

Arrangement of Facial Wrinkles and Influence of Certain Harmful Factors on their Appearance

Distribución de las Arrugas Faciales e Influencia de Ciertos Factores Nocivos en su Aparición

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PUPOVAC, N.; ERIC, M.; VUCINIC, N.; RADOSEVIC, D.; FEJSA LEVAKOV, A. S.; STOJANOVIC, N. & DURDEVIC, D. Arrangement of facial wrinkles and influence of certain harmful factors on their appearance. *Int. J. Morphol.*, 43(1):156-165, 2025.

SUMMARY: Changes in the skin are the most visible indicators of aging. Wrinkles are lines and folds that form due to physiological aging. This study aimed to determine the distribution and length of facial wrinkles and folds in females and males and to determine if there are statistically significant differences in relation to the age group and the influence of certain harmful factors on their formation. The study was conducted on 180 subjects (90 females and 90 males), divided into three age groups. Through questionnaire for each subject, we collected data on harmful habits (sun exposure, alcohol and cigarette consumption). Then, we took photographs of the subjects' faces and analyzed the distribution and length of wrinkles and folds. We drew visible wrinkles on a special facial scheme. Morphometric analysis was performed in the program ImageJ 1.48v. The number of wrinkles and length of nasolabial folds increases as the age group increases. In the 2nd age group, females had higher average values of the length of nasolabial folds compared to males of the same age group. Sun exposure, alcohol and cigarette consumption did not effect on the number of wrinkles in both genders, only smoking status affected on the length of nasolabial folds in male subjects. Men who were ex-smokers had higher values of the right nasolabial folds compared to non-smokers. Our results may improve knowledge about the development and patterns of wrinkles and inspire research on similar topics in other populations.

KEY WORDS: Wrinkles; Facial skin aging; UV rays; Smoking; Alcohol.

INTRODUCTION

Changes in the skin are the most visible indicators of aging. Clinical manifestations of these changes are discoloration, wrinkles and loss of skin texture. Unlike other organs, the skin is in direct contact with the external environment and it is aging, which is a consequence of the damage caused by the external environment. Hereditary and genetic influences, as it is now considered, contribute to this process with only 3 % (Robert *et al.*, 2009; Krutmann *et al.*, 2017). All five layers of the face - the skin, the subcutaneous layer, the musculoaponeurotic layer, the loose areolar layer, and the periosteum/ deep fascia layer - all play associated roles in the aging process (Liew *et al.*, 2016). Wrinkles are powerful psychosocial indicators of age. Two main types of wrinkles form in human skin: dynamic and static. Dynamic wrinkles appear at all ages during facial expression, i.e. the underlying skeletal muscles contract, causing large compressive strains and

mechanical buckling of the skin (Mazza *et al.*, 2007). Both the shape and depth of these “expressional lines” correlate with the level of muscle contraction (Hara *et al.*, 2017), and they go away when the underlying facial musculature relaxes. Static or permanent skin wrinkles persist regardless of muscle relaxation and usually emerge in the early 30s and grow in severity with aging. Static wrinkles are a natural part of aging. The skin produces less collagen as we age and becomes looser and less elastic over time, making it harder to keep wrinkles at bay (Kuwazuru *et al.*, 2008; Tsukahara *et al.*, 2012; Hara *et al.*, 2017).

Many factors contribute to, and in many cases accelerate the natural aging process. Long-term exposure to ultraviolet (UV) rays destroys the connective tissue of the skin, collagen and elastic fibers and leads to the accumulation of elastotic material in the dermis (solar

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elastosis) (Leung & Harvey, 2002). Solar elastosis is also a manifestation of premature skin aging caused by smoking. Tobacco smoke affects collagen production and increases the production of tropoelastin and matrix metalloproteinases (MMP). These degrade matrix proteins and produce abnormal elastic material in the dermis. Elastic fiber changes in smokers extend deep into the reticular dermis, whereas in sun damage, these changes are usually limited to the more superficial papillary dermis. Alcoholic drinks also affect the accelerated aging of the skin. Typical symptoms that reveal an alcoholic face are pronounced lines or redness between the eyebrows, drooping eyelids, enlarged pores, dehydrated skin with wrinkles that branch like feathers on the cheeks, redness on the cheeks and nose, and deep nasolabial folds (Goodman *et al.*, 2019).

Due to the increasing number of injectable fillers for wrinkles on the face, the need for objective measurement of the wrinkles, examination of the time of occurrence and the distribution scheme of wrinkles in different age groups in both sexes, as well as the influence of the factors that we consider to be the most important for their formation - exposure to ultraviolet (UV) radiation, smoking and alcoholism.

This study aimed to determine the arrangement and length of certain wrinkles and folds on the face in our population and to determine whether there are statistically significant differences in relation to the sex and age group of the subjects. Also, to determine whether exposure to UV rays, cigarette and alcohol consumption affect the appearance of wrinkles and folds on the face.

