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Effect of keraGEN IV Keratin oral supplementation on hair, skin, and nail attributes

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Abstract

Background: Skin attributes reflecting problems in the underlying structure can include a lack of elasticity and hydration, while problems with hair health may be indicated by hair loss. Hair anchoring is important in mitigating hair loss typical of that experienced during combing or styling, when hair is damaged through chemical treatments, coloring, or during peri- or post- menopause. Nail health, including nail keratin content, can be negatively affected by a variety of agents including nail cosmetics and chemicals. The unique properties of the structural protein keratin provides strength, resilience, and protection to skin, hair, and nails. In this article, we discuss the role of keratin in various dermatological conditions and evaluate the effect of ingestion of a novel keratin-based formulation on hair skin and nail health.

Materials and Methods: A randomized, double-blind, placebo-controlled non-invasive study was conducted in 65 female subjects aged 45-60 having healthy skin, but damaged or stressed hair. Instrumental measures of skin firmness and elasticity, hydration, and skin barrier function efficiency were taken along with a hair pull test, keratin quality assessments and participants' self-assessment of nail condition over the 60 days of the study.

Results: The investigational product's action of inducing collagen IV expression appears to translate to measurable improvement in hair anchoring, specifically a 43.1% reduction ($p \leq 0.01$) in hair loss in women aged 45-60 with stressed or damaged hair. Improvement in the hair keratin structure is further supported by a 17.61% increase ($p \leq 0.01$) in birefringence arising from increase in the hair cortex structural integrity following evaluation using polarimetric imaging analysis. The measured 12.5% reduction ($p \leq 0.05$) in trans epidermal water loss (TEWL) may arise from the improvement of skin structure and associated barrier function due to collagen IV expression induced by the investigational product - keraGEN IV®. Improvement in skin health and skin structure due to the investigational product is reinforced by the measured improvement in skin elasticity, increasing by 10.1% ($p \leq 0.001$). Evidence does not support a difference in either nail strength or overall condition based on whether the investigational product or the placebo was taken.

Conclusion: The use of oral keratin supplementation containing the investigational product keraGEN IV® resulted in improved skin structure, hair structure and reduction in hair loss from pull testing associated with the health of the underlying structures.

Keywords: keratin, nutraceuticals, supplements, health claims

1. Introduction

Skin, hair, and nails are external markers of health and well-being. These structures are partially composed of keratin, a family of fibrous proteins essential to the structural framework of the epidermis and its appendages. Keratin is a tough, insoluble protein that provides strength and resilience to these structures, protecting them from environmental stressors such as heat, cold, oxidative stress and mechanical trauma. In this article, we examine the importance of keratin in maintaining the health and integrity of skin, hair, and nails and investigate the effect of a novel keratin-based formulation on these structures.

Keratin production is influenced by various factors such as genetics and hormonal and nutri-

tional status, including sulfur amino acid metabolism. It's crucial to understand how to support the production and function of keratin since it plays both physical and biological roles in the body, affecting wound healing rates, stimulating protein synthesis, and building healthy skin structures[1, 2]. Therapies based on keratin have shown promise in treating dermatological conditions like epidermolysis bullosa[3], which causes the skin to become very fragile and leads to blistering lesions. Keratin also forms a protective barrier that prevents water loss and protects against environmental pollutants and pathogens. When added to an ointment base, keratin hydrolysates have been found to have humectant (increasing hydration in the stratum corneum) properties as well as occlusive (decreasing trans-epidermal water loss[4]) properties. With this promising baseline for both healing and protection, further research on the effect of keratin on skin characteristics is warranted.

Oxidized keratin promotes keratinocyte migration, which induces protein expression of collagen types IV and VII[5]. Unlike collagens I, II and III that are highly abundant in soft tissue and contribute to overall tissue strength and integrity, collagen IV is a junctional protein that is crucial in joining the epidermal and dermal layers of the skin and anchoring hair in the hair follicle. Low levels of collagen IV have been found to correlate with hair loss[6]. In some cases of alopecia areata, the breakdown of immune tolerance leads to destruction of the hair follicle and loss of keratinocytes resulting in hair loss[7]. Studies have shown that oral[8, 9, 10] and topical[11] keratin supplements can produce a positive effect on keratin biosynthesis and content, improving hair growth and quality.

Nail strength, hardness, flexibility, brittleness, and toughness are all characteristics of overall nail health. Nail cosmetics, chemicals, physical injury, generalized disease, and genetic disorders can negatively affect nail health and the keratin content of nails[12, 13]. Keratin-based formulations can improve the appearance and texture of nails, such as when affected by the common fungal infection onychomycosis[14, 15, 16].

Nutrients such as biotin, zinc, and sulfur are small molecules readily absorbed by the body and essential for the production of keratin. Keratin is made up of long, coiled polypeptide chains that form intermediate filaments uniquely rich in the sulfur amino acid cystine. The unique structure of keratin provides strength and elasticity to the skin, hair, and nails, allowing them to withstand repeated exposure to mechanical stressors such as bending, stretching, and twisting. Keratin protein has been established as a safe and effective source of cysteine with the ability to influence sulfur metabolism including glutathione and taurine levels when presented in a digestible form[17]. Once ingested, keratin in a digestible form is available to be broken down by digestive enzymes leading to absorption of bioavailable keratin peptides that are then have the potential to influence cell metabolism. Exploring the potential effects of keratin-based formulations on relatively healthy adults may lead to the development of new and effective treatments for health conditions in the long-term. In the short-term, it may help manufacturers develop products that maintain the health and integrity of these structures, which can be beneficial for consumers.

The use of dietary supplements for potential health benefits has increased in recent years, mainly due to the growing interest in health and wellness among consumers[18, 19]. When there is evidence to support their use in preventing, improving, and/or treating diseases or health conditions, dietary supplements are more accurately referred to as nutraceuticals[20]. These nutraceuticals are available in various forms, including tablets, capsules, gummies, and powders. In some cases, ingestible forms of these compounds may be more effective in delivering potential benefits than topical administrations[21]. Popular supplements for skin, hair, and nail health include multivitamins, biotin/B7, ascorbic acid/vitamin C, zinc, and collagen[22]. This study aims to investigate to what extent keratin also supports these structures.

The objective of the current study is to examine a novel proprietary keratin-based supplement's effect on user well-being, including:

- skin structure,
- hair follicle anchoring and hair condition,
- nail quality, and
- satisfaction and tolerance of the supplement.

2. Materials and Methods

The research was structured as a randomized, double-blind, placebo-controlled non-invasive study conducted at single site in France (Laboratoire BIO-EC) over 60 days in 65 subjects, aged 45-60. The interventional group comprised n=32 while 33 participants were in the placebo group; one volunteer assigned to the placebo group was excluded from the D30 analysis after not attending the D30 visit. Figure 1 presents the research stages.

In the period leading up to and including D0, study details were explained, participants were informed of possible adverse effects from using the product, and a correct understanding of the study was ascertained. Each participant then signed an informed consent document, including consenting to undergo a general clinical examination attesting to their ability to participate in the study.

Potential participants were evaluated by a dermatologist against inclusion and exclusion criteria set by the study. Inclusion criteria included that volunteers were healthy Caucasian women aged from 45 to 60 years old having healthy skin, but damaged or stressed hair. In accordance with recommendations of French law on biomedical research, participants needed to be affiliated to the French social security system and needed to understand the French language to be able to read the documents presented and freely adhere to what was explained.

In addition to not fitting the inclusion criteria or participating or intending to participate in another study, non-inclusion criteria included pregnancy, breastfeeding, or wishing to be pregnant during the study time frame; having surgery planned during the study; suffering from systemic diseases, pathologies, or any dermatosis likely to interfere with the study; having an allergy or hypersensitivity to food products or any component of the study product; or showing signs of recent intense sun or UV exposure or having recently carried out aesthetic treatments (e.g. scrub, peeling, self-tanning, depigmenting, etc.) that could confound study results. In addition, potential participants who could not be contacted urgently by phone, were unable to follow study protocol requirements, or were deprived of liberty or were otherwise protected by law were excluded from this study. Vegetarian and vegans were also excluded as the nature of the investigational product conflicts with these dietary choices.

Each participant meeting the inclusion criteria was weighed and given a clinical exam including recording general and dermatological features, hormonal status, and contraceptive method. A baseline assessment of hair was conducted.

At D2, biometrological measurements were taken, including measurements of skin firmness and elasticity, skin hydration measures, and skin barrier function efficacy measurements.

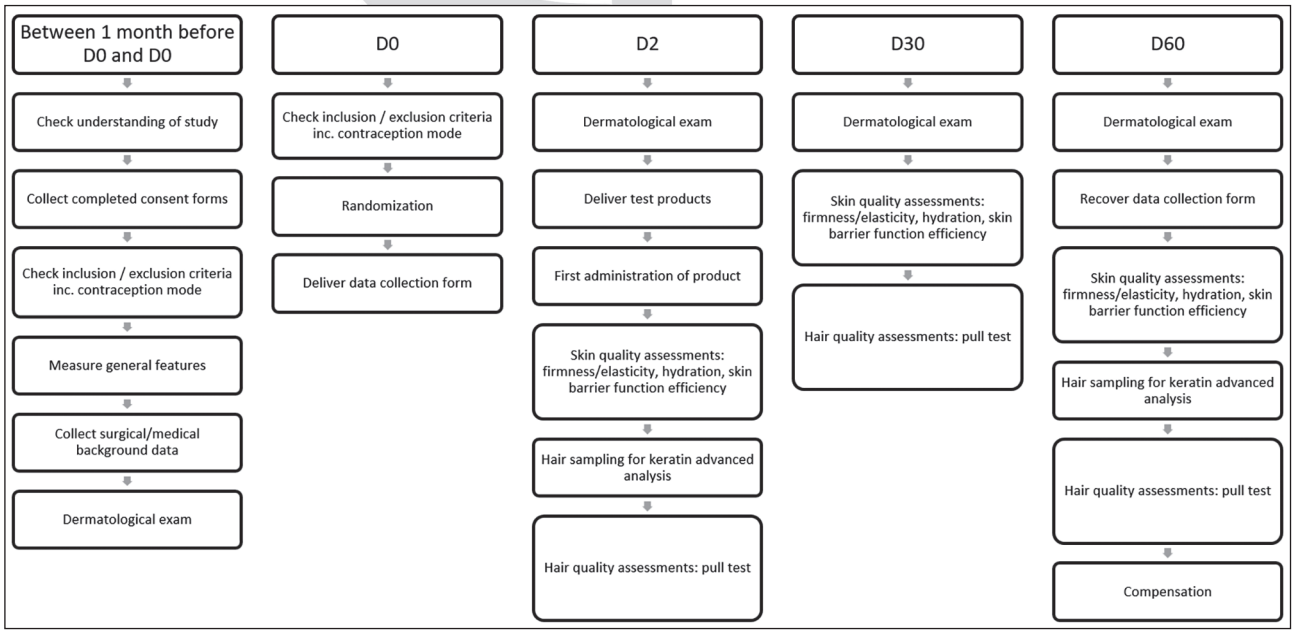

Figure 1. Clinical Design Flow Chart

The test product was given to the volunteer. The investigational keratin product (keraGEN IV®) tested is a highly bioavailable form of keratin supplied by Keraplast Manufacturing (Christchurch, NZ; patent application number US 20120219667A1). The investigational product consisted of 200 mg of keraGEN IV® and 550mg of microcrystalline cellulose; the placebo (also supplied by Keraplast Manufacturing) consisted of 750mg of microcrystalline cellulose. The product was in powder form contained in a transparent capsule packed in a white opaque bottle, to be kept at room temperature. Participants were asked to take two capsules once a day, every morning, swallowed with a glass of water, over the 60 day study period.

At D30, volunteers were weighed, and dermatological control (hair pull test, evaluation of hair and nail qualities) were performed to assess tolerance of the investigational product. Biometrological measurements were taken. During the final D60 visit, volunteers were weighed, biometrological measurements were taken, and the test product was returned, as well as a self-assessment questionnaire investigating the user's experience. Compensation was given to the volunteer.

Clinical assessment of hair and skin was done in seven different ways.

2.1 Hair pull test

The hair pull test helps evaluate diffuse scalp hair loss and follicle anchoring in the scalp. Gentle traction is exerted on small group of hairs (about 60) in three areas of the scalp (frontal, temporal, and occipital) and the number of extracted hairs is counted. The dermatologist takes a few strands between his/her thumb and forefinger and pulls them gently. In the anagen phase, growing hair should remain rooted in place while hair in the telogen phase will come out easily. If the number of lost hairs is greater than nine, the pull test is suggestive of telogen effluvium (temporary hair loss). Volunteers are asked to refrain from washing their hair for two to three days before the pull test.

2.2 Hair sampling for keratin analysis

Hair cross sections of 30µm thickness, cut at a 35◦ angle of two volunteers were sampled, one for the investigational product and one for the placebo. Thirty hair segments of 1 cm length per volunteer were analyzed both before and 60 days after supplementation. Polarimetric imaging analysis (performed by KAMAX company, France) allowed the effect of the investigational product and placebo on the keratin structure of the cortex of the hair at the molecular level to be evaluated by quantifying the birefringence of the sample. Greater keratin organization in the hair cortex results in greater birefringence and is indicative of high hair fibre structural integrity. The K_{index} value of the sample is a measure of how light is polarized as it is transferred through a hair segment, the birefringence. This is a quantification of keratin integrity in the hair cortex. The darkest hair shade accepted for analysis was dark brown as melanin interferes with the analysis.

2.3 Dermatological exam of hair quality

The hair appearance was evaluated by a licensed dermatologist who assigned a score of one to three based upon the person's hair brightness and luster. A score of 1 is dull and devoid of brightness, a score of 2 is basically dull and not so bright, and a score of 3 is shiny and bright.

2.4 Skin firmness/elasticity

Courage & Khazaka Electronic's MPA580 Cutometer® with a 2 mm diameter probe was used to evaluate the deformation of a skin area and its recovery skill after being subjected to mechanical suction stress. During the suction phase, the deformation of the skin by negative pressure measures first the elastic resistance, then the viscous component which, together, represent skin firmness. The immediate recovery of the skin measures sheer cutaneous elasticity, whereas the delayed return of the skin to its initial position measures the visco-elastic component. Skin firmness/elasticity measures were taken at the beginning of the study, and at 30 and 60 days.

2.5 Skin hydration

A probe linked to a condenser is the basic technology used by the Corneometer CM825TM by Courage & Khazaka Electronic. This technology was used at the study's beginning, and at 30 and

60 days to measure skin hydration by noting variation in electric capacity. Higher hydration is indicated by higher electric capacity values. Forearm skin was tested.

2.6 Skin barrier function efficacy

The Trans Epidermal Water Loss (TEWL) assessment evaluates the skin barrier function efficacy and is indicative of the skin structure and integrity of the stratum corneum. The TEWL value is inversely proportional to the barrier function. TEWL measures of the quantity of evaporated water (TEWL g/hm2) were taken with Courage & Khazaka Electronic's Tewamètre ® TM300 based on an open room diffusion technique. At the beginning of the study and at 30 and 60 days about twenty successive measures (one measure per second) were taken on the same area and the mean value was recorded. Forearm skin was tested.

