Original paper

Elevated circulating Krüppel-like factor 4 level as a novel independent marker of the proatherogenic risk in patients with psoriasis: a preliminary study

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Abstract

Introduction: Krüppel-like factor 4 (KLF4) is a transcription factor of anti-inflammatory and anti-thrombotic properties not studied in psoriasis yet.
Aim: To analyze the clinical value of the serum KLF4 level in psoriatics and elucidate the interplay between disease activity, metabolic or inflammatory parameters and systemic therapy.
Material and methods: The study enrolled thirty-four psoriatics and fifteen healthy subjects. Blood samples were collected before and after twelve weeks of treatment with methotrexate or acitretin. Serum KLF4 levels were measured using immune-enzymatic method.
Results: Serum KLF4 levels in psoriatic patients did not statistically differ comparing to the controls ($p > 0.05$). However, in severe psoriasis, KLF4 was significantly higher than in healthy ones before treatment and normalized after treatment to baseline levels of controls ($p < 0.05$, $p > 0.05$, respectively). KLF4 positively correlated with body mass index ($p = 0.038$) but not with psoriasis severity, nor inflammatory or metabolic markers. Interestingly, many pro-atherogenic parameters were shown as variables independently predicting the levels of KLF4. No significant effect of three-month systemic treatment on KLF4 was found.
Conclusions: KLF4 may be a novel independent indicator of the proatherogenic risk in psoriatics, especially with a severe form or obesity.

Key words: psoriasis, Krüppel-like factor 4, proatherogenic risk, antipsoriatic treatment.

Introduction

Psoriasis is a chronic, relapsing, inflammatory skin disease, affecting 0.91% to 8.5% of adults and estimated 125 million people globally [1, 2]. According to current knowledge, psoriasis is considered as systemic disease associated with a variety of comorbidities, including cardiovascular disease (CVD), metabolic syndrome, diabetes mellitus (DM) or non-alcoholic fatty liver disease (NAFLD) [3, 4]. The main basis of comorbidity in psoriasis is not only chronic systemic inflammation, but also angiogenesis, genetic and immune background, and also oxidative stress, numerous bioactive molecules or proatherogenic lipid profile [3, 4]. Despite a lot of effort that has been put into searching for markers of psoriasis, the knowledge and clinical usefulness remain inconclusive. Therefore it is purposeful to search for pathogenetic pathways lead-ing to development of psoriasis and other inflammatory diseases, as well as newer biomarkers, helpful in predicting and inhibiting the early cardiometabolic disorders (CMDs) and better therapeutic outcomes.

The Krüppel-like factors (KLFs) are a family of transcription factors regulating diverse biological processes that include proliferation, differentiation, survival, growth, development and responses to external stress [5, 6]. KLFs are deoxyribonucleic acid-binding transcriptional factors with the competency to bind CACCC or GT box deoxyribonucleic acid (DNA) elements. Furthermore, they can act as activators or repressors of transcription [6, 7]. The regions of KLFs that do not participate in DNA binding are highly different and take part in protein-protein interactions. Three conserved zinc-type Cys2-His2 zinc fibers at the carboxy-terminal end of the KLFs bind