MATERIAL AND METHOD

This study included 180 subjects (90 males and 90 females) with normal body mass index (BMI). All subjects were previously informed orally and in written form about the study and signed the "Informed Consent of Respondents" form. The subjects were divided into three age groups. The first group consisted of subjects aged 18 to 35 years (average age female 20, male 23 years), the second group of subjects aged 36 to 50 years (average age female 44, male 43 years) and the third group of subjects aged 51 and older (average age female 59, male 63 years). Each age group consisted of at least 30 subjects. The criteria for exclusion from the study were treatments with botox, fillers and other aesthetic corrections, as well as BMI above reference values. Through a specially designed questionnaire (Supplementary material 1), the following data were collected for each subject: sun exposure during the day, alcohol and cigarette consumption.

The face of the subjects was photographed with a Canon EOS 600D digital camera, at a distance of 3 steps/feet $3 \times 40 \text{ cm} = 120 \text{ cm}$ in good lighting. The subjects were photographed in a state of rest, without facial expressions and makeup. Each subject was photographed holding a vertically placed ruler in the right hand, next to the face - so that the ruler was at the height of the face and level with the face. The images were analyzed for the arrangement and length of some of the commonly present facial wrinkles and folds: frontal (F), periorbital (right and left - RPO/LPO), glabellar (vertical and horizontal - VG/HG) and nasolabial (right and left - RNL/LNL) (Fig. 1).

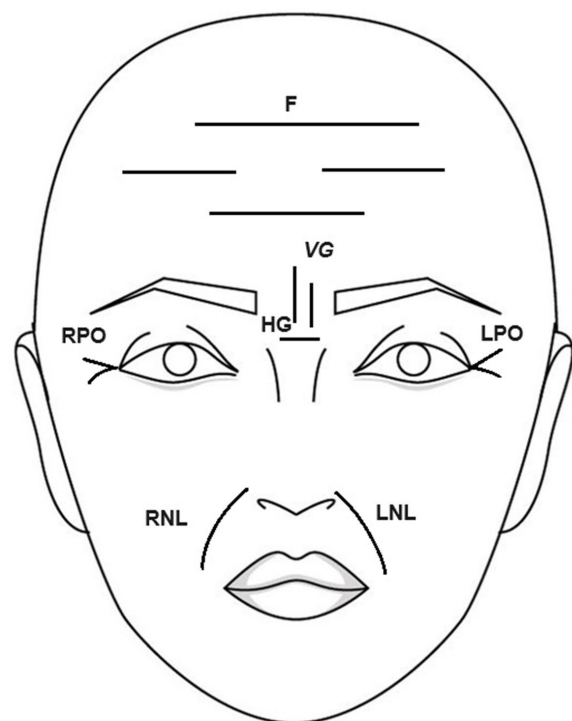


Fig. 1. Schematic view of the facial wrinkles and folds.

Visible wrinkles and folds for each subject are drawn on a special face schema (. Frontal, periorbital and glabellar wrinkles were measured numerically, while nasolabial folds were measured in millimeters. Morphometric analysis of wrinkles was performed in the software program "ImageJ 1.48v".

Sun exposure was classified as 'yes' (> 2h/day) or 'no' (< 2h/day). Alcohol consumption was classified as 'yes' (any amount, at least three times a week) or 'no' (no consumption or just occasional social use). According to smoking status subjects was classified into five groups: non-smoker, ex-smoker, smoker 1 pack/day, smoker 2 packs/day, smoker > 3 packs/day.

The statistical analysis of the obtained data was performed using the Statistical Package for Social Sciences-SPSS 21 program. The results are presented as parameters of descriptive statistics: mean value (\bar{x}), standard deviation (SD), minimum and maximum value (Min-Max). The comparison of numerical values between two groups will be performed using the Student's t test, i.e. the non-parametric Mann-Whitney test, while the one-way analysis of variance (ANOVA), i.e. the non-parametric Kruskal-Wallis test, will be used to compare the values between the three groups of data. In order to examine the influence of risk factors (sun exposure, alcohol use and smoking status) on the number and length of wrinkles, correlations and cross-tabulations were applied for categorical data (the conditions for the χ^2 test were not met), i.e., the t-test for independent samples and one-factor analysis of variance for quantitative data. A statistically significant difference was considered if $p < 0.05$. The results will be presented tabularly and graphically.

The research was approved by the Ethics Committee of the Faculty of Medicine in Novi Sad (date 26.12.2022; decision number: 01-39/302/1 and 01-39/303/1).

RESULTS

Figures 2, 3 and 4 show the percentage of presence of frontal, right and left periorbital, vertical and horizontal glabellar wrinkles and right and left nasolabial folds in subjects of both sexes in each of the three age groups.

Tables I and II show the number of female (male) subjects and the number of female (male) subjects who had frontal, right and left periorbital, vertical and horizontal glabellar wrinkles and their number (percentage) in each of the mentioned regions of the face in three age groups.