2.7 Dermatological Controls

A dermatological control was performed at the beginning of the study (D0), on day 30 (D30) and at the end of the study (D60) to assess users' tolerance of the products.

2.8 Statistical Analyses

Intra-product and inter-product comparisons over time were made using Student's t-test and the Wilcoxon signed rank test.

3. Results

3.1 Hair Assessments

3.1.1 Hair Pull Test

While neither the investigational product nor the placebo had a significant effect on hair loss after 30 days of use, volunteers taking the keraGEN IV® supplement demonstrated a substantial and statistically significant reduction in hair pull scores on day 60. Reduction compared to day 0 was -43.1% ($p \leq 0.01$). By day 60 the placebo recorded a modest but not statistically significant reduction in hair pull scores of -14.1% compared to day 0 ($p \geq 0.1$). Figure 2a shows intra- and inter-group percentage change in hair loss as a result of the hair pull test for keraGEN IV® and the placebo at day 30 and day 60 compared to day 0 and compared to each other at both days 30 and 60.

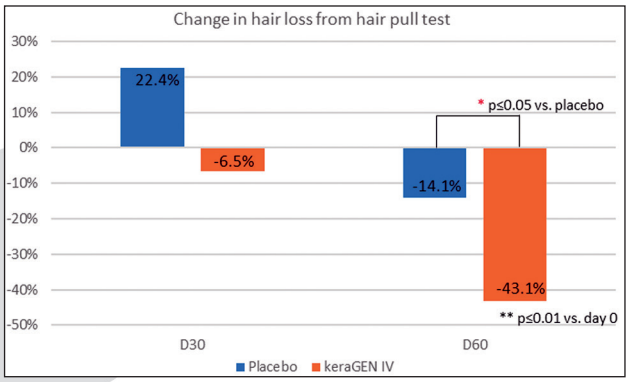

Figure 2a. Hair loss percentage change between keraGEN IV® and placebo at day 30 and day 60 compared to day 0

Compared to both baseline and the placebo, keraGEN IV® significantly decreased the hair pull test score thus significantly decreasing hair loss after 60 days of use.

3.1.2 Hair sampling for keratin analysis

The analysis of keratin fibers shown in figure 2b revealed an increase from 86.08($x10^{-4}$) to 101.24 ($x10^{-4}$), a 17.61% improvement in the treatment group volunteer's hair cortex after 60 days of supplementation, with statistical significance ($p \leq 0.01$). The Kindex mean value of the control group volunteer increased from 67.1($x10^{-4}$) to 71.76 ($x10^{-4}$), a variation of 6.94%, although this was not a statistically significant change. This suggests that KeraGEN IV® significantly increased the structural integrity of the hair cortex after 60 days of use.

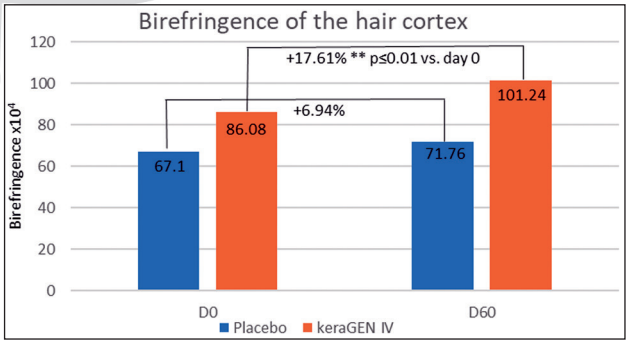

Figure 2b. Birefringence of hair cortex after 60 days of keraGEN IV® or placebo treatment

3.1.3 Dermatological exam of hair quality

Neither keraGEN IV® (p≥0.1) nor the placebo (p≥0.1) affected any significant change in hair luminosity and luster over the study time or compared to each other. Results from measurements done for hair are summarized in Table 1.

3.2 Skin Assessments

3.2.1. Skin firmness/elasticity

Neither the investigational product nor the control had an effect (p≥0.1) on skin firmness over the course of the study. As shown in figure 3a, neither supplement had an effect on gross elasticity (R7) at day 30 and the placebo still did not produce an effect at day 60. KeraGEN IV®, however, increased gross elasticity of the skin by 10.1% over the baseline (p≤0.001) by day 60; the effect was also significant compared to the placebo (p≤0.05).

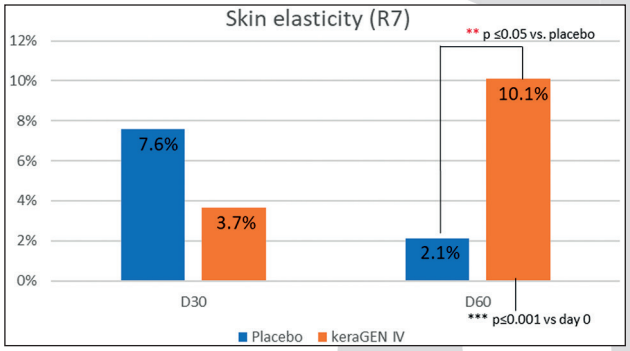

Figure 3a. Percentage change skin elasticity (R7) for keraGEN IV® and placebo at day 30 and day 60 compared to day 0. Statistical significance given vs. day 0 and vs. placebo at the same time point

3.2.2. Skin hydration

Neither the investigational product nor the control had a significant effect on skin hydration during the study compared to baseline or when compared to one another (p≥0.1).

3.2.3. Skin barrier function efficacy

TEWL measurements on day 30 volunteers taking the keraGEN IV® supplement were lower at -12.5% compared to day 0 with statistical significance (p≤0.05) (figure 3b).

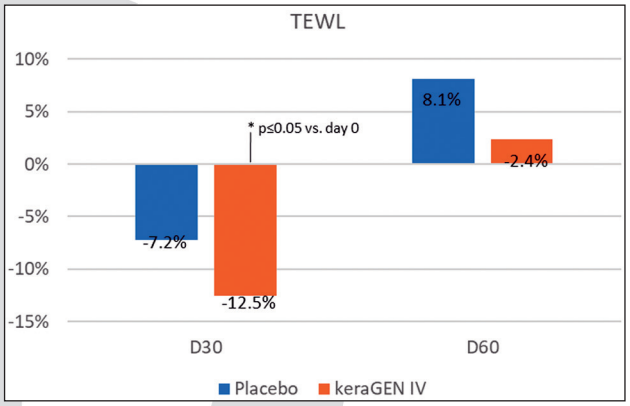

Figure 3b. Percentage change in trans epidermal water loss (TEWL) for keraGEN IV® and placebo at day 30 and day 60 compared to day 0. Statistical significance given vs. day 0 and vs. placebo at the same time point

Measurements on day 60 were not significantly different from day 0 (+2.4% increase, p≥0.1). Volunteers in the placebo group at day 30 also measured lower TEWL at -7.2% however this and

Table 1. Hair Results Summary

Assessment	Mean ± Standard deviation (1/cm²)			P values (+ Wilcoxon test, ++ Student's t test)	
	D0	D30	D60	D30	D60
Hair Pull Test					
keraGEN IV®	4.8 ± 4.2	4.5 ± 5.9	2.7 ± 3.0	0.3703 + Vs. placebo 0.1804++	0.0031 + ** Vs. placebo 0.0536++*
Placebo	2.9 ± 3.3	3.5 ± 5.8	2.4 ± 2.7	0.7186 +	0.9825 +
Hair sampling for keratin analysis					
keraGEN IV®	86.08 $^{10-4}$	N/A	101.24	N/A	0.0041 + **
Placebo	67.1$^{10-4}$	N/A	71.76$^{10-4}$	N/A	0.0707+
Hair quality (luminosity and luster)					
keraGEN IV®	1.8 ± 0.4	2.2 ± 0.5	2.3 ± 0.5	0.9615+ Vs. placebo 0.2571++	0.8340+ Vs. placebo 0.2900++
Placebo	1.9 ± 0.3	2.1 ± 0.4	2.2 ± 0.5	0.9993+	0.9414+

** Significant p≤0.05 **Significant p≤0.01 ***Significant p≤0.001*

the day 60 result (increase of +8.1%) was not statistically significantly different to day 0 ($p \geq 0.1$). Compared to placebo, the changes in skin barrier function at day 30 or day 60 did not achieve statistical significance.

Results from measurements of skin quality are summarized in Table 2.

3.3. User Experience / Questionnaire results

3.3.1. Dermatological control results

There was a very good tolerance of both keraGEN IV® and the placebo after 30 and 60 days of use. No adverse effects occurred during the study.

3.3.2. Skin self-assessment

An inclusion criterion for the study was that participants had "good" skin as evaluated by a dermatologist. The majority of participants echoed this evaluation by rating their skin appearance as either "good" (keraGEN IV® 15.6%, placebo 15.1%) or "average" (keraGEN IV® 81.3%, placebo 75.8%) with few rating their skin as "poor" (keraGEN IV® 3.1%, placebo 9.1%).

By the end of the study, the majority of those taking the investigational product felt that their skin had either significantly improved (3.1%) or improved (43.8%) with few feeling that their skin had gotten worse (3.1%). In the placebo group,

Table 2. Effect of the Investigational Product on Skin Quality

Assessment	Mean ± Standard deviation (1/cm²)			P values (+ Wilcoxon test, ++ Student's t test)	
	D0	D30	D60	D30	D60
Skin firmness (R0)/elasticity (R6)/ skin gross elasticity (R7)					
keraGEN IV® (n=32)	0.293 ± 0.034 (R0)	0.295 ± 0.037	0.293 ± 0.034	0.7534++ Vs. Placebo 0.3238++	0.9882++ Vs. Placebo 0.4159++
	41.39 ± 6.62 (R6)	40.60 ± 4.34	42.11 ± 5.97	0.5146++ Vs. Placebo 0.4906++	0.5867++ Vs. Placebo 0.4989++
	24.67 ± 4.93 (R7)	25.57 ± 6.47	27.16 ± 3.57	0.3616++ Vs. Placebo 0.4823++	0.0011++ ** Vs. Placebo 0.0189++*
Placebo (D30 n=32, D60 n=33)	0.305 ± 0.040 (R0, D30) 0.305 ± 0.039 (R0, D60)	0.301 ± 0.044	0.304 ± 0.041	0.4322+	0.8481++
	41.61 ± 5.32 (R6, D30) 41.51 ± 5.26 (R6, D30)	40.68 ± 7.17	42.77 ± 5.77	0.3865++	0.1952++
	26.71 ± 6.29 (R7, D30) 26.44 ± 6.38 (R7, D60)	27.42 ± 6.74	26.78 ± 6.40	0.3744+	0.2347
Skin hydration					
keraGEN IV® (n=30)	38.4 ± 8.7	39.4 ± 8.6	37.0 ± 7.5	0.3897+ Vs. Placebo 0.4232++	0.1553+ Vs. Placebo 0.3842++
Placebo (Day 30 n=32, Day 60 n=33)	38.8 ± 6.7 38.8 ± 6.6	39.6 ± 9.6	37.0 ± 6.2	0.8737++	0.1170++
Skin barrier function efficacy					
keraGEN IV® (n=30)	9.3 ± 2.7	8.1 ± 2.4	9.5 ± 1.8	0.0135++ * Vs. Placebo 0.3257++	0.6401+ Vs. Placebo 0.2071++
Placebo (n=32, n=33)	8.3 ± 1.6	7.7 ± 2.0	9.0 ± 2.2	0.2130++	0.2318+

** Significant p≤0.05 **Significant p≤0.01 ***Significant p≤0.001*

9.1% perceived a significant improvement, 36.4% felt that their skin had improved and 3.0% felt that their skin had worsened. Overall, the proportion of those taking keraGEN IV® (46.9%) and those taking the placebo (45.5%) who felt some level of improvement in their skin's appearance by the end of the study was virtually the same ($p>0.05$).

3.3.3. Hair self-assessment

Participants had more concerns about the baseline state of their hair relative to their skin, again reflecting study inclusion criteria. None of those in the test group and only 3.1% of those in the control rated their hair strength as good, 62.5% in test and 72.7% in control rated it as average, and 37.5% in the test and 24.2% in the control rated their hair strength as poor. Similar proportions of both groups (46.9% test and 48.5% control; $p>0.05$) perceived that their hair strength had improved to some degree over the course of the study.

At baseline 12.5% of the test and 21.2% of the control rated their hair growth as "good" while 50.0% and 57.6% rated it as average, and 37.5% and 21.2% of the test and control groups, respectively, rated their hair growth as poor. Half of the test and 45.5% of the control groups perceived that their hair growth had improved to some degree over the course of the study, a significant difference at $p\leq0.05$.

Over a third of both the test (37.5%) and control (33.3%) groups rated their hair loss as "poor" at the beginning of the study. By the end, over half of both groups perceived that their hair loss had improved to some level.

Every participant rated their overall hair condition as either "average" (59.4% test, 75.8% control) or "poor" (40.6% test, 24.2% control) at the start of the study. A significantly higher proportion of participants in the study group (9.4%) than in the placebo group (3.0%) perceived that their overall hair condition had significantly improved by the end of the study ($p\leq0.05$).

3.3.4. Nail self-assessment

While 37.5% of those in the test group and 39.4% of those in the control rated their baseline nail strength as "poor," over half (59.4%) of the keraGEN IV® users and 45.5% of those taking the placebo perceived that their nail strength had improved during the 60 days of the study. 56.2% of the test group and 51.5% of those in the control perceived that their overall nail condition had improved while taking the supplements. This evidence does not support that there is a difference in nail self-assessment of either strength or overall condition based on whether the investigational product or the placebo was taken.

