved in the regulation of the specific immune system by affecting macrophages, neutrophils, dendritic cells, B lymphocytes and T lymphocytes (Aranow, 2011). Vitamin D prevents the uncontrolled release of IFN-γ and TNF-α by preventing excessive activation of CD8+ cytotoxic T cells (Kongsbak et al., 2014). Similarly, 1,25(OH)D3 prevents the conversion of CD4+ helper T lymphocytes into Th17 cells and Th17 activation. In addition, it has been reported that 1,25(OH)D3 administration inhibits differentiation into Th1 cells and initiates the differentiation process into Th2 cells. In addition, vitamin D exerts anti-inflammatory effects by increasing the expression of IL-10 and FOXP3 towards the regulatory T cells (Treg) phenotype, which has an important role in the regulation of the immune system (van Hamburg et al., 2012). In summary, vitamin D suppresses the proliferation of T-lymphocytes, the secretion of immunoglobulins, and the transformation of B-lymphocytes into plasma cells. It prevents excessive host response and tissue damage by activating anti-inflammatory processes (Krishnan & Feldman, 2011). In an in vitro study investigating the anti-inflammatory effect of vitamin D, Porphyromonas gingivalis was applied to periodontal ligament cells. It was observed that less inflammatory cytokines were produced in the group treated with 1,25(OH)D3 with Porphyromonas gingivalis (Tang, Pan, & Zhao, 2013).

## Conclusıon

Recent studies show that low vitamin D levels affect the progression of periodontal disease (Agrawal et al., 2019; Isola et al., 2020; Ketharanathan et al., 2019). Vitamin D can act directly on the primary periodontal pathogen, Porphyromonas gingivalis, as well as in the prevention of periodontal disease through anti-inflammatory mechanisms (Han et al., 2019; Li, Zhong, Li, & Wang, 2019; Oh, Kim, & Kim, 2019; Q . Wang et al., 2020). Until today, the effect of vitamin D on bone metabolism has been emphasized. However, vitamin D is also effective on periodontal diseases as an immunomodulator. Although the mechanism of action has not been fully elucidated, more randomized controlled studies are needed.

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# Chapter 6

## THE IMPORTANCE OF VITAMINS IN COVID-19 DISEASE

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## 1. COVID-19 Disease

Coronaviruses within the family Coronaviridae are enveloped and single-stranded RNA viruses. It has been named as Coronavirus (Crowned Virus) because the rod-like extensions on the surfaces of the viruses resemble the shape of a crown. Looking at the literature, the first identification of corona viruses dates back to the 1960s. There are six human corona viruses identified up to date. Although four of these are more common in humans than the others, they have less disease-causing properties and their common features are that they cause symptoms similar to the common cold. The other two corona viruses are SARS-CoV (the Severe Acute Respiratory Syndrome corona virus) and MERS-CoV (the Middle East Respiratory Syndrome corona virus). These two types of corona viruses can cause much more serious respiratory diseases than others. ('Xinhua News Agency', 2020).

In December 2019, the virus that caused an epidemic of pneumonia in humans in the city of Wuhan, Hubei province of China, was defined as the new corona virus (SARS-CoV-2). This newly discovered virus is the first corona virus to pass from a human to human. ('Xinhua News Agency', 2020). The disease caused by the new type of virus has been named as COVID-19 (Coronavirus Disease-2019). Since there was not much information about the transmission route and treatment method of the virus when the disease first appeared, the disease spread to the world in a very short time and reached very serious dimensions. This epidemic was recognized as a 'pandemic' by the WHO (World Health Organization) on March 11, 2020. By March 14, 2022, the number of COVID-19 cases in the world reached 452,201,564 and the number of deaths reached 6,029,852. 14,487,482 cases and 96,094 deaths have been confirmed in Turkey by March 14, 2022 (WHO,2020a).

Studies have shown that the Sars-Cov-2 virus, which causes COVID-19 disease, is transmitted through droplets. The rate of spread of the disease can be reduced by having information about the transmission routes of the disease and taking the necessary preventive measures (WHO,2020b).