By comparing the average values of the length of the right and left nasolabial folds between the sexes, a statistically significant difference was found in the age group from 36 to 50 years, females had higher values compared to males, while in the other two age groups there was no statistically significant difference (Table III).

The results of the ANOVA test show that there are statistically significant differences between females of different ages regarding the average values of the right nasolabial folds ($F(2,87)=30.682, p < 0.001$) and the average values of the left nasolabial folds ($F(2,87)=36.033, p < 0.001$). Post hoc tests for multiple comparisons (Tuckey HSD) found that females aged 18 to 35 years were statistically significantly different in the mean value of the right nasolabial folds ($M=8.10$) compared to the other two groups of females - aged 36 to 50 years ($M=28.80$) and older than

Table I. Number of female subjects and the number of female subjects who had frontal, right and left periorbital, vertical and horizontal glabellar wrinkles and their number (percentage) in each of the mentioned regions of the face in three age groups.

Age group	Number of female subjects	Number of wrinkles (%)							
		1	2	3	4	5	6	7	
F	18-35 years	30	8 (26.67 %)	1 (3.33 %)	1 (3.33 %)	0	0	0	0
	36-50 years	30	23 (76.67 %)	7 (23.33 %)	5 (16.67 %)	6 (20.00 %)	0	1 (3.33 %)	0
	> 51 years	30	29 (96.67 %)	8 (26.67 %)	6 (20.00 %)	2 (6.67 %)	4 (13.33 %)	2 (6.67 %)	2 (6.67 %)
RPO	18-35 years	30	2 (6.67 %)	0	0	0	0	0	0
	36-50 years	30	18 (60.00 %)	10 (33.33 %)	2 (6.67 %)	0	0	0	0
	> 51 years	30	28 (93.33 %)	10 (33.33 %)	6 (20.00 %)	3 (10.00 %)	0	0	0
LPO	18-35 years	30	2 (6.67 %)	0	0	0	0	0	0
	36-50 years	30	19 (63.33 %)	9 (30.00 %)	2 (6.67 %)	0	0	0	0
	> 51 years	30	30 (100.00 %)	11 (36.67 %)	7 (23.33 %)	3 (10.00 %)	0	0	0
VG	18-35 years	30	1 (3.33 %)	0	0	0	0	0	0
	36-50 years	30	12 (40.00 %)	5 (16.67 %)	1 (3.33 %)	0	0	0	0
	> 51 years	30	25 (83.33 %)	17 (56.67 %)	2 (6.67 %)	1 (3.33 %)	0	0	0
HG	18-35 years	30	1 (3.33 %)	0	0	0	0	0	0
	36-50 years	30	9 (30.00 %)	8 (26.67 %)	0	0	0	0	0
	> 51 years	30	15 (50.00 %)	8 (26.67 %)	0	2 (6.67 %)	0	0	0

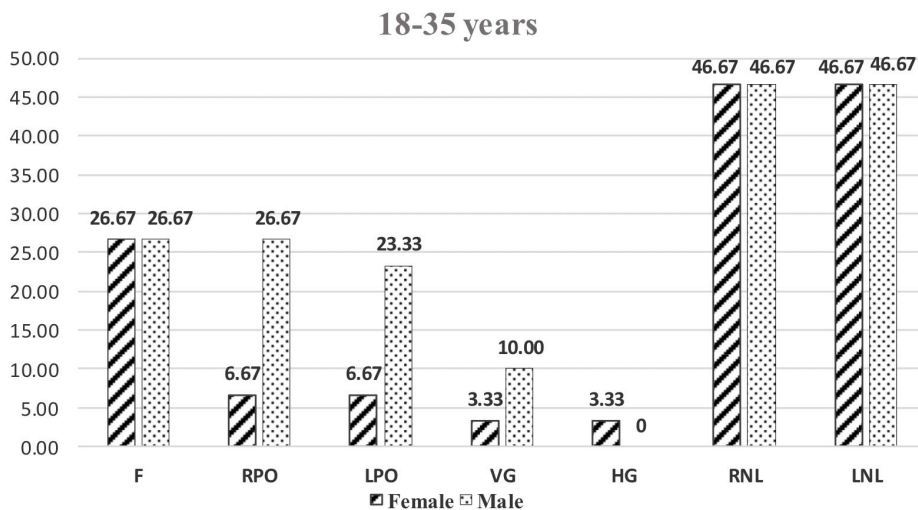


Fig. 2. Percentage presence of wrinkles and folds in females and males in the age group of 18-35 years.