4. Discussion and Conclusions

Prior work on keraGEN IV® using in vitro studies on human keratinocytes has shown that increased collagen IV expression is induced by the protein[23]. Further preclinical work using 16 ex vivo skin samples of facial skin removed during a face lift procedure demonstrated that collagen IV expression is induced around the hair follicle[24]. The known function of collagen IV as a junctional pro-

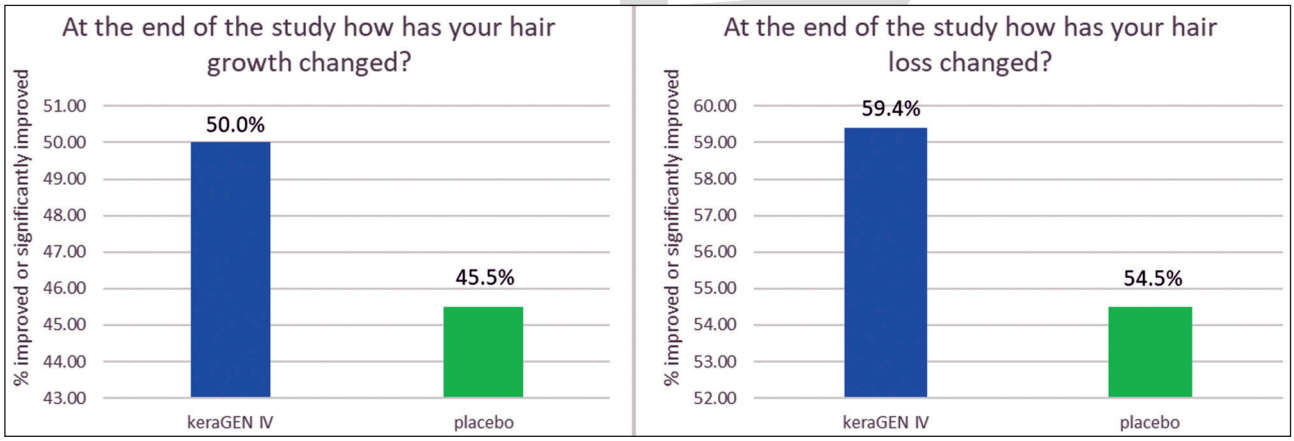

Figure 4. Self-assessment of hair after 60 days of keraGEN IV® or placebo treatment

tein is to build skin structure at the dermal-epidermal junction and further to bind hair follicles as the anchoring protein at the hair root, keeping hair well anchored to the head. This work now demonstrates in a randomised, double blind, controlled study that the keraGEN IV® action of inducing collagen IV expression translates to measurable improvement in hair anchoring, specifically a 43.1% reduction in hair loss in women aged 45-60 with stressed or damaged hair. The gentle pulling action triggering hair loss is typical of that experienced during combing or styling when hair is damaged through chemical treatments, colour, styling or during peri- or post- menopause. The development of healthy hair structure, assisting hair anchoring and importantly improving hair strength, arises from appropriate nutrition including bioavailable cystine and suitable proteins and peptides. The positive impact on cortex structure as a result of keraGEN IV® ingestion is apparent from the improvement in cortex birefringence measured in the study, reinforcing the bioavailable nature of the keraGEN IV® material. Alternative approaches to hair thinning in peri and post-menopausal women have used known anti-inflammatory materials, such as omega 3, omega 6 and lycopene. Published results have demonstrated improvements in follicle density and reduction in hair loss following 6 months intervention[25]. Anti-inflammatory materials impact hair follicle health from different metabolic pathways and may be complimentary to the collagen IV expression induced by keraGEN IV. Examining synergistic effects may be a valuable subject of future investigation.

The measured 12.5% reduction in TEWL may arise from the improvement of skin structure due to collagen IV expression induced by keraGEN IV®. Skin barrier function is an important indicator of skin health. Improvement in skin health and skin structure is also reinforced by the measured improvement in skin elasticity, increasing by 10.1%. Overall, it appears from the study that use of keraGEN IV® results in measurable improvement in skin structure.

COVID-19 has been reported as having a significant impact on hair and nail health, including in combination with isotretinoin therapy[26]. Statistically significant increases in hair loss associated with telogen effluvium have been noted in multicenter studies, as have nail disorders such as leukonychia[27, 28]. Telogen effluvium leads to diffuse hair loss as a result of changes to the growth phase of the hair from anagen to telogen and subsequent changes to hair follicle structure. Although not the subject of this study, increased collagen IV expression around the hair follicle may have an impact on the rate of hair loss following telogen effluvium due to improvement in hair anchoring. The impact of this on the occurrence of diffuse hair loss following COVID-19 is recommended as the subject of future investigation.

Based on the results of this study and recent in vitro and in vivo cytotoxicity assays reporting no adverse effects from keratin supplementation[29], further clinical analysis of keraGEN IV® is warranted. Given that the keratin structure of the hair differs based on ethnicity[30], for example, it would be interesting to expend the study to different ethnic groups. In addition, recruitment criteria included that potential participants had damaged hair but healthy skin; the cause of any nail damage was not investigated. Additional study on the possible effects of keraGEN IV® for those experiencing specific dermatological conditions, and specific nail and hair loss disorders is recommended.

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Serum Anti-Müllerian hormone (AMH) is a good secondary diagnostic parameter in the diagnosis and differentiation of phenotypic forms of PCOS

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Abstract

Introduction: Polycystic ovary syndrome (PCOS) is a multisystem endocrine-metabolic and reproductive disorder. Anti-Müllerian hormone (AMH) did not enter the Rotterdam criteria in the diagnosis of PCOS.

The aim of this study was to determine the diagnostic value of (AMH) in the diagnosis of PCOS and phenotypic forms of PCOS.

Subjects and methods: The study design is a retrospective-prospective case-control study. The study was conducted between May 2018. and December 2022. in the private health institution „dr. Hajder“ in Tuzla, Bosnia and Herzegovina. The study included a review of 99 women aged 19-40 years with a diagnosis of PCOS and 27 women aged 21-40 years without a diagnosis of PCOS, selected according to the 2003 ESHRE/ASRM criteria. Classified into 4 phenotypic forms according to Rott. criteria. Clinical and sonographic parameters, and hormonal analyses from the blood of the test subjects and the control group. The Mann-Whitney test was used for comparisons between two groups, and the Kruskal-Wallis test was used for comparisons between more than two groups. Spearman's test was used for bivariate correlations.

Results: In the PCOS group, serum AMH levels [median: 5.20 ng/ml (IQR: 3.30-8.60 ng/ml) versus median: 2.90 ng/ml (IQR: 2.40-3.20 ng/ml), p <0.001 significantly higher compared to the control group. Serum AMH levels were in phenotype A 8.11 ng/ml (IQR: 5.40-10.70) in and in phenotype B 2.90 ng/ml (IQR: 2.62-3.45-), phenotype C at 3.45 ng/ml (IQR: 2.80-3.99), phenotype D at 5.20 ng/ml (IQR: 4.13-8.87) and in the control group at 2.90 ng/ml (IQR: 2.40-3.20). A significant difference in median AMH was found between the 4 PCOS subgroups X2 =29.187 (df 3, n=99), p<0.001). A significant correlation (p<0.05) was found between AMH and androgens, LH/FSH ratio, ovarian volume, and number of antral follicles.

Conclusions: AMH is a good additional diagnostic parameter in women with PCOM without PCOS, in PCOS with present PCOM in phenotypic subgroups A, C, and D, while for phenotype B there is no significance. In combination with other Rott. criteria helps to differentiate the phenotypic forms of PCOS.

Keywords: polycystic ovary syndrome / phenotypic forms / Anti-Müllerian hormone

Introduction

Polycystic ovary syndrome (PCOS) is a very common complex endocrine metabolic disorder in women of reproductive age worldwide. About 70% of women are subfertile (1), 82% of total hyperandrogenism belongs to PCOS (2). The prevalence ranges from 6-20% in different countries (3). Diagnostic Rott. criteria for PCOS are: oligo-and/or anovulation (OA), clinical (hirsutism) and/or biochemical hyperandrogenism (HA), and polycystic ovarian morphology (PCOM). If two of the three Rott are present. criteria with the exclusion of Like PCOS, the diagnosis of PCOS is established (4). Symptoms of PCOS include: congenital adrenal hyperplasia (NCAH), hyperprolactinemia, Cushing's syndrome, thyroid dysfunction, neoplastic androgen secretion, or androgen excess caused by drugs (5). The presence of two of the three clinical parameters defines the 4 phenotypic forms of PCOS phenotype A (OA+HA+PCOM), phenotype B (OA+HA), phenotype C(HA+PCOM), and phenotype D (OA+PCOM) (4). Ultrasound-assessed morphology of polycystic ovaries was defined as the presence of 12 or more follicles of size 2-9 mm in diameter in each ovary and/or increa-

sed ovarian volume, greater than 10 cm^3, and such a finding on one ovary is sufficient (6). Regulation of ovarian AMH production has not been identified so far, but locally produced androgens and estradiol (7, 8) and gonadotropins (9) appear to be involved. The level of AMH is closely related to the number of antral follicles (AFC) (10,11). Serum AMH levels are higher in women with PCOS than in women with normal ovarian morphology. AMH in serum has been suggested as a surrogate marker of AFC (11). An AMH > 1 ng/ml represents a normal ovarian reserve, an AMH < 1 ng/ml for reduced and AMH > 5 ng/ml for PCOS (12).

This study aimed to determine the diagnostic value of AMH in the diagnosis and differentiation of phenotypic forms of PCOS.

Subjects and Methods

Study group: The study design is a prospective-retrospective case-control study. The research included 99 women of reproductive age with a diagnosis of PCOS and 27 women of reproductive age without a diagnosis of PCOS (control group), selected according to the ESHRE/ASRM criteria from 2003. the surveillance was conducted between May 2018 and December 2022 at PZU „Dr. Hajder“ in Tuzla. Inclusion criteria were included: women with PCOS aged 18-40 years selected according to ESHRE/ASRM(4) criteria. The exclusion criteria were women with PCOS older than 40 years, and patients with endocrinopathy suspected to be COS. The control group consisted of subjects without PCOS syndrome aged 20-40, and without endocrinological disorders.

Study protocol: The cohort of women of reproductive age (n=126) was divided into two groups: a group of women with PCOS (n=99) and a control group (n=27). The group of subjects with PCOS was additionally classified into 4 phenotypic forms according to the ESHRE/ASRM diagnostic criteria: Phenotype A with 40 subjects, phenotype B with 16 subjects, phenotype C with 14 subjects, and phenotype D with 29 subjects. We determined the following parameters for all women with and without PCOS: age, body weight, height, BMI, menarche, duration of menstrual cycles, glucose, and insulin levels, calculated HOMA-IR, LH, FSH, LH / FSH – quotient, testosterone, FAI, SHBG, and AMH.Ultrasound parameters (ovarian volume and AFC).

Definition of parameters: OA is defined by MC>25 days>90 days. Secondary amenorrhea is defined by the average length of the menstrual cycle longer than 90 days. Biochemical hyperandrogenemia is defined as a total testosterone value > 2.5 nmol/l and/or an androstenedione value of ≥ 10.0 ng/ml. HOMA-IR > 2.00 (13) is a sign of insulin resistance and is calculated using the formula: HOMA-IR = insulin (mIU/l) × glucose (mmol/l) / 22.5 (14). FAI is calculated as follows: FAI=Testosterone (nmol/l) × 100 To date, there is no agreed cut-off value for AMH in relation to the diagnosis of PCOM.: FAI = Testosterone (nmol/l) × 100 / SHBG (nmol/ l) (15). FAI value > 3 indicates hyperandrogenemia. Ultrasound criteria for the diagnosis of PCOM are the presence of more than 12 follicles 2-9 mm in diameter per ovary and/or ovarian volume > 10^3 ml, based on at least one ovary. We calculated the volume of ovaries in both collectives using the formula: V=ovary length x ovary width x ovary thickness x 0.524 (16). Suggested values of > 4.7 ng/ml indicate PCOM or PCOS (17). The cut-off value for AMH is 3.92 ng/ml with a specificity of 97.5% and a sensitivity of 84.2% (20).

Methods: Body weight and height were measured on a scale at the examination. BMI is calculated with a calculator using the following formula: BMI (kg/m^2) = weight (kg)/height/m^2. Blood samples for determining basal hormone values were taken by medical staff between the 2nd and 5th day of the menstrual cycle. In women diagnosed with PCOS who did not have spontaneous bleeding for >90 days, they were treated with a progestogen. Hormone parameters were determined by the following analyses: Testosterone (ElecsysTestosterone II test, Rotkreuz, Switzerland), androstenedione (DRG Androstenedione ELISA, Marburg, Germany), SHBG (Elecsys SHBG test, Rotkreuz, Switzerland), FSH (Elecsys FSH test, Mannheim, Germany), LH (Elecsys LH-Test, Mannheim, Germany), glucose (GLUC3 / Glucose HK Gen.3, Mannheim, Germany), insulin (Elecsys Insulin-Test, Mannheim, Germany) and AMH (Elecsys AMH-Test, Rotkreuz, Switzerland). Analyzes were performed in the Dia Lab laboratory in Bijeljina, Bosnia and Herzegovina. Ultrasound measurements were performed

with a GE Voluson E8 ultrasound device with a 7 MHz transvaginal probe (General Electric Medical Systems). Transvaginal ultrasound was performed on all subjects by an experienced examiner.

Statistical analysis: Data analysis was performed using the SPSS statistical version (version 22.0; SPSS Inc., Chicago, IL, USA). The parameters did not follow a normal distribution and are expressed as non-parametric. A median with an interquartile range is given. The nonparametric Mann-Whitney test was used for comparisons between two groups, and the Kruskal-Wallis test was used for comparisons between more than two groups. In all cases, a p-value <0.05 was considered significant. The nonparametric Spearman test was used for bivariate correlations. A p-value < 0.05 was also considered statistically significant here.

Results

The highest representation was phenotypic subgroup A, and the smallest subgroup B. PCOS type A accounted for 40 (40.40%), PCOS B 16 (19.19%), PCOS-type C 14 (14.14%) and PCOS-type D 29 (29.29%)) (Figure 1).

In PCOS women had significantly higher median values (p<0.05) of TT, BMI, fasting insulin, fasting glucose, HOMA-IR, longer MC, and earlier menarche, compared to the control group without PCOS. The results indicated that the medians were: OV 8.32 vs. 6.45, p<0.001; AFC 11.00 vs. 7.00, p<0.001; were significantly higher in the PCOS group compared to the control non-PCOS group (Table 1).

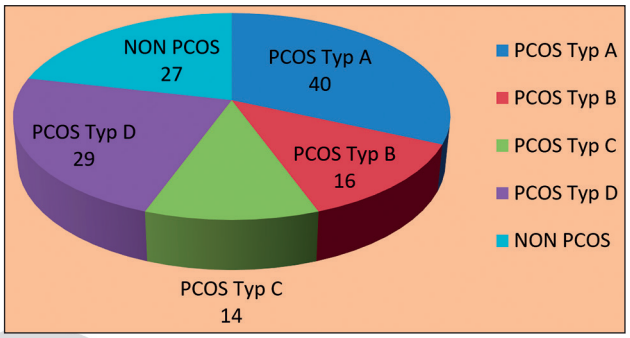

Figure 1. Representation of phenotypic forms of PCOS and non-PCOS.

The results of basal hormone values indicated that the medians were: testosterone 2.20 (1.4-2.9) vs. 1.3 (0.9-1.5, p<0.001; FAI 6.21 (2.6-9.1) vs. 1.83 (1.3-2.8), p<0.001; androstenedione 3.01 (2.2-4.2) vs. 1.77 (1.5-2.1), p<0.001; LH 7.30 (5.8-9.8) vs. 5.7 (4.5-6.8), p<0.001; LH/FSH ratio 1.19 (0.9-1.6) vs. 0.87 (0.6-0.9), p<0.001; AMH 5.20 (3.3-8.6) vs. 2.90 (2.4-3.2), p<0.001 were significantly higher, while SHBG 35.30 (29.6-51.2) vs. 62.30 (51.6-74.3), p<0.001; FSH 5.0 (5.2-6.2) vs. 6.10 (5.8-7.3); p<0.009 were significantly lower in the PCOS group compared to the control non-PCOS group (Table 2).