Vaccines are the most effective way to prevent viruses. As of March 12, 2022, a total of 10,712,423,741 doses of vaccine have been administered in the world (WHO, 2020a). Although the vaccine prevents the disease from being severe, the best method after vaccination is to strengthen the immune system, since viruses are constantly mutating and there is no proven antiviral drug developed against the SARS-CoV-2 virus yet. Very serious immune abnormalities appear to occur in patients infected with the SARS-CoV-2 virus. These abnormalities can cause infections and later mortality due to many reasons. Therefore, there is a very important relationship

between the severity of the COVID-19 disease, its progression rate, and immune system characteristics. For this reason, it has been reported that strengthening the immune system can be considered as a treatment method against SARS-CoV-2 (Yang, C. W.,2020).

It is stated that metabolic events at the cellular level in the immune system cause changes and affect homeostasis. For instance, the risk of infection increases with the deterioration of immunity in malnutrition or the activation of the inflammatory response caused by overnutrition, the increase of oxidative stress and the suppression of the adaptive immune system (Butler J. M.,2020; Khaled BM, 2020; Sanders J, 2010). Therefore, maintaining nutritional balance is also important in maintaining systemic homeostasis (Alwarah Y, 2018). However, the fact that a lot of people experience the disease in a mild asymptomatic phase while others experience a severe symptomatic phase awaits scientific response regarding the disease (Shi Y, 2020).

The most effective way to fight the virus is to strengthen the immune system. It has been known since long time ago that vitamins are very important in strengthening the immune system. As the mechanism of action of each vitamin is different from each other, their effect status in COVID-19 is also different from each other. In this study, by summarizing the general structures of vitamins, a compilation of recent studies associated with COVID-19 was made and it was aimed to draw attention to the use of vitamins in COVID-19 disease.

## 2.Vitamins

Vitamins are organic compounds that must be present in the humans body as catalysts and regulators in order for humans to perform their vital functions (Bingöl G,1977). Vitamins are generally classified under two headings: vitamins A, K, E, D, which are fat-soluble vitamins; and vitamins C and B, which are water-soluble vitamins (Holick MF, 2007). Although most of the vitamins are supplied to our body from external sources through food, there are few vitamins that can be synthesized in the body, such as vitamin D (Bingöl G, 1977).

### 2.1. Vitamin A and Its Relationship with COVID-19

Vitamin A (Retinoids) is not produced endogenously, it is taken into the body exogenously. Vitamin A exists in three forms; retinol (alcohol form), retinal (aldehyde form) and retinoic acid (acid form), and its metabolically active form is retinoic acid (Pino-Lagos K, 2020).

It is obtained from herbal foods as carotenoids and from animal foods as retinol. The 2 most important forms of vitamin A are ATRA (All-Trans Retinoic Acid) and 9-cis retinoic acid. Both forms regulate the transcription

of more than 500 genes that are important in cell proliferation, cell differentiation, and gene expression.

Vitamin A is important in the realization of various physiological functions from embryonic development to visual functions, from differentiation and maturation of epithelial tissue cells to brain functions (Pino-Lagos K, 2010; Huang Z, 2018; Debelo H, 2017).

Studies have shown that vitamin A has immunomodulatory effects in inflammatory diseases (Oliveira LdM, 2018). The relationship between vitamin A deficiency and the immune system has been a subject of interest for many researchers for many years. It has been revealed that vitamin A plays a role in cellular and humoral immune processes and its deficiency causes deterioration in immune system responses. Its deficiency has been reported to increase keratomalacia, xerophthalmia, epithelial cell dysfunction, gastrointestinal system diseases, and some infections (Huang Z, 2018).

It has been determined that vitamin A plays an important role against pneumonia and when vitamin A supplementation is given in children with its deficiency, both infection and mortality rates are reduced by strengthening the immune system (Tian Y, 2020).

In a different study, 100 patients in the mild to moderate COVID-19 treatment group (50 patients on placebo and 50 patiens on supplement) were given 2 doses of vitamin A (200,000 IU) for 2 days, after 2 days of supplementation, it was shown that the symptoms of the patients who took the supplement decreased, while the symptoms did not decrease in the placebo group (Al-Sumiadai MM, 2021).

In addition, in a clinical study, vitamin A was included in the treatment process of 90 patients to restore the loss of smell caused by the disease due to the decrease in olfactory cells because of vitamin A deficiency. Vitamin A given pharmacologically or in high doses has been shown to increase the formation of regulatory T cells while inhibiting the activity of T cells that can induce disease progression and the release of proinflammatory cytokines (Mucida D, 2007; Xiao S, 2008).