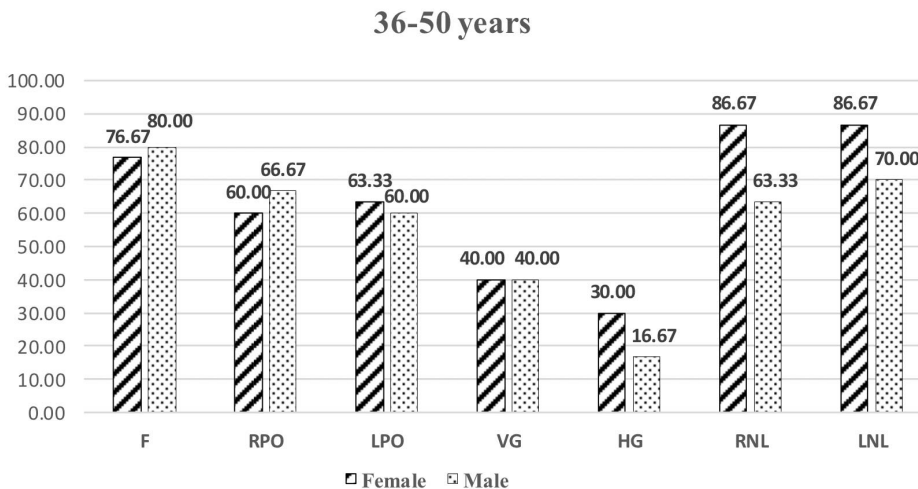


Fig. 3. Percentage presence of wrinkles and folds in females and males in the age group of 36-50 years.

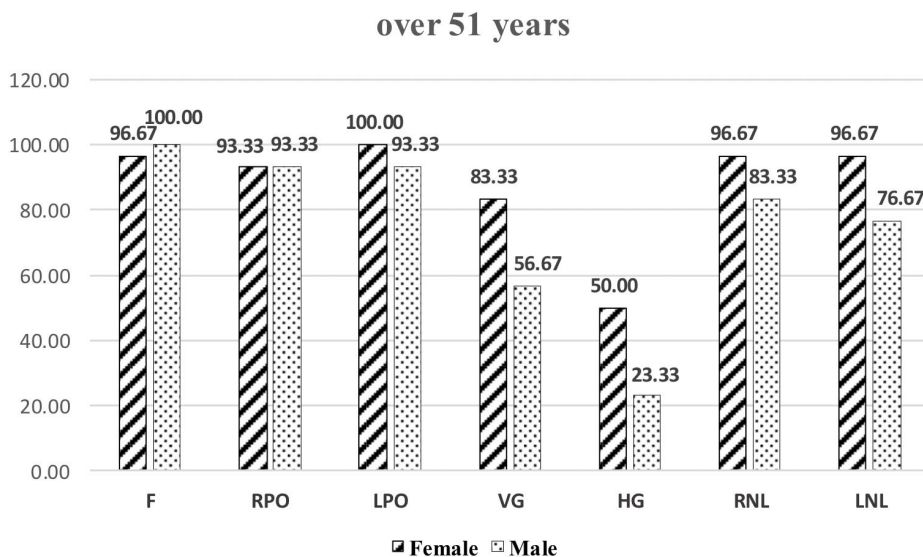


Fig. 4. Percentage presence of wrinkles and folds in females and males in the age group over 51 years.

Table II. Number of male subjects and the number of male subjects who had frontal, right and left periorbital, vertical and horizontal glabellar wrinkles and their number (percentage) in each of the mentioned regions of the face in three age groups.

Age group	Number of male subjects	Number of male subjects with wrinkles	Number of wrinkles (%)						
			1	2	3	4	5	6	
F	18-35 years	30	8 (26.67 %)	4 (13.33 %)	3 (10.00 %)	1 (3.33 %)	0	0	0
	36-50 years	30	24 (80.00 %)	5 (16.67 %)	9 (30.00 %)	6 (20.00 %)	2 (6.67 %)	1 (3.33 %)	1 (3.33 %)
	> 51 years	30	30 (100.00 %)	1 (3.33 %)	8 (26.67 %)	6 (20.00 %)	6 (20.00 %)	11 (36.67 %)	4 (13.33 %)
RPO	18-35 years	30	8 (26.67 %)	4 (13.33 %)	4 (13.33 %)	0	0	0	0
	36-50 years	30	20 (66.67 %)	13 (43.33 %)	4 (13.33 %)	3 (10.00 %)	0	0	0
	> 51 years	30	28 (93.33 %)	8 (26.67 %)	11 (36.67 %)	8 (26.67 %)	0	0	0
LPO	18-35 years	30	7 (23.33 %)	5 (16.67 %)	1 (3.33 %)	1 (3.33 %)	0	0	0
	36-50 years	30	18 (60.00 %)	8 (26.67 %)	8 (26.67 %)	2 (6.67 %)	0	0	0
	> 51 years	30	28 (93.33 %)	4 (13.33 %)	13 (43.33 %)	10 (30.00 %)	1 (3.33 %)	0	0
VG	18-35 years	30	3 (10.00 %)	3 (10.00 %)	0	0	0	0	0
	36-50 years	30	12 (40.00 %)	9 (30.00 %)	3 (10.00 %)	0	0	0	0
	> 51 years	30	17 (56.67 %)	7 (23.33 %)	7 (23.33 %)	3 (10.00 %)	0	0	0
HG	18-35 years	30	0	0	0	0	0	0	0
	36-50 years	30	5 (16.67 %)	3 (10.00 %)	2 (6.67 %)	0	0	0	0
	> 51 years	30	7 (23.33 %)	7 (23.33 %)	0	0	0	0	0

Table III. Comparison of average values of right and left nasolabial folds between female and male subjects in all three age groups (mm).