The Kruskal Wallis test revealed a statistically significant difference in AMH levels in four different PCOS phenotypic subgroups (Pg 1, n=40: PCOS typus A, Pg 2, n=16: PCOS typus B, Pg 3, n=14: PCOS typus C, Pg 4, n=29: PCOS typus D), X2 =29.187 (3, n=99), p<0.001. PCOS typus A has a higher median score (Md = 8.11) than the other three phenotypic subgroups, whose medians are

Table 1. Comparison of clinical and sonographic parameters between PCOS and non-PCOS groups

Parameters	Kohorta PCOS (n=99)	Non-PCOS (n=27)	p-value
	Median (Q1-Q3)	Median (Q1-Q3)	
Age (years)	29.00 (25.0-33.0)	30.00 (25.0-36.0)	0.350
Weight (kg)	74.40 (68.0-88.0)	66.20 (57.0-72.3)	0.001
Height (cm)	168.90 (165.0-172.0)	167.00 (163.0-171.4)	0.220
BMI (kg/m^2)	26.30 (23.4-30.0)	22.74 (21.2-25.2)	0.001
HOMA-IR	2.26 (1.8-3.2)	1.48 (1.38-1.81)	0.001
Menarche (years)	13.00 (12.0-14.0)	13.00 (12.0-14.0)	0.001
Menstrual cycle (days)	35.00 (25.1-41.2)	29.00 (28.0-34.0)	0.001
Ovarijan volume (OV)	8.32 (6.6-11.4)	6.45 (5.8-7.7)	0.001
AFC	11.00 (7.0-15.0)	7.00 (6.0-9.0)	0.001

Note: parameters are expressed as median (range Q1-Q3); MC, BMI, Body mass index; AFC, antral follicle count; HOMA-IR, homeostasis model assessment for insulin resistance index; Two-sample Wilcoxon rank-sum (Mann-Whitney) test, p<0.05, PCOS vs. non-PCOS.

Table 2. Comparison of basal values of hormone parameters between PCOS subjects and a control group without PCOS

Parameters	PCOS (n=99)	Non-PCOS(n=27)	p-value
	Median (Q1-Q3)	Median (Q1-Q3)	
Testosterone (nmol/L)	2.20 (1.4-2.9)	1.30 (0.9-1.5)	0.001
SHBG (nmol/L)	35.30 (29.6-51.2)	62.30 (51.6-74.3)	0.001
FAI	6.21 (2.6-9.1)	1.83 (1.3-2.8)	0.001
Androstendione (nmol/L)	3.01 (2.2-4.2)	1.77 (1.5-2.1)	0.001
FSH (U/I)	5.80 (5.2–6.2)	6.10 (5.8–7.3)	0.009
LH (U/I)	7.30 (5.8-9.8)	5.7 (4.5-6.8)	0.001
LH/FSH Ratio	1.19 (0.9-1.6)	0.87 (0.6-0.9)	0.001
AMH (ng/ml)	5.20 (3.3-8.6)	2.90 (2.4-3.2)	0.001

Note: Parameters are expressed as median (range Q1-Q3); FAI, free androgenic index, SHBG, sex hormone binding globulin). FSH, follicle-stimulating hormone; LH, luteinizing hormone; AMH, anti-Mullerian hormone, Two-sample Wilcoxon rank-sum (Mann-Whitney) test, p<0.05, PCOS vs. non-PCOS. (1ng/dl= 28.65 nmol/l).

2.90; 3.45; 5.20. In conclusion, it can be concluded that the difference in median AMH between the 4 PCOS groups is significant (Table 3).

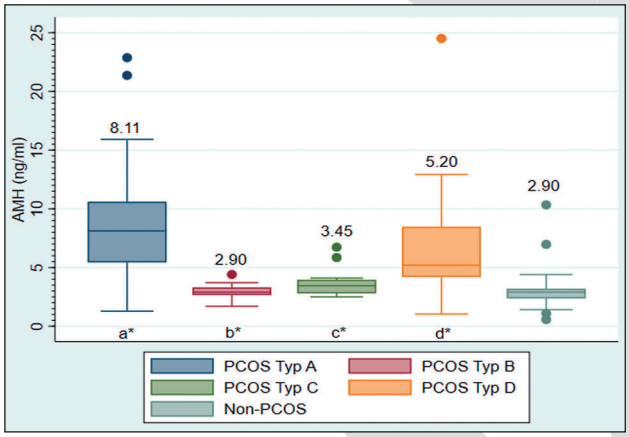

Figure 2. Comparative analyses of AMH in phenotypic subgroups of the PCOS cohort

*Note: Values are median (Q1-Q3), PCOS, Polycystic Ovary Syndrome; AMH, Anti Mullerian hormone Two-sample Wilcoxon rank-sum (Mann-Whitney) test, p<0.05, a*p= 0.001 for PCOS phenotypes A vs. PCOS phenotypes B, a*p= 0.001 for PCOS phenotypes A vs. PCOS phenotypes C,a*p= 0.001 PCOS phenotypes A vs. Non-PCOS; b*p=0.001 for PCOS phenotypes B vs. PCOS phenotypes D; c*p=0.026 for PCOS phenotypes C vs. Non-PCOS; d*p=0.001 for PCOS phenotypes D vs.Non-PCOS.*

Figure 2 shows comparative analyses of AMH in PCOS phenotypic subgroups. The results indicated that the median AMH was significantly higher in PCOS type A (8.11 vs. 2.90, p<0.001) compared to PCOS type B; PCOS type A (8.11 vs. 3.45, p<0.001) compared to PCOS type C; PCOS type A (8.11 vs. 5.20, p<0.001) compared to PCOS type D, while the median is significantly lower in PCOS type B (2.90 vs. 3.45, p<0.001) compared to PCOS type C and the median of PCOS type C (3.45 vs. 5.20, p<0.026 in relation to PCOS tpus D. The highest value was recorded in PCOS typus A, while the lowest value was in PCOS typus B, which has a regular ovarian morphology.

Spearman's linear correlation of AMH parameters in a cohort of PCOS reproductive women

Spearman-Rho correlation between the AMH parameter in relation to other parameters of PCOS women is shown in Table 4. A weak positive correlation was found between AMH and testosterone (ro=0.195, n=99, p<0.028); strong positive correlation between AMH and FAI (ro=0.181,n=99, p<0.043); strong positive correlation between AMH and LH (ro=0.254, n=99, p<0.04); strong

Table 3. Comparisons of median AMH in 4 different phenotypic subtypes of PCOS

Parameters	Subgroup	n	Mean rank	Chi-Square	df	p-value
AMH	PCOS Type A	40	64.30	29.187	3	0.001
	PCOC Type B	16	25.22			
	PCOC Type C	14	29.86			
	PCOC Type D	29	53.67			

Note: Values are median (range Q1-Q3), PCOS, Polycystic Ovary Syndrome; AMH, Anti- Mullerian hormone; n- number of respondents; Mean rank- mean rank value; Kruskal-Wallis equality-of-populations rank test, p<0.0001.

positive correlation between AMH and LH/FSH ratio (ro=0.243, n=99, p<0.006); strong positive correlation between AMH and OV. (ro=0.462, n=99, p<0.001); strong positive correlation between AMH and AFC (ro=0.538, n=99, p<0.001); strong positive correlation between AMH and DHEAS (ro=0.413, n=99, p<0.001).

Table 4. Spearman-Rho correlation between the AMH parameter and other PCOS parameters

Parameters PCOS	AMH (ng/ml)	
	rho	p-value
BMI (kg/m^2)	0.175	0.043
HOMA-IR	0.134	0.256
Menarche (years)	0.19	0.048
FAI	0.181*	0.043
LH (U/L)	0.254**	0.004
LH/FSH ratio	0.243**	0.006
Ovarialn Volume	0.462**	0.001
AFC	0.538**	0.001
DHEAS (µmol/L)	0.413**	0.001

*Note: BMI, body mass index; HOMA-IR, homeostasis model assessment for insulin resistance index; FAI; free androgen index; LH, luteinizing hormone; AFC, antral follicular count; AMH; anti-Mullerian hormone; *Weak correlation; **strong correlation; DHEAS, Dehydroepiandrostenedione sulfate; Spearman rho correlation*p<0.05.*

Discussion

In this study, we investigated whether AMH can be a valid diagnostic marker of PCOS and a marker in the differentiation of phenotypic forms of PCOS. The diagnosis of PCOS is based on the ESHRE/ASRM criteria based on a positive 2 out of 3 criteria with the exclusion of PCOS-like. The presence of two out of three clinical parameters defines the 4 phenotypic forms of PCOS (A, B, C, D,) (5). Of the three clinical parameters (OA, HA, PCOM), a combination of seven different clinical forms is possible, which complicates the diagnosis (18). A meta-analysis was published on the cut-off values of AMH, specificity, and sensitivity in women with PCOS (7,18,19,20,21). The results of cut-off levels of AMH in different studies are given in Table 5 (17).

The results of the AMH cut-off value so far show poor specificity and sensitivity, leaving one-third of women with PCOS undiagnosed (17). Consensus on AMH cut-off levels will be difficult to reach until a standard AMH analysis is established (22). The cut-off AMH level was 4.7 ng/ml with a density of 79.45 and a specificity of 82.9% (17). The two companies have recently merged and introduced a new commercially available test, the AMH Gen II test, which provides new analytical methods and reference cut-off values with high sensitivity and specificity (23). The diagnostic value of AMH in the diagnosis of PCOS is significantly increased when combined with other diagnostic Rott. criteria. Due to the heterogeneous nature of PCOS, they argued that no single value could define the PCOS phenotype, but that AMH could only replace polycystic ovarian morphology (18). Dewailly et al. (2014) pointed out that the AMH threshold of 3.92 ng/ml with a specificity of 97.5% and sensitivity of 84.2% define the form of ovarian morphology PCOM with PCOS phenotype A, C, and D and PCOM-like. The anti-Mullerian hormone is correlated with AFC, AFC values were >12 per ovary in phenotype A, C and D, except in phenotype B (20). The results of this study indicated that AMH in women with PCOS was 2.13 times higher compared to the non-PCOS group. PCOS phenotypic subgroups A, C, and D had significantly higher (p<0.05) AMH values, while phenotypic subgroup B had no significant differences compared to non-PCOS. The results of this study indicated that AMH is a good biochemical marker in the diagnosis of phenotypic forms of

Table 5. Cut-off AMH sensitivity (%) and specificity (%) A meta-analysis of other studies

Study	Country	AMH (ng/ml)	Sens. (%)	Spec. (%)
Pigny et al. 2006 [7]	France	8.40	67	92
Dewailly. 2011 [18]	France	5.00	92	97
Eilertsenet et al. *(2012)* [19]	Norway	2.80	97.1	94.6
Dewailly et.al. *(2014)* [20]	France	3.92	84.2	97.5
Saxena et al., 2018 [21]	Indija	3.44	77.78	68.89
Iliodromiti et al. (2013)[17]	Meta-anal.	4.70	79.4	82.9

Note: AMH, anti-Mullerian hormone.

PCOS (A, C, D), but has no diagnostic significance in PCOS phenotype B.

The results of this study can be explained by the pathophysiological mechanisms of AMH on the hypothalamus-pituitary-ovary axis (24), (25), (26), (27), (24), (26). AMH also correlates with oligoamenorrhea and hyperandrogenism. The level of AMH is strongly correlated with the number of antral follicles (AFC) and can serve as an alternative for PCOM in the diagnosis of PCOS (26). An elevated AMH level increases the risk of developing PCOS but does not affect the risk of developing insulin resistance and metabolic syndrome (28).

The results of this study are consistent with the results of other authors in AMH values in phenotypic subgroups, with small percentage differences. A possible reason for AMH levels is the use of different methods for AMH analysis. In general, in all studies, the highest AMH values were recorded in phenotype A, and the lowest in phenotype B, because phenotype B has a regular ovarian morphology (31, 32, 33, 20, 30, 29) (Table 6).

The results of earlier studies showed a good agreement between serum levels of AMH and the results of ultrasound measurements for the diagnosis of PCOS (18) (9,34).

In conclusion, AMH is a good additional diagnostic parameter in women with PCOM without PCOS, in PCOS with present PCOM in phenotypic subgroups A, C, and D, while for phenotype B it is not significant. In combination with other Rott. criteria helps to differentiate the phenotypic forms of PCOS.

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Table 6. Median AMH(ng/ml) in PCOS phenotypic subgroups in studies

Studies	Country	Total (n)	PCOS Phenotype			
			A %	B %	C %	D %
Wiweko et al, 2018) [31]	Indonesia	406	11.7	9.9	7.5	7.14
Bhide et al.(2017) [32]	Great Britain	418	9.3	4.1	5.9	7.1
Sahmay et al (2013) [33]	Turkiye	560	9.5	3.1	6.1	8.1
Dewailly et al. (2014.)[20]	France	1212	7.13	-	4.5	5.9
Lizneva et al. (2016)[30]	MCS	13796	50	13	14	17
Panadisa et al. ([29])	Greece	1212	48.2	30.7	9.7	11.4
Our study	Bosnia and Hrz.	99	8.1	2.9	3.5	5.2

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Native collagen type II (NCTII) plays an important role in osteoarthritis: a critical meta-analysis on collagen sources

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Abstract

Summary: Collagen, including hydrolyzed type I and native (undenatured) collagen type II sources, is recognized as a safe food ingredient, whose combination of amino acids stimulates collagen synthesis in cartilage and the extracellular matrix of other tissues.

Objective: The aim of the study corresponded to carry out a critical meta-analysis of the literature on the action of collagen on bone and cartilage tissue and its functional purposes in osteoporosis and osteoarthritis.

Method: The research was carried out in the PubMed databases; MEDLINE; LILACS; SciELO. Articles published between 1994 and 2023, in English and Portuguese, were considered.

Results: The final sample was composed of sixteen experimental articles with in vivo (animal and human) and in vitro (human cells) models, which describe the use of different doses and types of collagens associated with the maintenance of bone composition and strength and proliferation and growth cartilage cell.

Conclusion: Native collagen type II (NCTII) has a positive therapeutic function in osteoporosis and osteoarthritis with a potential increase in bone mineral density, a protective effect on articular cartilage and mainly in symptomatic relief in pain.

Key words: collagen; osteoarthritis; native collagen type II; in vivo; in vitro; meta-analysis.

Introduction

The human organism goes through several phases: childhood, puberty, maturity or stabilization and aging. Aging is marked by several changes starting in the second decade of life. At first, these changes are barely noticeable, however, at the end of the third decade it presents important functional and/or structural changes (GOTTLIE *et al*., 2007).

Evidence indicates that many chronic diseases result from the interaction of genetic, environmental and lifestyle factors, being classified as modifiable, highlighting smoking, alcohol intake, eating habits, sedentary lifestyle, stress, and non-modifiable, such as heredity, gender, and age (CASADO, VIANNA, THULERL, 2009).