It supports the idea that vitamin A can be seen as an alternative treatment for COVID-19 disease, with its features such as increasing the body's immunity by increasing IgM and IgG levels, activating T lymphocytes (Lu H, 2020; Stephensen CB, 2001) and contributing to curing pneumonia at a level that can be demonstrated by clinical studies (Zhang P, 2018; Li R, 2020).

According to a study related to this idea, the use of vitamin A as a clinical COVID-19 treatment has been proven and explained by cell

signaling pathways and different biological processes (Xing Y,2020).

Like other vitamins, vitamin A, especially as a result of its role in the immune system, is seen to have curative effects in COVID-19 disease, which has such a high mortality rate and still causes deaths. In this situation, the importance of vitamin intake in fighting this disease may be greater than thought.

### 2.2. Vitamin E and Its Relationship with COVID-19

Vitamin E is a fat-soluble vitamin consisting of eight isoforms as α, γ, β and δ, including 4 tocopherols and 4 tocotrienols. These isoforms are not interchangeable and only α-tocopherol meets human vitamin E requirements. The main source of vitamin E is found in nuts such as almonds and hazelnuts, peanuts, as well as legumes, avocados and sunflower seeds (Jovic TH, 2020). In humans, vitamin E deficiency is rare except in individuals with malabsorption disorders (Iddir M, 2020).

Vitamin E cells have the antioxidant function required to maintain the membrane integrity and bioactivity of the cells. Along with that, vitamin E has anti-inflammatory and immunomodulatory properties. Vitamin E inhibits the activity of protein kinase C-α (PKC) by increasing its phosphorylation. In this way, it causes inhibition of thrombocyte aggregation, decrease in vascular smooth muscle cell proliferation, and decrease in superoxide production in macrophages and neutrophils (Lee GY, 2018).

Recent studies have reported that vitamin E plays an immunomodulatory role in inflammation and neurological damage (Khadangi F, 2019).

Although it has been shown that the effects of vitamin E on the immune system occur through many different mechanisms, the most studied is that it enhances the T cell-mediated immune response (Lee GY, 2018). Studies have shown that the activation of T cells, which decreases with aging, can be increased with vitamin E (De la Fuente M, 2008; Marko MG, 2007; Meydani SN, 2018). Considering the general effects on the immune response, it can be said that it is important to pay attention to adequate intake, especially in the elderly population, where the immune system is weakened and respiratory tract infections are common (Meydani SN, 2018).

In addition to its antioxidant effect, there are studies showing that supplementing with vitamin E reduces the risk of respiratory tract infection (Hemilä H, 2016; Meydani SN, 2004). In a different study, the ratio of plasma tocopherol concentrations to circulating lipids in ARDS patients compared to the control group was examined. It was reported that the level of tocopherol showed a specific deficiency in ARDS patients

compared to the control group (Bertrand Y,1989). It has been reported that serum vitamin E levels are lower in critically ill patients with acute respiratory distress syndrome (ARDS), which is one of the most important side effects of COVID-19 (Bertrand Y,1989).

In a study, it was determined that in the pathogenesis of influenza, which causes inflammation-induced lung damage and cell damage in the bronchi and bronchioles due to viral effects, vitamin E indirectly plays a protective role against damage to the lung with its antioxidant effect (Mileva M, 2018). In a randomized controlled study, it was determined that 600 IU/day vitamin E supplementation provided a 10% reduction in the risk of chronic lung disease in women (38).

According to Meydani and colleagues' study in the elderly, it was found that 135 mg of vitamin E supplementation per day reduced the risk of upper respiratory tract infections (Agler AH, 2011). In a different study conducted in elderly individuals, it was reported that 50 mg of vitamin E supplementation per day reduced the incidence of pneumonia by 69 % (Lee GY, 2018). Again, in a different study conducted with elderly individuals, it was shown that 200 mg daily vitamin E supplementation did not have an effect on the severity or incidence of respiratory tract infections (Graat, JM, 2002). It is stated that the inconsistent results may be due to differences in the administration of vitamin E, in addition, polymorphisms in genes related to vitamin E metabolism such as lipoprotein lipase, apolipoprotein E, and alpha-tocopherol transfer protein may affect the function of vitamin E (Arslan E. 2021).

Studies show that vitamin E can be effective in preventing the severity of COVID-19 disease and induction of acute respiratory distress syndrome, thanks to its both T cells and strong antioxidant properties.