	18-35 years		36-50 years		> 51 years		t	p
	Female x±SD	Male x±SD	Female x±SD	Male x±SD	Female x±SD	Male x±SD		
RNL	17.28±5.27	15.80±6.99	33.21±12.20	22.46±10.10	35.33±13.03	30.66±9.14	1.49	0.14
LNL	21.16±8.66	15.36±8.77	34.64±9.72	21.75±9.42	39.55±10.48	35.03±6.38	1.81	0.07

51 years (M=34.13). Also, females aged 18 to 35 years were statistically significantly different in the mean value of the left nasolabial folds (M=9.90) compared to the other two groups of females - aged 36 to 50 years (M=30.00) and older than 51 years (M=38.33). Statistically significant differences were found between females aged 36 and 50 and those older than 51.

The results of the ANOVA test show that there are statistically significant differences between males of different ages regarding the average values of the right nasolabial folds (F(2,55)=12.580, p<0.0003) and the average values of the left nasolabial folds (F(2,55)=28.63, p<0.0001). Post hoc tests for multiple comparisons (Tuckey HSD) found that males aged 18 to 35 years were statistically significantly different in mean value of the right nasolabial folds (M=6.99) compared to males aged over 51 years (M=9.32) and that males aged 36 to 50 years (M=10.10) were statistically significantly different in mean value of the right nasolabial folds compared to males aged older than 51 years (M=9.32). Also, males aged 18 to 35 years were statistically significantly different in the mean value of left nasolabial folds (M=8.77) compared to males aged over 51 years (M=6.39), and males aged 36 to 50 years (M=9.42) were statistically significantly different in the mean value of the left nasolabial folds compared to males aged older than 51 years (M=6.39).

There were no statistically significant associations between sun exposure, alcohol and cigarette consumption and the number of wrinkles in females and males (p>0.05) (Table IV).

No statistically significant differences were found between the female and male subjects who were exposed to the sun and those who were not and who consumed alcohol and those who were not in terms of the length of the right and left nasolabial folds ($p>0.05$) (Table V).

There were no statistically significant differences between the female subjects according to smoking status regarding the length of the right and left nasolabial folds ($p>0.05$) (Table VI).

The results of the ANOVA test show that there are statistically significant differences between five groups of male subjects (according to smoking status) regarding the average values of the right nasolabial folds ($F(3,86)=4.872, p<0.004$). Post hoc tests for multiple comparisons (Tuckey HSD) found that ex-smokers were statistically significantly different in the mean value of the right nasolabial folds ($M=27.73$) compared to non-smokers ($M=11.86$). There were no statistically significant differences between the five groups of male subjects (according to smoking status) regarding the average values of the left nasolabial folds ($p>0.05$) (Table VII).

Based on the common mean values of the number of wrinkles and folds, we created a "photo robot" for each of the three groups of female and male subjects in order to show in an illustrative way the changes in the appearance, development and characteristics of wrinkles and folds in different periods of life (Fig. 5).

Table IV. Association between sun exposure, alcohol and cigarette consumption and the number of wrinkles.

	F		RPO		LPO		VG		HG	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
Sun exposure										
Spearman Correlation	0.099	0.053	0.087	0.124	0.028	0.164	0.083	0.144	0.071	0.071
Sig. (2-tailed)	0.353	0.621	0.417	0.242	0.794	0.123	0.437	0.177	0.507	0.507
N	90	90	90	90	90	90	90	90	90	90
Alcohol										
Spearman Correlation	-0.109	0.198	-0.125	-0.051	-0.105	-0.012	-0.030	0.088	-0.068	-0.068
Sig. (2-tailed)	0.306	0.061	0.242	0.636	0.326	0.913	0.778	0.408	0.524	0.524
N	90	90	90	90	90	90	90	90	90	90
Cigarette										
Spearman Correlation	-0.133	-0.055	-0.138	-0.085	-0.185	-0.056	-0.182	-0.190	-0.136	-0.136
Sig. (2-tailed)	0.213	0.607	0.194	0.426	0.081	0.601	0.087	0.073	0.202	0.202
N	90	90	90	90	90	90	90	90	90	90

Table V. Comparison average values of the length of the right and left nasolabial folds of female and male subjects who were exposed to the sun and those who were not and who consumed alcohol and those who did not (mm).