Osteoporosis (OP) is a multifactorial cause of skeletal disease, characterized by reduced bone mass and deterioration of the anatomical and structural integrity of the bone, leading to increased bone fragility and susceptibility to fractures. The group most affected by OP are elderly women whose decreased estrogen production after menopause accelerates bone loss (INDERJEETH, POLAND, 2014).

Among joint diseases, osteoarthritis (OA) is the most prevalent and progresses slowly over decades with episodes of pain until loss of joint function. Inconclusive studies indicate that bone changes can initiate or influence cartilage degradation. Despite many efforts, to date there is no cure for OA and available treatments, pharmacological and non-pharmacological, work to reduce symptoms, mainly pain, inflammation and immobility (HENROTIN *et al*., 2014; TONGE, PEARSON, JONES, 2014).

Nutraceuticals are substances that can act as adjuvants in the prevention and treatment of chronic diseases, especially OA. The term nutraceutical comes from the combination of the words “nutrition” and “pharmaceutical”. It corresponds to food or product that provide health benefits and, according to definition and regulatory laws, are devoid of adverse effects. Collagen (HC) is recognized as a safe food with minimal adverse effects, whose amino acid composition has high levels of glycine and proline, which, when well metabolized, accumu-

lates, preferentially, in bone (type I) and cartilage (type II) (HENROTIN *et al*., 2014).

Both aging and poor diet can affect the body's demand for collagen. These changes are not noticeable in the early stages of life, but become evident in maturity, a phase in which food intake does not meet the recommended needs for both energy and macro and micronutrients (FRANZEN; SANTOS; ZANCANARO, 2013).

Also at this stage, the chances of developing bone and joint dysfunction are greater. Balanced nutrition is essential not only to prevent chronic diseases, but also to maintain the health of the body and ensure its proper functioning (COSGROVE *et al*., 2007).

Although auxiliary methodologies for OA treatment can significantly relieve the pain of OA, they have disadvantages such as low potency, low tolerability, and a large dosage. The consumption of various forms of collagen, such as native (undenatured) collagen type II (NCTII), has also been studied (LUO *et al*., 2022) for their potential benefits during OA management in pre-clinical and clinical studies, demonstrating positive results with substantially lower therapeutic doses (CROWLEY *et al*., 2009; LUGO, SAIYED, LANE, 2016).

Therefore, the objective of this study was to carry out a systematic review of the literature on the action of collagen on bone and cartilage tissue and its therapeutic purposes in osteoporosis and osteoarthritis.

Methods

A meta-analysis of pre-existing scientific literature was carried out, searching for scientific articles that had as their object of study the action of collagen on cartilage and bone, in addition to possible therapeutic support in cases of osteoarthritis and osteoporosis.

The databases PubMed, MEDLINE; LILACS and SciELO, and the descriptors used for research were collagen (hydrolyzed and native), combined with osteoporosis, osteoarthritis, bone, cartilage, aging, intake and supplement. The search period was from January 1994 to October 2023. The review was carried out from July to October 2023.

The inclusion criteria were: experimental articles, in English and Portuguese, published between January 1994 to October 2023, with the object of study being the action of collagen on bone and cartilage tissue, as well as its therapeutic purposes in osteoporosis and osteoarthritis.

Notes, and clinical case reports, these were excluded; that involved other etiologies of bone and/or joint diseases; who associated drugs with oral collagen supplementation; and duplicate articles, indexed in more than one of the selected databases.

The process for including articles in the study was carried out by reading the titles and abstracts, by three independent reviewers, who applied the inclusion and exclusion criteria. In case of disagreement, the study was selected for evaluation of the full text.

Results

The initial search based on the combination of terms identified 252 articles. After checking for duplication, 63 were excluded. Considering the titles and abstracts for a broad selection of likely works of interest, 72 articles were excluded, leaving 68 records identified, 47 in the PubMed database and 21 in the MEDLINE, LILACS and SciELO databases.

Articles that met the eligibility criteria were retrieved for reading the full text, aiming for new evaluation. At this stage, another 59 publications that did not meet the objective of this research were excluded.

Sixteen experimental articles were then defined as the basis for discussion of this review, nine of which were research studies with human models, five with animal models and two articles that evaluated, respectively, in vitro models (human cells) and animal models.

Table 1 presents some of the main experimental data of the articles included in this systematic review.

Discussion

Due to the progressive decrease in the adaptive responses of the elderly body in relation to environmental factors, it is possible that aging is accompanied by chronic diseases, which generally require continuous treatments, functional limitations, and some level of dependence. In several countries, population aging receives attenti-

Table 1. Distribution of articles according to author, sample, method, results and conclusion

Author	Sample	Method	Results	Conclusion
Hays *et al.*	Humans nine women elderly (between 65-85 years old)	Whey protein (0.85 g/kg/weight/day) 15 days CH (0.81 g/kg/weight/day) 15 days	Whey protein group: Less weight; greater excretion of nitrogen CH Group: Weight maintenance; less excretion of nitrogen	Maintenance of weight and muscle mass
Guillerminet *et al.*	*In vivo* Mice	CH 10 or 25 g/kg/weight/day 4-12 weeks Bone mineral density	Greater growth and differentiation of osteoblasts; increased growth and differentiation of osteclasts	Osteoprotective action
Jackie *et al.*	Rats, six groups	CH 50 mg; 100 mg, control (gelatin) Sample of the femur and spine	Supported load four times greater; higher percentage of bone protein; greater bone mineralization	Greater conservation of bone composition and strength
Kim *et al.*	*In vitro* Human cells	COL1A1 gene	Enhancement of osteoblastic differentiation by gene expression	Osteoprotective action
	In vivo Rats	CG 150; 500 mg/kg/weight/day 12 weeks Lumbar vertebrae	Increased bone mineral density	
Clark *et al.*	Humans 147 athletes	CH 10 g placebo (xanthan gum) Inflammation, mobility and joint pain	Significant improvement in pain (knee arthralgia)	Pain reduction and cartilage protection
Sugihara *et al.*	Humans five individuals	CH 8 g Blood samples 0.5; 1; two; 4 hours Pro-Hyp; Hyp-Gly	Increased AA, di- and tripeptides in peripheral blood	Cell proliferation and growth and cartilage protection
Hartog *et al.*	Rats	CH 12.5; 25; 50 mg Three consecutive days Inflammation induced in the ear blood sample	Higher concentration of glycine in plasma; reduction of edema; proinflammatory cytokines	Potential pain reduction (hip and knee)
Shigemura *et al.*	Humans healthy volunteers n = 4	Different doses of CH/kg/weight (30.8; 153.8; 384.6 mg/kg/weight) Blood sample before, 15, 30, 60, 120, 240, 360 minutes after ingestion	Dose-dependent increase of 6.43; 20.17; 32.84 nmol/ml in plasma Hyp concentration, respectively.	Increased plasma Hyp and amino acid absorption potential
Bruyère *et al.*	Humans 200 patients over 50 years old	CH 12 g Placebo (gel capsules)	6th month of treatment, improvement in symptoms according to the VAS-D scale; tolerability	Efficacy and safety of supplementation
Marone *et al.*	*In vitro* and *in vivo*	Type II collagen	A dose-dependent 90-day sub-chronic toxicity study revealed no pathologically significant changes	Safety of supplementation

Bagchi *et al.*	Humans n = 5 Patients between 58 and 78 years old	Type II collagen 10mg Placebo	42-day treatment, significant pain reduction; tolerability	Efficacy and safety of supplementation
Crowley *et al.*	Humans 52 patients Osteoarthritis (OA)	Type II collagen 40mg G+C capsules	90 days of treatment; reduction of all assessment (OA)	Efficacy and safety of supplementation
Lugo *et al*.	Humans 191 volunteers	Type II collagen Placebo Glucosamine + chondroitin (G+C)	180-day period; improvement of knee joint symptoms	Efficacy and safety of supplementation
Luo *et al*.	Humans 85 individuals Osteoarthritis (OA)	Type II collagen Placebo Glucosamine + chondroitin (G+C)	12 weeks of investigation; better effect and safety profile for OA patients	Efficacy and safety of supplementation
Srivastava et al.	Humans 60 patients Osteoarthritis (OA)	Type II collagen Placebo Glucosamine + chondroitin (G+C)	12-week study; improvement of the joint health and quality of life	Efficacy and safety of supplementation

on through new forms of treatment, in addition to preventive care that adjusts to the profile of the elderly, in order to avoid unnecessary hospitalizations with a consequent increase in healthcare costs (LUIZ, FARIA, 2017).

It is important to highlight that the aging process is not directly related to disabling diseases, but chronic degenerative diseases are often associated with the effects of age (ALVES *et al*., 2007).

OP, as it is asymptomatic, is often underdiagnosed and undertreated. The consequences of osteoporotic fractures include increased morbidity and mortality and impact social, emotional, and financial quality of life. Among the fractures with the greatest impact on mobility, the hip fracture is considered the most devastating type of osteoporotic fracture, as, in addition to the loss of mobility, it increases the need for long-term care (ALVES *et al*., 2007; LEWIECKI, 2009).

Other types of fractures can also impact quality of life, such as multiple or severe vertebral fractures, associated with significant pain, reduced lung function, decreased height, and kyphosis, which can restrict movement and increase the risk of further falls and fractures (LEWIECKI, 2009).

Bone is a mineralized and complex tissue, whose main function is to resist mechanical forces. To this end, it presents specific characteristics, not only in the quantity of bone tissue, but also in its quality, specifically in terms of geometry and shape, trabecular microarchitecture, deposition of minerals and the quality of collagen in the organic matrix (LOTZ *et al*., 2013).

OA is a degenerative joint disease, characterized mainly by a slow and progressive destruction of cartilage with narrowing of the joint space, formation of osteophytes, bone sclerosis and synovitis (STAINES *et al*., 2013; SUANTAWEE *et al*., 2013) and its exact cause is still unknown (VERMEIJ *et al*., 2014).

It generally affects adults of middle age and although it is one of the main causes of chronic disability, conventional therapeutic treatments are still limited, as the results are minimal and prolonged use of these drugs can cause toxicity. Because of this, the dietary supplement industries have been investing increasingly in the development of supplements with the aim of delaying the disease, directly providing natural components to inhibit or reinforce the role of biological mediators to preserve the structural integrity of the joint (VISTA, LAU, 2011).

The collagen molecule is basically composed of a repeated sequence of three amino acids (Gly-X-Y), where Gly is the amino acid glycine; X is

almost always proline and Y is hydroxyproline or hydroxylysine. In general, collagen contains about 30% glycine, 12% proline, 11% alanine, 10% hydroxyproline, and 1% hydroxylysine (PRESTES, 2013).

From a nutritional point of view, collagen is considered a protein of inferior quality, as there is a predominance of the described amino acids and minimal or absent quantities of the majority of essential amino acids, such as tryptophan, methionine, cystine and tyrosine (PRESTES, 2013; ROMAN, SGARBIERI, 2007).

Despite this, its nutritional importance is established by its atypical amino acid profile that stimulates collagen synthesis in cartilage and the extracellular matrix of other tissues (ROMAN, SGARBIERI, 2007).

Collagen, like other ingested proteins, is not absorbed as collagen. Most protein digestion, around 80%, occurs in the duodenum and jejunum through the action of pancreatic juice and only 10-20% in the stomach through the action of hydrochloric acid and pepsin (ROMAN, SGARBIERI, 2007; FRENHANI, BURINI, 1999).

In the small intestine, luminal hydrolysis of proteins and polypeptides occurs into free amino acids (AA) and small peptides through the action of enteropeptidase, which, at neutral pH, activates trypsinogen and trypsin, which, in turn, promotes the activation of other propeptidases of pancreatic juice. AA and small peptides are hydrolyzed by brush border peptidases to AA, di- and tripeptides, which are absorbed mainly by the proximal jejunum by simple diffusion, facilitated diffusion or active transfer by co-transport. AA is intended for numerous functions, including the synthesis of collagen itself (FRENHANI, BURINI, 1999).

Experiments with rats to quantify the distribution of radioactive collagen peptides, indicated that after intestinal absorption, peptides from HC accumulate, preferentially, in cartilage and bones (OESSER *et al*., 1999).

In connective tissue, type I collagen or tropocollagen is the most abundant and from it partially hydrolyzed collagen (gelatin) and hydrolyzed collagen are obtained. The difference between hydrolyzed collagen and gelatin is that hydrolyzed collagen dissolves in water or brine, making it easy to digest and absorb, as well as the production of collagen by the body from free amino acids (PRESTES *et al*., 2013).

The most important characteristic of hydrolyzed collagen is the prevalence of glycine and proline in its composition. These amino acids are essential for the stability and regeneration of cartilage (SILVA, PENNA, 2014).

Although OA and OP are diseases related to skeletal dysfunction, epidemiological surveys rarely associate one disease with the other. On the contrary, the presence of one can be considered a protective factor for the other, as the increased bone conformity in OP would keep the articular cartilage preserved (BOBINAC *et al*., 2013).

There are few reports on OA in the early stages, however, recent investigations have reported serious microscopic changes in the cartilage bone in advanced stages of OA, such as increased volume of subchondral bone, low bone mineralization and mechanical stiffness, as well as considerable deterioration in the articular cartilage, suggesting development of OA in patients with OP and that treatment of OP may help prevent the progression of OA (KAMIMURA *et al*., 2013).

A clinical trial was carried out in order to test supplementation in women aged between 65 and 85 years. The nitrogen balance was compared from the supplementation of two protein compounds: "whey protein" and hydrolyzed collagen. Although the amount of protein was the same for both supplements, women who consumed the whey-based supplement experienced a reduction in weight without changes in their body profile, suggesting a loss of lean mass. For women who took the collagen supplement there were no significant changes in body weight; Furthermore, nitrogen excretion was lower for hydrolyzed collagen than for whey, maintaining nitrogen balance and lean mass (HAYS *et al*., 2009).

Also, according to the aforementioned study, the data from the aforementioned study, combined with previous estimates of protein requirements in the diet of older people, strongly indicate that the current recommended dietary intake (RDA) may be inadequate or marginal, even in normocaloric diets They also observed that, although hydrolyzed collagen is deficient in essential amino acids, associated with a diet with adequate amounts of protein could promote nitrogen balance (HAYS *et al*., 2009).

Type I collagen makes up 25% of the body's total protein and 80% of connective tissue in humans. Type I collagen synthesis also plays an important role in osteoblast differentiation, improving bone mineral density, bone mineral content and increasing the amount of type I collagen in the bone matrix (TAKEDA *et al*., 2013).

Bone loss is due to an imbalance between bone formation and reabsorption, especially in postmenopausal women. This imbalance is characterized by excessive activity of osteoclasts over osteoblasts, inducing increased bone remodeling. For the effect of administering type I collagen to be positive, the source needs to be hydrolyzed (GUILLERMINET *et al*., 2010).

In the in vivo studies with mice, they observed that proteins are essential for bone health and prevention of OP. Collagen modulates bone formation and mineralization of the bone matrix with an increase in the growth and differentiation of osteoblastic cells and a reduction in osteoclastic cells (GUILLERMINET *et al*., 2010).