### 2.3. Vitamin C and its Relationship with COVID-19

Vitamin C (ascorbic acid) is an essential vitamin that plays an important role as an antioxidant and as a cofactor in various biosynthetic pathways in the immune system, cannot be synthesized by the human body, water-soluble and its excess is excreted through urine (Linster CL, 2007).

Vitamin C, also known as ascorbic acid, is well known for protective properties against free radicals and its anti-inflammatory (Carr AC, 2019; Mousavi S, 2019). It is stated that vitamin C has an important role in neutralizing the harmful effects of reactive oxygen species produced during infections (47). In addition to the antioxidant properties of vitamin C, its role in the function and regulation of the immune system is very important. Also, vitamin C can increase vasopressor and cortisol synthesis, affect leukocyte functioning, thereby strengthening the immune system

against various pathogens, including viruses. (Mousavi S, 2019; Teng J, 2018; Carr AC, 2015). It enhances chemotaxis and phagocytosis ability of neutrophils, activation of macrophages, production of interferon, maturation of T-lymphocytes and inhibits the replication of viruses (Hemila H,2017 ). Other effects of vitamin C on inflammatory regulation include modulation of NFkB (nuclear transcription factor kappa B) and reduction of proinflammatory cytokine production (Carr AC, 2017).

It has been reported that there is a decrease in vitamin C levels in plasma and immune cells during various infections, including the common cold and pneumonia, and its need is increased in infected individuals (Carr AC, 2017). The use of vitamin C to prevent and/or treat infections has long attracted the interest of researchers (Farjana M, 2020). Since it is a water-soluble vitamin, it is thought that it can not be stored, reducing the side effects. There is little information about the side effects of vitamin C in the literature, and epidemiological data show that it may be associated with diarrhea, abdominal bloating, and kidney oxalate stone formation in men (Ferraro PM, 2016; Thomas LD, 2013). In a phase 1 study evaluating the safety of intravenous ascorbic acid administration in severe sepsis patients, it was stated that doses of 50 and 200 mg/kg per day were safe, well tolerated, and could positively affect biomarkers of inflammation and endothelial damage (Fowler AA, 2014). Similarly, in a study evaluating 17 COVID-19 patients published recently, it was stated that no side effects were observed with intravenous vitamin C administration, though a significant decrease in inflammatory marker levels was observed (Hiedra R, 2020). In another study conducted in Shanghai, it was reported that the use of high-dose IV vitamin C in the treatment of moderate-to-severe COVID-19 patients was beneficial in terms of improving the inflammatory response, immunity and organ functions (Zhao B, 2021).

In a randomized controlled study conducted with 150 patients aged 52-53 years, who had severe COVID-19 disease, patients were divided into 2 groups according to a study in Pakistan. While one group received standard treatment (control group), the other group received 50 mg/kg/day intravenous (IV) vitamin C supplementation in addition to this standard treatment, and the other group received only standard treatment. Data such as results like number of days required for treatment, length of hospital stay, need for ventilation, and mortality as well as age, gender, vital values and biochemical values were compared and recorded between the two groups. COVID-19 patients who received IV vitamin C supplementation became symptom-free earlier and spent fewer days in hospital compared to the control group. However, there was no statistically significant difference between the two groups in terms of mechanical ventilation need (p value: 0.406) and mortality (p value: 0.31). According to this study,

while vitamin C can significantly improve clinical symptoms in patients affected by COVID-19, no effect has been reported on mortality and need for mechanical ventilation (Kumari P., 2020).

A meta-analysis of 12 studies with 766 patients on vitamin C found that it shortened the length of stay in ICU by an average of 8%. (Hemilä H, 2020) A meta-analysis of 8 studies found that vitamin C shortened the duration of mechanical ventilation in patients requiring ventilation (55). In addition, Zabet et al. (Zabet MH,2016) reported that vitamin C reduced mortality by 78% in 28 sepsis patients. Fowler et al. (Fowler AA, 2019) reported that vitamin C reduced mortality by 35% in 167 patients with ARDS and sepsis.

Administration of vitamin C in patients with sepsis, pneumoni and ARDS (acute respiratory distress syndrome) has shown potential benefits, such as reducing hospital and intensive care unit length of stay and mortality (Holford P, 2019). Sepsis, ARDS and pneumonia are common complications of patients with severe COVID-19, and in March 2020, the WHO highlighted vitamin C as a potential adjunctive therapy for patients with critical COVID-19 (WHO, 2020c). However, larger-scale studies are needed to further evaluate the role of vitamin C in the treatment of COVID-19.