	Sun exposure	N	x±SD	t	p	Alcohol	N	x±SD	t	p
Female	yes	22	25.77±15.71	0.64	0.52	yes	11	19.45±18.61	-0.86	0.39
	no	68	23.00±18.17			no	79	24.33±17.55		
LNL	yes	22	27.23±17.15	0.35	0.73	yes	11	21.27±19.91	-0.96	0.33
	no	68	25.71±18.15			no	79	26.82±17.66		
Male	yes	41	16.57±14.95	-0.50	0.61	yes	43	17.54±15.57	1.11	0.26
	no	49	15.00±14.36			no	47	14.04±13.55		
LNL	yes	41	18.63±15.74	-1.26	0.21	yes	43	16.75±15.80	1.19	0.84
	no	49	14.56±14.85			no	47	16.11±15.02		

Table VI. Comparison of five groups of female subjects (according to smoking status) regarding the average value of the length of the right and left nasolabial folds.

		Sum of Squares	df	Mean Square	F	Sig
RNL	Between Groups	2089.968	3	696.656	2.359	0.077
	Within Groups	25400.518	86	295.355		
	Total	27490.486	89			
LNL	Between Groups	2183.203	3	727.734	2.404	0.073
	Within Groups	26030.255	86	302.677		
	Total	28213.457	89			

Table VII. Comparison of five groups of male subjects (according to smoking status) regarding the average value of the length of the right and left nasolabial folds.

		Sum of Squares	df	Mean Square	F	Sig
RNL	Between Groups	2746.568	3	915.523	4.872	0.004
	Within Groups	16159.247	86	187.898		
	Total	18905.815	89			
LNL	Between Groups	1692.573	3	564.191	2.528	0.063
	Within Groups	19195.040	86	223.198		
	Total	20887.613	89			

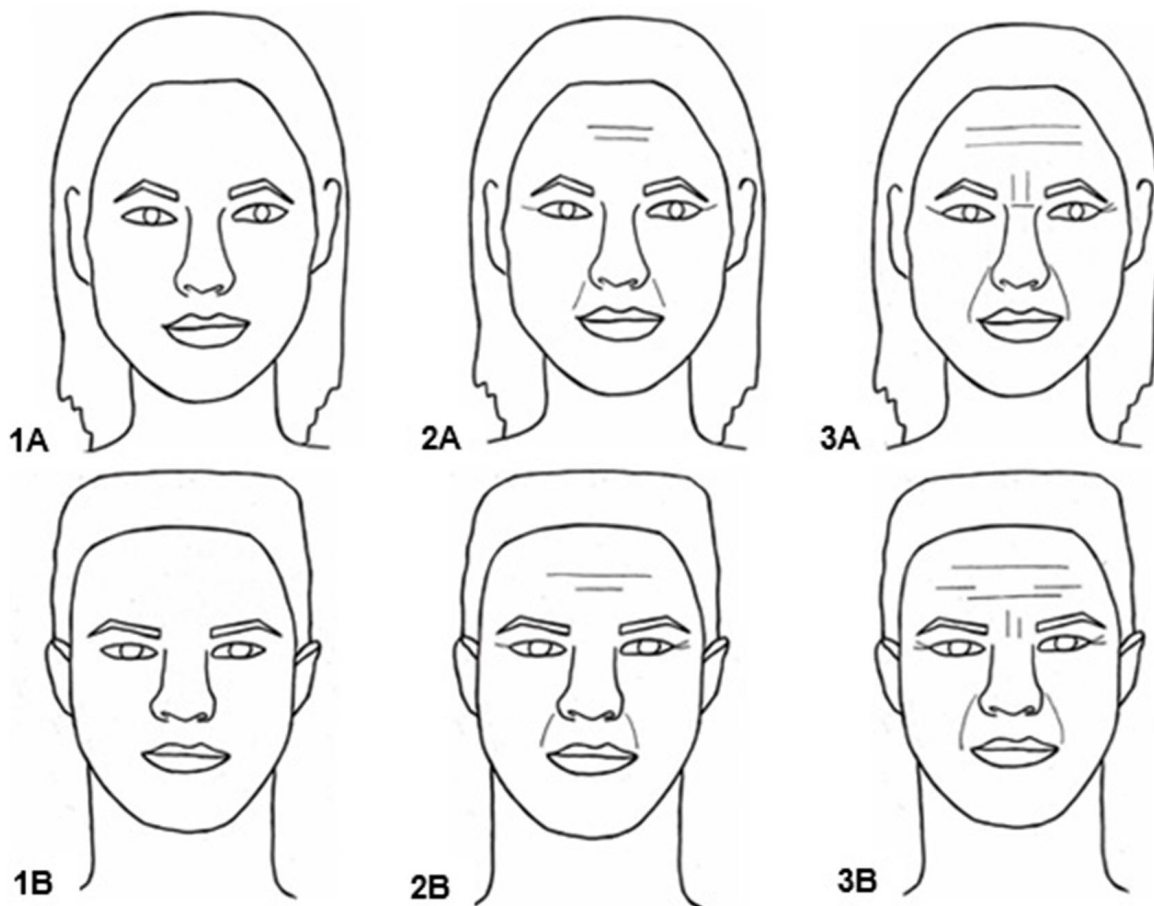


Fig. 5. Facial scheme of the average number of wrinkles and folds in three age groups: 1. Group 18-35 years old - female (1A), male (1B); 2. Group 36-50 years old - female (2A), male (2B); 3. Group over 51 years old - female (3A), male (3B).