All collagens tested were able to increase osteoblast activity. These results, as well as previous observations, show that the structure and quantity of peptides originating from collagens after oral administration depend not only on the origin of the collagen, but on the molecular size of the hydrolyzed collagen, suggesting that not every collagen molecule interacts with cells bones (GUILLERMINET *et al*., 2010).

A clinical study demonstrated hydrolyzed collagen contributed to greater bone conservation, composition, and strength. The results of collagen application to neutralize the effects of ovariectomy on bone mass, biomechanical strength, protein content and serum osteocalcin levels were evaluated in six groups of eight rats: three ovariectomized groups, a negative control group submitted to sham surgery and two intact (JACKIE *et al*., 2010).

One month after surgery, the rats received a diet supplemented with gelatin (control) or HC at two levels, (1) an amount equivalent to five times that recommended for humans (10g/day), and another (2) with a level (ten times) largest, according to the following criteria: two intact groups, gelatin and HC (ten times); three ovariectomized groups, gelatin, HC (five times) and HC (ten times); a group with HC sham surgery (ten times) (JACKIE *et al*., 2010).

After eight weeks, samples from the femur, spine and blood were evaluated. The group that received the highest dosage of HC supported a load four times greater, in addition to having a higher percentage of bone protein, mineral content and osteocalcin content than the other groups (JACKIE *et al*., 2010).

The group with the highest level of supplementation (OVX-HC10) showed divergence in osteocalcin levels. Regarding the results of alkaline phosphatase, an increase was identified in this group, but the relevance in this experiment was limited, as the increase in alkaline phosphatase may be associated with enzymatic activity as a compensatory reaction to surgery (JACKIE *et al*., 2010).

Some authors consider bone loss to be a non-uniform process, as cancellous bone, the main component of the vertebrae, represents a greater risk than cortical bone, the main component of the femur. Therefore, the lumbar vertebrae play a key role in monitoring OP (KIM, KIM, LEEM, 2013).

In their study, they demonstrated functional effects of hydrolyzed collagen in vitro and in vivo. In the in vitro test, they observed that HC increases osteoblastic differentiation in human cells through the expression of the COL1A1 gene involved in collagen synthesis; in vivo, they found a significant increase in bone mineral density in the lumbar vertebrae, as well as in the entire body of ovariectomized rats (OVX) treated for 12 weeks with diets containing 0.3% and 1% HC, 150mg/kg, and 500mg/ kg, respectively (KIM, KIM, LEEM, 2013).

These results suggest that HC exerts an osteoprotective action and may be a therapeutic alternative for the treatment and prevention of OP. Elevated levels of bone markers in OVX rats may mask additional effects of HC treatment. Furthermore, measurements performed at a single time point may not determine subtle effects between treatment and bone marker responses (KIM, KIM, LEEM, 2013).

The positive effect of protein on bone constitution is related to the composition, that is, 50% of the bone is made up of collagen and the other half is made up of calcium. Therefore, a diet inadequate not only in calcium but also in protein would limit bone reconstruction (MONTILLA, ALDRIGHI, MARUCCI, 2004).

Researchers highlighted in their research a concept of a cause other than aging for OA, whose therapeutic proposal must cover all aspects of the disease. The pathogenesis of OA results from inflammatory and mechanical factors: inflammatory, in chondrocyte- and synoviotic-mediated responses; mechanics, associated with movement and physical strength concentrated mainly in the joints (REZENDE, GOBBI, 2009; REZENDE, CAMPOS, 2013).

OA would be the result of joint inflammation in an attempt to correct abnormal mechanical stress. Furthermore, for them, inflammatory responses are greater in patients with OA and increased with aging, while mechanical responses comprise a combination of physiological and genetic factors, and in both, obesity would be an aggravating factor (REZENDE, GOBBI, 2009; REZENDE, CAMPOS, 2013).

Obesity increases the load on the joint and activates the production of pro-inflammatory adipokines in receptors present on the surface of chondrocytes, osteoblasts, synovial and subchondral membranes (KING, MARCH, ANANDACOOMARASAMY, 2013).

As postulated by experts, there is a consensus that the effects promoted by the ingestion of collagen peptides are related to their hydrolyzed form. For the authors, dietary and pharmacological supplementation of HC are justified because they have beneficial biological functions far beyond reducing pain in patients with OA (ZAGUE *et al*., 2013).

In addition to being involved in the synthesis of cartilage matrix, some collagen peptides exhibit antihypertensive and cardioprotective activity, through the regulation of nitric oxide and the intercellular adhesion molecule and inhibition of the angiotensin I converting enzyme, in addition to antioxidant activities in different oxidative systems (ZAGUE *et al*., 2013).

Researchers followed 147 athletes for 24 weeks. Although there was no evidence of joint disease, it was a group considered to be at high risk. The individuals were divided into two groups, one group received a formulation of 25ml of liquid containing 10g of hydrolyzed collagen, and the other group received a placebo consisting of 25ml of liquid with xanthan gum (CLARK *et al*., 2008).

Parameters including inflammation, mobility and joint pain were evaluated; pain when walking, standing, at joint rest, carrying objects and when lifting. There was a significant improvement in the group supplemented with collagen in terms of pain, in all parameters evaluated, especially in the subgroup with knee arthralgia (CLARK *et al*., 2008).

Some authors suggested that collagen stimulates collagen biosynthesis in chondrocytes, joint cells responsible for the synthesis, organization and maintenance of the ECM extracellular matrix (OESSER, SEIFERT, 2003).

Changes in the composition of the ECM cause collagen turnover that stimulates chondrocyte activity, inducing synthesis and continuous remodeling. Based on experiments and review of the literature, it was concluded that hydrolyzed collagen administered orally could accumulate in cartilage, in addition to stimulating a significant increase in the synthesis of ECM macromolecules by chondrocytes (BELLO; OESSER, 2006).

With the hypothesis that some amino acids play active roles in bone tissue, the levels of hydroxyproline (Pro-Hyp) and hydroxyglycine (Hyp-Gly) present in the blood of five healthy individuals after oral ingestion of HC were evaluated. The volunteers ingested 8g of CH dissolved in 100ml of water and blood samples were collected beforehand; 30 minutes, 1, 2 and 4 hours after ingestion (SHIGEMURA *et al*., 2014).

The concentration of Hyp-Gly and Pro-Hyp in plasma reached its peak after one hour, in a proportion of 6.3% to 22.1%, respectively. After oral ingestion of HC, not only amino acids but di- and tripeptides are assimilated and remain for a relatively long period in human peripheral blood. It is estimated that these peptides promote cell proliferation and growth, synthesis of hyaluronic acid in cultures of dermal fibroblasts and synovial cells, in addition to a chondroprotective effect on articular cartilage (SHIGEMURA *et al*., 2014).

A limitation of this study refers to the lack of standardization in relation to sporting activities. More reliable results could be obtained by including athletes involved in similar sports, for example, football or basketball (SHIGEMURA *et al*., 2014).

With the aim of evaluating the anti-inflammatory potential of glycine, HC was administered in different amounts, inducing inflammation in the ear

of mice. CH was administered daily via oral tube in the following amounts: control (0); 12.5, 25 and 50mg for three consecutive days, and inflammation was induced on the third day by intra-dermal injection of zymosan (HARTOG *et al*., 2013).

The plasma level of glycine, in the blood samples collected, increased according to the concentration of CH applied, suggesting its ability to neutralize locally induced inflammation, in accordance with the reduction of ear edema, as well as the reduction of IL-6 and lipopolysaccharide (LPS) production. Glycine is a non-essential amino acid found in many different proteins and is one of the main structural units of collagen, making up about 30% of the amino acids (HARTOG *et al*., 2013).

The effects of glycine in inhibiting the expression of pro-inflammatory cytokines have been studied in vitro and confirmed in different animal models, mainly for beneficial effects in reducing pain in hip and knee OA (HARTOG *et al*., 2013).

In addition to glycine, there is a relationship between collagen intake and hydroxyproline levels in human plasma. Hydroxyproline is an amino acid present specifically in collagen, and studies have demonstrated that its presence in plasma inhibits the mineralization of chondrocytes and modulates the expression of the Runx1 (runt-related transcription factor 1) and osteocalcin genes, stimulating the production of hyaluronic acid in cultures of synovial cells and increases the production of skin fibroblasts in mice (SHIGEMURA *et al*., 2014).

In order to estimate an effective dose for beneficial effects on human health, the concentration of Hyp in human plasma from different doses of CH with an interval of one week between each intake was measured. Four healthy adults, with a mean age of 27 years, ingested 2, 10, and 25 g per 65 kg of body weight of HC and venous blood samples were collected before, 15, 30, 60, 120, 240 and 360 minutes after administration (SHIGEMURA *et al*., 2014).

According to the analyses, the concentration of Hyp peptides increased in a dose-dependent manner from 30 minutes after ingestion and reached a maximum level after two hours, and although the Hyp level reduced to two thirds of its maximum six hours after ingestion, it was still significantly higher than before HC administration. The results showed that higher doses of CH cause an increase in the concentration of Hyp in plasma and the absorption potential of these amino acids (SHIGEMURA *et al*., 2014).

Some authors evaluated the efficacy and safety of HC supplementation in a randomized, double-blind study with 200 patients of both sexes, aged 50 years or over and who had joint pain (BRUYÈRE *et al*., 2012).

For six months, half of the group of individuals received a daily dose equivalent to 1,200mg of HC and the other half received a placebo (gel capsules). In terms of safety and tolerability, no difference was observed between the placebo group and the HC group (BRUYÈRE *et al*., 2012).

Regarding the clinical response, in the third month of treatment there was no significant difference, however, in the sixth month, the improvement was significantly greater in the group that ingested the HC capsules (BRUYÈRE *et al*., 2012).

Despite expectations regarding the positive results of studies (BELLO; OESSER, 2006; SCHADOW *et al*., 2013) concluded in their clinical trials with radioactive proline in in vitro models that collagen, even at high doses (10mg/mL), does not exert a stimulatory effect on collagen biosynthesis by human cartilage, regardless of the degree of OA alteration. Divergent results may occur between studies due to differences in applied analytical methods, species, age, and joint health.

Furthermore, determining the incorporation rate of radioactive proline without specific separation of total proteins does not reflect the true rate of collagen synthesis, since the enrichment of proline in collagen compared to other proteins is not attributed (SCHADOW *et al*., 2013).

However, it was stated that there may be collagen hydrolysate preparations with therapeutically active peptides, but exhaustive studies are needed, as well as clinical trials, before they can be applied as nutraceuticals (SCHADOW *et al*., 2013).

In addition, other authors highlighted the good tolerability and safety in the use of type II collagen, with less than 5% of patients with adverse effects and significant results in the WOMAC and VAS indices in evaluations of 30, 60 and 90 days of use in patients with osteoarthritis of knee (MEHRA *et al*., 2019).

These data were also confirmed by other study (CROWLEY *et al*., 2009), demonstrating improvement in the various aspects of pain (VAS) when compared to type II collagen and G+C, also showing an improvement in the Lequesne's functional scale when compared to the control.

These results confirm once again what in vitro studies (BAGI *et al*., 2017) where rats submitted to partial meniscectomy had less joint wear and more function in the operated limb when using type II collagen compared with not using. The rats in the model showed less loss of cartilage matrix and less cartilage degradation when using the type II collagen.

Also, it was evidenced in the work carried out in horses with moderate to severe osteoarthritis, with symptoms such as difficulty walking, stiffness and swelling of the joint and joint pain, that the effectiveness of using type II collagen is superior in the treatment of OA in relation to the use of glucosamine and chondroitin (GUPTA *et al*., 2009).

Positive results started to be observed from 30 days after the beginning of the administrations and after 5 months of treatment the horses were very active and performing their daily activities normally (BAGCHI *et al*., 2002).

The study was carried out with five women between 58 and 78 years who suffered from significant joint pain and a daily dose of 10 mg/day of type II collagen was used for 42 days. This can lead to a significant reduction in pain, morning stiffness, stiffness after rest periods, pain that worsens with the use of the affected joint, and loss of range of motion and joint function (BAGCHI *et al*., 2002).

In tests in female rats, the capacity for acute oral toxicity by the administration of oral type II collagen was studied in relation to mortality, signs of toxicity and changes in the behavior of these animals (MARONE *et al*., 2010).

After 14 days of observation and administration of 5,000 mg/kg, none of the rats showed changes with the use of the drug at this dosage. Thus, in addition to being an effective therapy, the use of type II collagen seems to be safe in its indicated doses (MARONE *et al*., 2010).

It was noticed, as a limitation, a small number of references found that met the criteria of a randomized controlled clinical trial, as well as access to confidence intervals and requested data.

However, the good results of the application of type II collagen in OA could be seen through the WOMAC and VAS questionnaires. Furthermore, the studies were carried out in patients with knee osteoarthritis, which limited our perception to the involvement of only one joint, despite this being the most incident of the pathology in the world.

Thus, it is relevant to produce more studies that assess the effectiveness of type II collagen in the treatment of osteoarthritis in order to constantly seek a better quality of life for the large number of patients who suffer from the disease.

Compared with the placebo, the NCTII collagen group had a significantly reduced total score after 4 weeks. Subsequently, after 12 weeks, the NCTII collagen group showed a gradual and greater reduction in the total score. A meaningful reduction of the total scores from baseline was seen in all treatment groups ($p < 0.05$) at the end of week 4, 8, and 12 (LUO *et al*., 2022).

The within-group analysis demonstrated a statistically significant difference in each group for all study visits compared with the baseline, with the extent of reduction increased with the longer treatment duration (LUO *et al*., 2022).

However, the intergroup analysis showed that while the absolute change in the WOMAC-P scores for the association of glucosamine and chondroitin (G+C) was statistically significant compared to the placebo for all study visits. No statistically significant difference was observed for the absolute change in the scores between collagen and G+C groups on all visits, which shows that NCTII collagen was as effective as the positive control in relieving joint pain (LUO *et al*., 2022).

After a treatment period of 12 weeks, a significant difference was seen within all quality-of-life domains and the VAS score compared to the baseline. Furthermore, the absolute change within the NCTII collagen was statistically significant compared to that in the placebo for 4 of the 5 domains and the VAS score, except for the "anxiety or depression" domain (LUO et al., 2022). The same trend was observed within the G+C group scores; however, collagen demonstrated a more significant change in the "usual activities" than in the G+C group. Overall, NCTII was able to significantly improve the joint health and quality of life of participants, compared to placebo and to G+C (LUO et al., 2022).

Conclusion

Hydrolyzed collagen has a positive therapeutic function in osteoporosis and osteoarthritis, with a potential increase in bone mineral density, a protective effect on articular cartilage and mainly in symptomatic relief in pain.