### 2.4. Vitamin B12 and its Relationship with COVID-19

B vitamins are water-soluble molecules that play an important role in cellular metabolism (Poudel-Tandukar K, 2016). B vitamins are known as coenzymes that play a role in cell energy metabolism and organic molecule synthesis (Mikkelsen, K.,2019). In HIV (Human Immunodeficiency Virus) infected patients, niacin, pyridoxine and cobalamin intakes, respectively, have been reported to be significantly associated with reduced CRP. A study conducted in 2016 found a correlation between vitamin B deficiencies and inflammation in HIV-positive patients (Layden AJ, 2018). Considering other vitamins, it is thought that group B vitamins may also be associated with COVID-19 disease.

Vitamin B12 (cobalamin) , is a water-soluble vitamin obtained from animal products such as red eggs, milk and meat, When vitamin B12 is absorbed, it is used as a cofactor for enzymes involved in the synthesis of DNA, fatty acids, and myelin. In the case of vitamin B12 deficiency, cell-mediated immunity and humoral immunity are compromised and various effects are seen on immune cells (Fritz J, 2018). B12 is stored in excess in the liver; however, in cases where B12 cannot be absorbed for a long time (eg, dietary insufficiency, malabsorption, intrinsic factor deficiency), liver stores are depleted and deficiency occurs. B12 deficiency can lead to hematological and neurological symptoms Miller JW, 2018; Arslan E, 2021; Kandeel M, 2020).

Studies have shown that vitamin B12 plays an immunomodulatory role (Tamura J, 1999). It has been reported that when B12 is given to patients with vitamin B12 deficiency, the ratio of CD4+ to CD8+ and NK cell activity increase (Gutiérrez R, 2017). A different study investigating the relationship between vitamin B12 and monocytes reported increased TNF-α (tumor necrosis factor-α) synthesis in macrophage cells of vitamin B12-deficient mice (Mikkelsen K, 2019).

Vitamin B12 supplementation has been shown to be effective in reducing viral and bacterial infections in animal models (Iddir M, 2020; Agyemang-Yeboah F, 2013).

In line with the studies, some researchers stated that Ribavirin (broad spectrum antiviral), Telbivudine (anti-hepatitis B virus), vitamin B12 and nicotinamide can be used for the treatment of Covid-19 (Poudel-Tandukar K, 2016).

Rodriguez and colleagues demonstrated that 2% of patients admitted to the Intensive Care Unit (ICU) were Vitamin B12 deficient (Agyemang-Yeboah F, 2013).

It can be said that a strong immune system can help prevent or treat Covid-19 infection and the use of vitamins in general and vitamin B12 in particular can have an effect. However, strong data on the efficacy of vitamin B12 supplementation in the prevention and treatment of patients with Covid-19 are not yet sufficient.

**3. Conclusion and Discussion**

COVID-19 is a disease with a high mortality rate that has affected the whole world in a very short time. Although there is no specific drug developed against the virus yet, drug studies are still ongoing. The best way to protect yourself from infectious agents such as viruses is to pay attention to the contamination with the virus and avoid contact. For this reason, attention should be paid to the mask, distance, cleaning rule. However, due to the necessities of social life, how much we try to stay away from it, it somehow causes us to come into contact with the virus. Because of this, vaccine studies are very important. Nevertheless, strengthening the immune system is extremely important both to increase the effectiveness of the vaccine and to be strong against mutated viruses.

Numerous studies have been done on strengthening the immune system all over the world, and they keep continuing too. In this article, the relationships of vitamins A, C, E and B12 in COVID-19 disease have been revealed together with the studies.

While drawing attention to the importance of vitamin D on COVID-19 disease in many previous studies we did, we wanted to evaluate the studies on other vitamins in this study. Even though vitamins act in different ways, they are very important in protection against both the Sars-Cov-2 virus that causes COVID-19 disease and other viruses.

Although vitamins have a great role in strengthening the immune system, it is not recommended to use them unconsciously. Vitamins should be taken as supplements under the control of a doctor in the light of biochemical data. However, it is also extremely important to diversify the foods that help us meet our daily energy needs by increasing the variety of food, taking into account the protective roles of vitamins.

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