DISCUSSION

Wrinkles are a subject of great interest for many scientists because of their varied position on the face, which has a great influence on the aesthetics of the face. Wrinkles on the face appear with skin relaxation caused by retraction of papillae and degeneration of elastic and collagen fibers at the junction of the epidermis and dermis. This degeneration begins around the age of 30 and increases over time (Robert *et al.*, 2009).

In our study, we analyzed the number of frontal, periorbital and glabellar wrinkles, as well as the length of nasolabial folds, in order to determine possible differences in relation to the three age groups for the appearance of facial wrinkles in females and males, because the general impression is that the mentioned wrinkles and folds are the most frequently present and the most pronounced.

According to Braz & Sakuma (2010), the arrangement of frontal wrinkles is most often along the entire length of the forehead, sometimes medially and rarely laterally, which correlates with the results of our study. In our population, in the group of subjects aged 18 to 35, about 73.33 % of females and males do not have a single frontal wrinkle, while among subjects aged 36 to 50, the majority are without wrinkles or with two or four wrinkles in females and with two or three wrinkles in males. As expected, females older than 51 years usually have two frontal wrinkles, then three wrinkles, while a quarter of them have even more than five wrinkles. In males aged over 51 years there were mainly those with four wrinkles (36.67 %).

Periorbital wrinkles are present even in some teenagers when they laugh. Since the late thirties, most people have had these wrinkles even at rest. Factors that influence the appearance of these wrinkles are sun - damaged skin, skin thickness, and amount of subcutaneous adipose tissue, smoking status and amount of contraction orbicularis oculi muscle during smiling (Kane, 2003). In our study, about 6.67 % of females from the first group have periorbital wrinkles. In the second group, about 63.33 % have wrinkles, mostly one or two. In the third group, most females have one or two wrinkles; 20.00 % of them have three, and 10.00 % even have four periorbital wrinkles. In the first group of males, about 26.67 % of them have wrinkles, mostly having one or two. In the second group, about 66.67 % of them have wrinkles, mostly having one or two wrinkles. In the third group, most males have one or two, and only 3.33 % have more than three wrinkles. In the study by Akiba *et al.* (1999), who conducted research on 195 men in Japan, aged 20-29 and 50-59, periorbital and frontal wrinkles appear equally quickly at around 20 years of age.

Less than 5.00 % of females in the first group have vertical glabellar wrinkles, while in the second group, 40.00 % of them have wrinkles, mostly one or two wrinkles. In the oldest age group, over 83.33 % of females have wrinkles, 56.67 % have two and 10.00 % have even three or four wrinkles. As for horizontal wrinkles, less than 5.00 % of the youngest group has wrinkles. In the second age group, 70.00 % of females have no wrinkles, while a quarter have one. In the oldest group, 50.00 % have no wrinkles, a quarter has one wrinkle, and about 15 % have two horizontal wrinkles. In the first group of males, 10.00 % of them have wrinkles, mostly one. In the second group, 40.00 % of them have wrinkles, and a third of them have one wrinkle. In the third group, 56.67 % have wrinkles, mostly one or two. As for horizontal wrinkles, we had no male subjects in the youngest group with these wrinkles. In the second age group, 16.67 % of them have wrinkles, mostly having one or two, and in the oldest group, 23.33 % of them have wrinkles, mostly one. In a study Luebberding *et al.* (2014), whose research was conducted on 150 men in Germany, aged between 20-70 years, showed that glabellar wrinkles did not manifest until the 40 age.

The results of our study showed that there is a statistically significant difference in the length of the right and left nasolabial folds among female and male subjects of different age groups. The oldest group of female and male subjects had significantly higher average values of the length of the right and left nasolabial folds compared to younger and middle-aged subjects. A sudden increase in the length of wrinkles occurs precisely in the fifth decade of life, which also shows the difference in our results (Paes *et al.*, 2009).

By comparing the average values of the length of the right and left nasolabial folds between the sexes, a statistically significant difference was found in the age group from 36 to 50 years, females had higher values (right 33.21 ± 12.20 mm and left 34.64 ± 9.72) compared to males (right 22.46 ± 10.10 mm and left 21.75 ± 9.42 mm). Chung *et al.* (2001), reported that women have a greater risk of developing wrinkles than men after controlling for sun exposure and smoking, and considered post-menopausal decreases in female hormones as a contributory factor. Female hormonal decreases heighten the UV sensitivity of the skin and wrinkle progression is significantly increased with long-term UV exposure, suggesting that female hormones may suppress UV-induced increases in wrinkle development (Tsukahara *et al.*, 2004).