Although there is no consensus in the researched scientific literature on the dosage of hydrolyzed collagen to be administered, with supplementation of 8g daily an increase in the concentration of glycine and proline in plasma is observed and doses equivalent to 12g daily promote significant improvement in the symptoms of osteoarthritis and osteoporosis. As an alternative to high dosages of hydrolyzed collagen, it is possible for consumers to supplement themselves with a source of native type II collagen or, in a shorter form, type II collagen, such as NCTII, whose application depends on only 40mg per day, facilitating adherence of individuals, both healthy and those with symptoms of joint and bone diseases.

In the other hand, the use of type II collagen alone (LUO et al., 2022) or in combination with other products, such as manganese ascorbate has also shown statistical improvements in the management and treatment of OA compared to placebo (DAS, HAMMAD, 2000).

Other studies were conducted to evaluate the efficacy of type II collagen LUGO, J.P.; SAIYED, Z.M.; LANE, 2016; SRIVASTAVA *et al*. 2023). Also, it was observed that the WOMAC score for the undenatured type II collagen was reduced by 33% from baseline. In comparison, it was reduced by only 14% in participants in the G+C group after a treatment period of 90 days, the reduction between both groups being statistically significant (CROWLEY *et al*., 2009).

Based on these trials, it can be concluded that type II collagen (NCTII) has a proven efficacy towards OA symptomatic relief better than G+C, as was seen due to the statistically comparable reduction in scores. Additionally, it is effective at low doses and more potent than G+C (LUO *et al*., 2022).

Therefore, it is possible to define, from this analysis, that NCTII plays a very important role in individuals who suffer from the aforementioned pathophysiological conditions and live with mobility difficulties and pain, based on its intended use proven in different trials, with authorized patents across the globe, demonstrating an unique effect compared to other sources within the first month of supplementation, that is, a significant effect in 28 days of treatment.

However, more studies are needed to determine the pathogenic factors involved in osteoporosis and osteoarthritis, their early diagnosis, and at what stage of life the start of supplementation and the appropriate dosage would be recommended to achieve significant therapeutic potential.

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Validation of the translation of the Barthel index (BI) in Bosnian language for assessment of activities of daily living and outcomes among post-ischemic stroke patients in family medicine teams

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Abstract

Background: The Barthel Index (BI) for assessing activities of daily living (ADL) has not been translated into Bosnian, nor has a study been conducted on a sample of post-ischemic stroke (IS) patients in family medicine teams to examine the instrument's reliability and validity. Thus, the aim of this study was to examine the reliability and construct validity of the Bosnian version of the BI among patients after IS.

Study design: Cross-sectional descriptive study provided among post-ischemic stroke patients in family medicine teams, using BI in Bosnian language.

Methods: Translation of BI in Bosnian is performed according to the proposed set of standardized guidelines. The respondents were patients who are in the registry of patients with ischemic stroke in eight family medicine teams in Sarajevo Canton, Bosnia and Herzegovina. The examination and survey of the respondents were carried out in the outpatient clinic, and for immobile patients in home conditions. The collected data were tested for reliability and validity based on statistical models proposed in the existing literature.

Results: We included 184 patients for construct validity and a subsample of 40 patients for reliability analyses. We observed adequate reliability (intraclass correlation coefficient 0.997 for rater 1 and 0.995 for rater 2) and internal consistency (Cronbach's alpha 0.84 for rater 1, and 0.83 for rater 2). There were adequate correlations between the Barthel Index and the mRS (rho was -0.706, $p<0.001$ for rater 1, and -0.723, $p<0.001$ for rater 2).

Conclusions: The Bosnian version of the Barthel Index presents adequate test–retest and inter-rater reliability, acceptable internal consistency, and valid construct for measuring the activities of daily living (ADL) among post-ischemic stroke patients in family medicine teams.

Keywords: ischemic stroke, Bathel index, validity, family medicine, Bosnian

Introduction

Stroke is a major cause of disability worldwide. Every year, over 12.2 million new strokes occur in the world, of which over 7.6 million are new ischemic strokes (IS). In Europe, approximately 1.1 million people suffer from a stroke annually. About 26% of people after IS remain dependent in basic activities of daily living (ADL), while 50% have reduced mobility due to hemiparesis (1-5).

The family physician has a key role in monitoring the recovery of patients who have experienced an IS and an effective assessment of ADL can provide evidence to the family physician to make decisions about further treatment and care of people after IS.

There are many functional outcome assessment scales that can be used after IS, and for the purpose of ADL assessment they are used: Functional Independence Measure (FIM), Stroke Impairment Assessment Set (SIAS), Barthel Index (BI), Modified BI (MBI), Modified Rankine scale (mRS). The two most commonly used scales in stroke, but also in patients with other diseases and conditions, are BI and mRS (6-12).

The BI was first published in 1965 by Mahoney and Barthel, and was created to measure disability in patients whose rehabilitation affects the use of their limbs to perform daily life activities. There are currently two modifications of the original 10-item BI: the Collin and Shah versions. All three versions are used today both in clinical practice and in research. The ten individual items that make up the BI focus on activities of daily living. Each item is scored based on whether the person can perform the task or activity independently, with help, or is completely dependent. The points are distributed so that bathing and grooming for independence carry 5 points each. Feeding, dressing, bowels and bladder control, toilet use and stairs climbing 10 points each, and transfers (bed to chair and back) as well as mobility (on level surfaces) for independence carry 15 points each. The time required for a BI assessment is less than ten minutes and no formal training is required to perform any version of the BI. Instead, the examiner only needs to be familiar with the ten functional skills being assessed and the scoring system used. Points can be assigned through direct observation or from interviews with the patient, family, caregiver, or health care staff. The total score is calculated by adding up the individual scores, and ranges from 0 (total dependence) to 100 (total independence). BI allows the examiner to measure a patient's functional disability by quantifying their performance (9,10,12).

The range of results is interpreted so that 0-20 points are complete dependence, 21-60 severe dependence, 61-90 moderate dependence, 91-99 low dependence and 100 points independence in ADL. Most studies use a score of 60/61 (moderate dependence) as a cut-off point. BI for ADL assessment can be used in any adult with a decline in ADL performance, patients with neuromuscular or musculoskeletal conditions, and is especially recommended in the assessment of people with IS, Parkinson's disease, oncology patients, COVID-19 patients, patients admitted to intensive care units, and in the elderly (13-20).

The Rankin Scale was published in 1957 by Dr. John Rankin and is a global outcome scale where patients are assigned to one of five classes. The modified Rankin scale (mRS) describes various derivatives of the original. The 1988 version by Bonito, the most widely used, adds two categories: dead and asymptomatic. It was originally designed as a scale that assesses neurological deficits, and today it is considered more of a disability scale and serves to assess the remaining possibilities of functioning (21-24).

The aim of this study was to examine the reliability and validity of the Bosnian version of BI among patients after IS in family medicine.

Methods

The study was divided into two phases. First, the English version of the 10-item BI was translated into Bosnian. The Maryland State Medical Society owns the copyright to the BI, but may freely use it for non-commercial purposes (25, 26). The translated BI was then tested for validity and reliability in a cross-sectional, descriptive study.

For the purposes of this study, the questionnaire (BI) was translated from English to Bosnian according to accepted translation standards (27,28), independently by two family medicine specialists. Differences in the translation were analyzed in detail and resolved by consensus, and the translation was harmonized into the first version. Then the so-called back-translation into English by a professional English translator who is familiar with medical terms and semantics and had no insight into the original version of the questionnaire. An English-speaking doctor compared the translated version with the original English version of the questionnaire, in order to determine that the meaning of certain questions had not been changed.

After accepting the final version, the questionnaire was tested within a group of six doctors working in the family medicine service in order to prove the clarity of the questions.

Items of the Modified Rankin Scale (mRS) were also translated into Bosnian. The translated question has 7 choices for selection: the first one, which is coded as 0, refers to no signs or symptoms and the last one refers to death (the same as the original mRS scale).

The validity and reliability testing process was based on the data of 184 patients from the registry of patients suffering from ischemic stroke in eight family medicine teams in Sarajevo Canton, Bosnia and Herzegovina. The study was approved

by the Ethics Committee of the Sarajevo Cantonal Health Center (decision number 01-06-33-2-1459-2/23 dated June 8, 2023) and was conducted during the period June - December 2023.

All patients were informed about the study and signed an informed consent before inclusion. The inclusion criteria were patients who have recovered from IMU, are treated in one of the eight selected family medicine teams and voluntarily agree to participate in the study. Exclusion criteria were patients who had suffered a hemorrhagic stroke, were not treated in one of the eight selected family medicine teams, or did not sign an informed consent to participate in the study.

BI was applied voluntarily by two raters, one family medicine doctor and one nurse. The examination and survey of the respondents were carried out in the outpatient clinic, and for immobile patients in home conditions.

Assessment of test-retest reliability, was performed by filling out the questionnaire twice in a period of seven to eleven days with 40 patients by the same raters.

All data were statistical analyzed using the Statistical Package for the Social Sciences (SPSS) version 27 for Windows. The description of the variables was carried out using frequency tables, mean values and standard deviation (SD).

Reliability refers to the consistency of a measure. Psychologists consider three types of consistency: over time (test-retest reliability), across different researchers (inter-rater reliability), and across items (internal consistency) (29).

Assessing **test-retest reliability** requires using the measure on a group of people at one time, using it again on the same group of people at a later time, and then looking at test-retest correlation between the two sets of scores. Test-retest reliability coefficients (also called coefficients of stability) vary between 0 and 1, where 1 represents perfect reliability, ≥ 0.9 excellent reliability, ≥ 0.8 good reliability < 0.9, ≥ 0.7 acceptable reliability < 0.8, ≥ 0.6 questionable reliability < 0.7, ≥ 0.5 poor reliability < 0.6, < 0.5 unacceptable reliability, and 0 no reliability.

On this scale, a correlation of 0.9 (90%) would indicate a very high correlation (good reliability) and a value of 10% a very low one (poor reliability). This is typically done by graphing the data in a scatterplot and computing Pearson's r (Pearson Correlation Coefficient). Intraclass Correlation can also be used, and has the advantage it doesn't overestimate relationships for small samples. In general, a test-retest correlation of ≥0.80 is considered to indicate good reliability (30).

Inter-rater reliability is a measure used to examine the agreement between two raters on the assignment of categories of a categorical variable. It is measurement of the extent to which data collectors (raters) assign the same score to the same variable. Inter-rater reliability is an important measure in determining how well an implementation of some coding or measurement system works. A statistical measure of inter-rater reliability is Cohen's Kappa which ranges generally from 0 to 1.0 (although negative numbers are possible) where large numbers mean better reliability (0.81 – 1.00 almost perfect agreement). Values near or less than zero suggest that agreement is attributable to chance alone (31,32).

In results of the inter-rater analysis with Kappa we can find a 95% confidence interval. Some statisticians prefer the use of a weighted Kappa, particularly if the categories are ordered. The weighted Kappa allows "close" ratings to not simply be counted as "misses" (32).

A second kind of reliability is **internal consistency**, which is the consistency of people's responses across the items on a multiple-item measure. Like test-retest reliability, internal consistency can only be assessed by collecting and analyzing data. One approach is to look at a split-half correlation. A split-half correlation of +0.80 or greater is generally considered good internal consistency.

Validity is the extent to which the scores from a measure represent the variable they are intended to.

Validity, often called construct validity, refers to the extent to which a measure adequately represents the underlying construct that it is supposed to measure. Validity can be assessed using theoretical or empirical approaches, and should ideally be measured using both approaches.

Theoretical assessment of validity focuses on how well the idea of a theoretical construct is translated into or represented in an operational measure. This type of validity is called translational validity (or representational validity), and consists of two subtypes: face and content validity.

Translational validity is typically assessed using a panel of expert judges, who rate each item (indicator) on how well they the conceptual definition of that construct, and a qualitative technique called Q-sort.

While translation validity examines whether a measure is a good reection of its underlying construct, criterion -related validity examines whether a given measure behaves the way it should, given the theory of that construct. This assessment is based on quantitative analysis of observed data using statistical techniques such as correlational analysis and factor analysis.

Content validity is an assessment of how well a set of scale items matches with the relevant content domain of the construct that it is trying to measure. As with face validity, an expert panel of judges may be employed to examine content validity of constructs (33).

An Exploratory Factor Analysis (EFA) was performed with the principal component and maximum likelihood extraction methods, followed by varimax rotation. The sample adequacy was assessed by the Kaiser-Meyer-Olkin (KMO) and Bartlett's test of sphericity. Factors with eigenvalues higher than 1.0 and items with loadings greater than 0.4 were accepted (34).

Results

Study participants (184) were patients who had recovered from IS, aged from 37 to 95 years with an average age of 71.63 (SD±11.13) years. Most of the participants are female (n=126; 68.5%) and retirees (n=143; 77.7%). More than half of the participants had a college education (n=107; 58.2%), while almost a third had a university education (n=57; 31.0%). Most of the participants were married (n=149; 81.0%) and had a negative family history of stroke (n=150; 81.5%). About a third of the participants were smokers at the time of the research (n=61; 33.2%), and 5.4% consumed alcohol (Table 1).

Table 1. Demographic characteristics of patients

Age (mean ± SD) years	71.63 (±11.13)	37 to 95 years
Female n (%)	126 (68.5%)	
Retirees n (%)	143 (77.7%)	
College education n (%)	107 (58.2%)	
University education n (%)	57 (31.0%)	
Married n (%)	149 (81.0%)	
Negative family history of stroke n (%)	150 (81.5%)	
Smoking n (%)	61 (33.2%)	
Alcohol use n (%)	10 (5.4%)	

The translated BI was completed by two raters for 184 patients. The characteristics of the raters are shown in Table 2.

Table 2. Characteristics of raters

Rater	1	2
Sex	Female	Female
Age	48	43
Occupation	Family medicine doctor	Nurse
Work experience (years)	17	24

Forty out of the 184 included patients were submitted to test–retest reliability procedures. Correlation and Intraclass correlation tests were used for test-retest reliability. Results for rater 1 were Pearson correlation (r) 0.997 (p=0.000), ICC= 0.997 (95%CI: 0.995-0.999), *p*=0.000. Results for rater 2 were Pearson correlation (r) 0.996 (p=0.000), ICC= 0.995 (95%CI: 0.991-0.997), *p*=0.000 (Table 3).

Table 3. Test-retest reliability among raters

Rater	ICC	95%CI	p
R1	0.997	0.995-0.999	0.000
R2	0.995	0.991-0.997	0.000

The results inter-rater reliability among raters are reported in Table 4.

As a measure of concurrent validity correlation between BI-BH scores and mRS scores was calculated. Spearman's rho (Spearman's correlation coefficient) was -0.706, $p<0.001$ for rater 1, and -0.723, $p<0.001$ for rater 2.

Table 4. Inter-rater reliability among raters

Statistical measure	R1	R2
Mean (standard deviation) of the test	1.46 (±1,50)	1.44 (±1.46)
Correlation (Spearman's r, 95%CI)	0.999	
Cohen's Kappa	0.972 (0.95-1.00)	
Cohen's Kappa w	0.995 (0.99-1.00)	
Percent Agreement	97.80%	

Table 5. Item-Total Statistics for rater 1

Item	Scale Mean if Item Deleted	Scale Variance if Item Deleted	Corrected Item-Total Correlation	Squared Multiple Correlation	Cronbach's Alpha if Item Deleted
Feeding	84.33	106.770	0.418	0.263	0.84
Bathing	88.37	103.255	0.798	0.737	0.80
Grooming	88.09	113.704	0.655	0.637	0.82
Dressing	83.87	97.872	0.700	0.593	0.80
Bowel	82.86	128.078	0.250	0.151	0.84
Bladder	83.14	120.721	0.340	0.209	0.84
Toilet use	83.16	108.685	0.677	0.645	0.81
Transfers bed-to-chair-and-back	78.17	113.600	0.549	0.507	0.82
Mobility on level surfaces	78.42	103.002	0.548	0.364	0.82
Stairs climbing	84.61	93.491	0.590	0.435	0.82

Table 6. Item-Total Statistics for rater 2

Item	Scale Mean if Item Deleted	Scale Variance if Item Deleted	Corrected Item-Total Correlation	Squared Multiple Correlation	Cronbach's Alpha if Item Deleted
Feeding	84.3914	103.892	0.414	0.237	0.83
Bathing	88.4375	100.503	0.791	0.722	0.79
Grooming	88.0921	113.704	0.600	0.467	0.82
Dressing	83.9309	95.054	0.698	0.594	0.80
Bowel 2	82.9276	124.735	0.254	0.157	0.84
Bladder	83.2072	117.340	0.347	0.209	0.83
Toilet use	83.2237	105.960	0.666	0.628	0.81
Transfers bed-to-chair-and-back	78.2401	110.874	0.536	0.463	0.82
Mobility on level surfaces	78.4868	99.585	0.559	0.358	0.81
Stairs climbing	84.6711	90.576	0.592	0.429	0.82

Internal consistency was tested by Cronbach's alpha and was 0.84 for rater 1, and 0.83 for rater 2.

The results of the statistical measures for item total BI for rater 1 and 2 are presented in Tables 5 and table 6.

The dimensionality of the BI-BH instrument and its 10 items was analysed using principal components analysis (PCA). The Kaiser-Meyer-Olkin measure confirmed the sampling adequacy for the analysis, with a KMO value of 0,916. Bartlett's test of sphericity, $\chi^2(45) = 1819{,}999$, $p < 0.001$, indicated that the correlation structure was suitable for factor analysis. A maximum likelihood factor analysis was performed with a cut-off point of 0.40 and Kaiser's criterion of eigenvalues greater than 1, which resulted in a one-factor solution. This was determined to be the best fit for the data, with an eigenvalue of 7,137, accounting for 71,37% of the variance (see Figure 1).

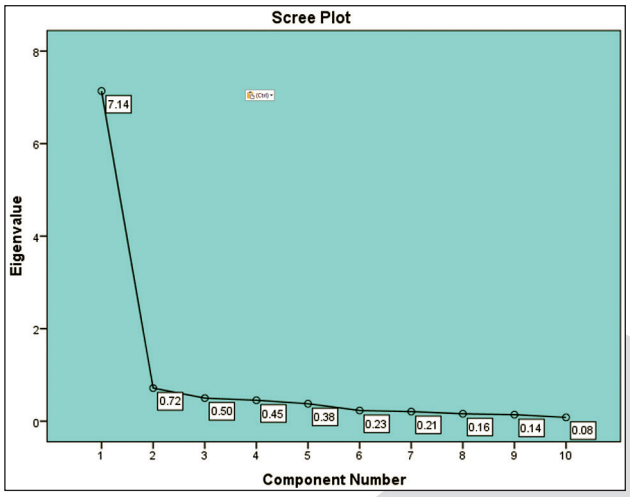

Figure 1. Eigenvalues of PCA Components of BI-BH reflects the analysis of the variance accounted for by each principal component in a data set

The results of the factor analysis, including the factor loadings, are presented in Table 7. These results indicate that all items have relatively high loadings (all above 0.7), suggesting that each item contributes significantly to the identified component.

Table 7. Exploratory Factor Analysis of the Items of the BI-BH

Item	Component
	1
Feeding	.724
Bathing	.831
Grooming	.842
Dressing	.896
Bowel	.842
Bladder	.798
Toilet use	.925
Transfers bed-to-chair-and-back	.867
Mobility on level surfaces	.887
Stair climbing	.818

Extraction Method: Principal Component Analysis.
a. 1 components extracted.

Discussion

This is, to the best of our knowledge, the first study that reports translation and validation of the BI in Bosnian language, and evaluate features of the Bosnian version of the BI in a sample of post-ischemic stroke patients in family medicine teams. Our research was motivated by the need for the family medicine team to monitor the recovery of patients who have experienced a stroke, and the effective assessment of ADL can provide evidence for making decisions about further treatment and care of people after IS. The objective of the present study was to examine the reliability and validity of a Bosnian translation of the BI. Translation and adaptation in Bosnian language was performed applying internationally recognized methods (27, 28), and under the supervision of a panel of experts that ensured the maintenance of the original meaning of the items.

The mean age of the 184 patients (126 women, 58 men) was 71.63 years (SD ±11.13), which is a similar result to the research by Ohura et al. (71.9, SD ±10.5) (35).

For test-retest reliability were used Correlation and Intraclass correlation tests. Our results for rater 1 were Pearson correlation (r) 0.997 (p=0.000), ICC= 0.997 (95%CI: 0.995-0.999), p=0.000, and for rater 2 were Pearson correlation (r) 0.996 (p=0.000), ICC= 0.995 (95%CI: 0.991-0.997), p=0.000. A correlation of 0.9 (90%) would indicate a very high correlation. In general, a test-retest correlation of ≥0.80 is considered to indicate good reliability (30). The Italian version of the BI translation had a discretely lower ICC value of 0.983 (95%CI: 0.967 – 0.992) (36).

The results inter-rater reliability among raters were: Correlation (Spearman's r, 95%CI) 0.999, Cohen's Kappa 0.972 (0.95-1.00), Cohen's Kappa w 0.995 (0.99-1.00) and percent agreement 97.80%. Large numbers mean better reliability (0.81 – 1.00 almost perfect agreement) (31,32). The high results indicates that scores of patients remain stable after repeated measurement and high reliability BI-BH, implying that the BI-BH scale adequately reflects the performances of the original English version.

Correlation between BI-BH scores and mRS scores was calculated. Spearman's rho (Spearman's correlation coefficient) was -0.706, p<0.001 for rater 1, and -0.723, p<0.001 for rater 2, and presented a strong correlation between the two scales. Spearman's a value is from -1 to 1, where +1 a perfect positive correlation between ranks, -1 a perfect negative correlation between ranks, and 0 means was no correlation between ranks. In the validation of the Italian version of the BI, the internal consistency was also calculated on the 180 included cases. Both Pearson and Spearman tests revealed a strong correla-

tion between each item and the entire scale (rho>0.7 p<0.01), just as shown in our results (36).

Internal consistency, tested with Cronbach's alpha, was 0.84 for rater 1, and 0.83 for rater 2. The original BI has been shown to have a Chronbach's alpha of 0.87 (10). The BI was translated and validated in many languages, such as Italian, Japanese, Persian, Turkish, Greek, Brazilian. The Italian translated version of the BI has been reported to have a Chronbach's alpha of 0.94 (p<0.001), Japanese 0.93, Turkish 0.88, Brazilian 0.97. Most of those studies have also been performed on stroke patients (36-41).

Certain authors argue that BI lacks a hierarchical structure and is not grounded in a specific theoretical dimension (42). This index is one-dimensional, according to the BI's authors (9). Our research yields comparable findings. The unidimensionality of the BI has been rejected by researchers in Turkey and Greece, leading to the adoption of a two-factor solution (39,40). A study conducted in Spain has determined that BI is most suitable for hospitalized patients in three specific dimensions (43). Several studies propose that it may be necessary to revise the unidimensional structure of the BI, as these factors may vary depending on the specific characteristics of the patient and setting of the research (44).

This study has some limitations. The research was carried out in the Sarajevo Canton, potentially constraining the applicability of the findings to other areas characterized by distinct healthcare systems or demographic attributes. The generalizability of the assessment's reliability may be limited to the specific settings of the study. The testing was conducted exclusively in a controlled setting, specifically involving family physicians and nurses. This limited scope may not accurately capture the range of assessments made by other health or social care professionals, or the potential complications that may arise from self-reported measures. The applicability of the BI-BH is limited to specific patient groups. This study specifically excluded patients who had experienced coma, hemorrhagic stroke, cognitive impairments, or psychiatric diseases. The BI-BH's applicability in these patient populations, which are frequently encountered in clinical settings, is constrained. The study focuses on the BI without comparing its effectiveness and reliability against other functional assessment tools in the same population. This could limit understanding of whether the BI is the most suitable tool for this purpose.

The aforementioned limitations indicate that although the study offers valuable insights into the efficacy of the BI-BH for a particular group of patients, additional research may be necessary to substantiate its applicability in a wider array of settings and demographics.

Conclusions

The Bosnian version of the BI presents adequate test–retest and inter-rater reliability, acceptable internal consistency, and valid construct for measuring the activities of daily living (ADL) among post-ischemic stroke patients in family medicine. Our research provides a new tool for assessing functional impairment in Bosnian-speaking post-stroke patients in primary care settings along the continuum of care.

Given that family medicine physicians have a key role in the follow-up of patients who have experienced a stroke, the BI-BH could be used in the assessment of ADL in these patients, but also in various future studies on IS in family medicine.

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Appendix 1.

The Barthel index (BI-BH)
Ime i prezime pacijenta / *Patient Name and Surname*: _____
Ime i prezime ocjenjivača / *Rater Name and Surname:* _____
Datum / *Date:* _____

Aktivnost *Activity*		**Rezultat** *Score*
Hranjenje ***Feeding***	0 = nesposoban / *unable* 5 = treba mu pomoć pri sječenju, razmazivanju putera i sl. / *needs help cutting, spreading butter etc.* 10 = neovisan / *independent*	
Kupanje ***Bathing***	0 = ovisan / *dependent* 5 = neovisan / *independent*	
Lična higijena ***Grooming***	0 = potrebna pomoć pri obavljanju lične higijene / *needs to help with personal care* 5 = samostalno umivanje, češljanje, pranje zuba, brijanje /*independent face/hair/ teeth/shaving*	
Oblačenje ***Dressing***	0 = ovisan / *dependent* 5 = potrebna mu je pomoć, ali može bar pola samostalno / *needs help but can do about half unaided* 10 = neovisan (uključujući dugmadi, feršlus, pertle i sl.) / *independent (including buttons, zips, laces, etc.)*	
Pražnjenje crijeva ***Bowels***	0 = inkontinentan (ili ima potrebu za laksativima) / *incontinent (or needs to be given enemas)* 5 = povremeno inkontinentan / *occasional accident* 10 = kontinentan /*continent*	
Pražnjenje bešike ***Bladder***	0 = inkontinentan ili plasiran kateter i nije sposoban da sam isprazni / *incontinent, or catheterized and unable to manage alone* 5 = povremeno inkontinentan / *occasional accident* 10 = kontinentan / *continent*	
Upotreba toaleta ***Toilet use***	0 = ovisan / *dependent* 5 = potrebna mu je neka pomoć, ali nešto može i sam / *needs some help, but can do something alone* 10 = samostalan (oblačenje/svlačenje/brisanje) / *independent (on and off, dressing, wiping)*	
Prelazak sa stolice na krevet i obratno ***Transfers (bed to chair and back)***	0 = nesposoban, nema balans sjedenja / *unable, no sitting balance* 5 = veća pomoć (1 ili 2 čovjeka, fizička), može da sjedi / *major help (one or two people, physical), can sit* 10 = manja pomoć (verbalna ili fizička) / *minor help (verbal or physical)* 15 = neovisan / *independent*	
Pokretljivost na ravnim površinama ***Mobility (on level surfaces)***	0 = nepokretan ili manje od 50 m / *immobile or < 50 yards* 5 = neovisan u invalidskim kolicima, uključujući uglove, > 50 m / *wheelchair independent, including corners, > 50 yards* 10 = hoda uz pomoć druge osobe (verbalne ili fizičke) > 50 m / *walks with help of one person (verbal or physical) > 50 yards* 15 = neovisan (može koristiti neku pomoć, npr. štap) > 50 m / *independent (but may use any aid; for example, stick) > 50 yards*	
Hod uz stepenice ***Stairs***	0 = nesposoban / *unable* 5 = potrebna pomoć (verbalna, fizička) / *needs help (verbal, physical, carrying aid)* 10 = neovisan / *independent*	

UKUPNI REZULTAT (*Total score)* (0-100)_____

0-20 = potpuna ovisnost, complete dependence
21-60 = teška ovisnost, severe dependence
61-90 = umjerena ovisnost, moderate dependence
91-99 = mala ovisnost, low dependence
100 = neovisnost u svakodnevnim životnim aktivnostima, independence in activities of daily living

Instructions for the authors

All papers need to be sent to e-mail: healthmedjournal@gmail.com

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Abstract

In this paper the instructions for preparing camera ready paper for the Journal are given. The recommended, but not limited text processor is Microsoft Word. Insert an abstract of 50-100 words, giving a brief account of the most relevant aspects of the paper. It is recommended to use up to 5 key words.

Key words: Camera ready paper, Journal.

Introduction

In order to effect high quality of Papers, the authors are requested to follow instructions given in this sample paper. Regular length of the papers is 5 to 12 pages. Articles must be proofread by an expert native speaker of English language. Can't be accepted articles with grammatical and spelling errors.

Instructions for the authors

Times New Roman 12 points font should be used for normal text. Manuscript have to be prepared in a two column separated by 5 mm. The margins for A4 (210×297 mm2) paper are given in Table 1.

Table 1. Page layout description

Paper size	A4
Top margin	20 mm
Bottom margin	20 mm
Left margin	20 mm
Right margin	18 mm
Column Spacing	5 mm

Regular paper may be divided in a number of sections. Section titles (including references and acknowledgement) should be typed using 12 pt fonts with **bold** option. For numbering use Times New Roman number. Sections can be split in subsection, which should be typed 12 pt *Italic* option. Figures should be one column wide. If it is impossible to place figure in one column, two column wide figures is allowed. Each figure must have a caption under the figure. Figures must be a resolution of 300 DPI, saved in TIFF format, width 10 cm min. For the figure captions 12 pt *Italic* font should be used. (1)

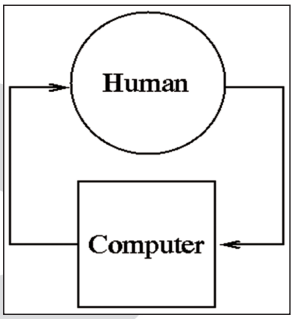

Figure 1. Text here

Conclusion

Be brief and give most important conclusion from your paper. Do not use equations and figures here.

Acknowledgements (If any)

These and the Reference headings are in bold but have no numbers.

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