Wrinkles are not necessarily the result of aging, but the appropriate muscle activity of the face can also cause irreversible folds in the skin. There is an opinion that the movement pattern of the mimic muscles of each person is unique, like a fingerprint (Kane, 2003). Sun exposure has

been shown to be associated with premature skin wrinkling or facial ageing (Leyden, 1990). However, in our study, for both sexes, no statistically significant correlations were found between sun exposure and the number of wrinkles, as well as between sun exposure and the length of nasolabial folds. This could be explained by the fact that both of our subjects had worked indoor types of jobs (economists, doctors, lawyers) and a small number of them had been exposed to the sun during working hours. From this, we could assume that UV rays may not affect the number and length of wrinkles but only the earlier appearance of those wrinkles, probably with the help of other risk factors.

A large number of epidemiological studies have shown that smoking is a strong independent predictor of facial wrinkle formation and early skin ageing. Moreover, recent in vivo studies confirmed that smoking causes premature skin ageing and also revealed some of the molecular changes occurring in the skin in response to smoking (Kadunce *et al.*, 1991; Leung & Harvey, 2002; Mnogoma *et al.*, 2024). Leung & Harvey (2002) found in a British population that smoking 20 cigarettes per day had an effect equivalent to almost ten years of chronological ageing. Koh *et al.* (2002) found that current cigarette smokers had more facial wrinkles than non-smokers and ex-smokers in a Korean population. Raitio *et al.* (2004) reported that in a study of a Finnish population, smokers had greater skin ageing than non-smokers, but a significant difference in facial ageing between smokers and non-smokers was not found. In our study, no statistically significant differences were found between the female subjects according to smoking status and the number of wrinkles and length of the folds. Also, there is no significant effect of smoking status on the number of wrinkles in males, but it found that men who were ex-smokers had higher values of the right nasolabial folds, 27.73 ± 13.59 mm compared to non-smokers, 11.85 ± 12.66 mm. However, in this study, fewer women were smokers and women smoked less than men, which may also explain why the effect of smoking is not statistically significant for women.

This discrepancy in our results and results in other study may be due to different methods of assessing facial aging, fewer variables and different statistical methods, the small number of subjects in our study, population differences and different geographic locations. Beside this, limitations of the study are relatively small sample size and self-reported data of included subjects.

For both sexes, there is no significant effect of alcohol consumption on the number of wrinkles and length of folds. Schou *et al.* (2017), show that heavy alcohol use and smoking are associated with generally looking older than one's actual age.

In recent decades, with the development of aesthetic medicine, there has been a need for detailed knowledge of the most minor structures of the face. Therefore, further examination of wrinkles and their depth is necessary because the skin, as our largest organ, reflects health. Our results may improve knowledge about the development and patterns of wrinkles, and inspire research on similar topics in other populations.

PUPOVAC, N.; ERIC, M.; VUCINIC, N.; RADOSEVIC, D.; FEJSA LEVAKOV, A.S.; STOJANOVIC, N. & DURDEVIC, D. Distribución de las arrugas faciales e influencia de ciertos factores nocivos en su aparición. *Int. J. Morphol.*, 43(1):156-165, 2025.

RESUMEN: Los cambios en la piel son los indicadores más visibles del envejecimiento. Las arrugas son líneas y pliegues que se forman debido al envejecimiento fisiológico. Este estudio tuvo como objetivo determinar la distribución y la longitud de las arrugas y pliegues faciales en mujeres y hombres y determinar si existen diferencias estadísticamente significativas en relación con el grupo de edad y la influencia de ciertos factores nocivos en su formación. El estudio se realizó en 180 sujetos (90 mujeres y 90 hombres), divididos en tres grupos de edad. A través de un cuestionario para cada sujeto, se recogieron datos sobre hábitos nocivos (exposición al sol, consumo de alcohol y tabaco). A continuación, se tomaron fotografías de las caras de los sujetos y se analizó la distribución y longitud de las arrugas y los pliegues. Se dibujaron las arrugas visibles en un esquema facial especial. El análisis morfométrico se realizó en el programa ImageJ 1.48v. El número de arrugas y la longitud de los pliegues nasolabiales aumentan a medida que aumenta el grupo de edad. En el segundo grupo de edad, las mujeres tenían valores medios más altos de la longitud de los pliegues nasolabiales en comparación con los hombres del mismo grupo de edad. La exposición al sol, el consumo de alcohol y tabaco no afectaron al número de arrugas en ambos sexos, solo el estado de tabaquismo afectó a la longitud de los pliegues nasolabiales en los hombres. Los hombres que eran fumadores tenían valores más altos de los pliegues nasolabiales derechos en comparación con los no fumadores. Nuestros resultados pueden mejorar el conocimiento sobre el desarrollo y los patrones de las arrugas e inspirar la investigación sobre temas similares en otras poblaciones.

PALABRAS CLAVE: Arrugas; Envejecimiento de la piel del rostro; Rayos UV; Tabaquismo; Alcohol.

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