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to sites rich in GC in the promoter of target genes. The transactivation and the transrepression domains are located at the amino-terminus of KLF proteins. The name "Krüppel-like factor" was derived from the homologous Drosophila melanogaster protein Krüppel, which means "cripple" and is related to development [7]. The original member of the mammalian KLF family was discovered in red blood cell lineage and plays the main role in the β-globin expression and erythrocyte development. KLF subtypes have different biological functions and impact on numerous diseases. To date, 18 KLFs have been identified, which are divided into three main groups depending on structural features and transcription roles. They have gained a lot of attention due to their participation in metabolic pathways and maintenance of homeostasis of many organs [7]. KLFs are critical regulators of physiological systems including cardiovascular, digestive, respiratory, hematopoietic and immune ones. Their altered expression or activation has been linked with metabolic abnormalities, and has been shown to be involved in the development of diseases such as obesity, CVD, DM, NAFLD or cancer including their key role in the control of vascular inflammation [7, 8]. An increased KLF2 expression has anti-inflammatory and anti-atherosclerotic effects but also inhibits angiogenesis, which in turn is crucial in psoriasis [1, 7]. KLF4 was first described in 1996 as gut-enriched Krüppel-like factor (GKLF) [9]. It is expressed in immune cells, takes part in cell cycle arrest and suppresses apoptosis, however may change its function to pro-apoptotic [6]. Furthermore, it is mainly produced in epithelial cells, not only of the gastrointestinal tract, but also in the epidermis, taking part in differentiation and maintaining the skin barrier [10]. Segre et al. observed deaths of the KLF4 knockout mice due to the rapid loss of body fluids highlighting the role of KLF4 in regulating cell proliferation of the skin epithelium [11]. Madonna et al. noted a reduced expression in psoriatic keratinocytes comparing to healthy ones [12]. Kim et al. demonstrated a significantly higher elevated KLF4 expression in psoriatic lesional skin vs. non-lesional or healthy skin [10]. They suggested the outcomes as a physiological mechanism to maintain the homoeostasis but also due to the immunological interplay with interferon γ (IFN-γ) and tumour necrosis factor α (TNF-α), finally one of the key cytokine in psoriasis pathogenesis [10]. In 2012, Tsoi et al. demonstrated a list of notable genes from the newly identified psoriasis loci and one of them was KLF4 (9p.31.2) [13]. Recently, in 2020, an upregulation of the KLF4 gene in psoriatic keratinocytes has been proved [14]. Therefore the studies cited encourage to deepen research on KLF4 in psoriasis. Other link in that interplay is an important role of the factor in vascular wall biology. KLF4 exerts a function similar to KLF2 protective function in endothelial cells, but even more intensely through different mechanisms such as inhibition of endothelial inflammasome activation or cholesterol flux [8]. It stimu-

lates anticoagulant activity and fatty acid oxidation, followed by the activation of peroxisome proliferator activated receptors (PPARs), which are also highly relevant in psoriasis [7, 8]. Enhanced insulin resistance, vascular inflammation and diet-induced obesity have been demonstrated in animal models with myeloid-specific deletion of KLF4 [15]. Furthermore, a decreased KLF4 expression in monocytes of patients with coronary artery disease (CAD) was demonstrated [16]. Increased serum KLF4 levels were significantly related with the presence and severity of CAD [17]. It can be suspected that KLFs may be another link between CVD and psoriasis. Further, KLF4 induces the IL-17 expression which also takes part in immunological pathways of psoriasis [18].

There are promising reports on the use of various therapeutic methods (including mTOR pathway inhibitors, statins, retinoid agonists and PPARs) which, by modulating the expression of KLFs, may reduce the risk of CVD [19]. The described relationships of Krüppel-like factors with systemic diseases indicate that they might be potential risk indicators for the development of CMDs in psoriasis. However, there is a lack of data explaining the precise role of KLFs in that dermatosis.

The numerous biological functions of KLF4 mentioned above underline our desirability of deepening research into the predictive and diagnostic value of selected KLFs in psoriatics.

Aim

This is the first study that explored serum KLF4 levels in patients with active plaque-type psoriasis and its relations with the disease intensity, inflammation or metabolic indicators and also determined the impact of systemic therapy. Additionally, we attempted to assess the relationship of KLF4 with parameters predicting CMDs occurrence in psoriatics, improving or monitoring the effectiveness of treatment or identifying novel therapeutic targets.

Material and methods

The case control study was conducted on thirty-four patients with active psoriasis vulgaris and fifteen sex-, age- and body mass index (BMI)-matched healthy volunteers. The patient group consisted of 22 males and 12 females, at the mean age of 47.6 ±18.12 years. Persons with other types of psoriasis, pregnancy, inflammatory conditions, autoimmune, oncological or cardiometabolic diseases were excluded from the study. None of the participants was under any dietary restriction. Psoriatics were not on any systemic antipsoriatic treatment 3 months prior to the enrollment. The same investigator assessed the psoriasis area and the severity index (PASI) in all subjects. The study group was divided according to severity of psoriasis into two subgroups: mild-to-moder-

ate (PASI I) with PASI < 10–20 including 23 psoriatics and severe form (PASI II) with PASI ≥ 20 achieved by 11 subjects. All participants were also divided into subgroups depending on BMI, group 0 meant the healthy controls, BMI 1 was related to normal weight (BMI 18.5–24.9 kg/m^2) and consisted of 8 psoriatics, group 2 – overweight (BMI 25–29.9 kg/m^2) present in 18 subjects, BMI 3 (BMI > 30 kg/m^2) comprised 8 obese patients. C-reactive protein (CRP), complete blood count (CBC), serum glucose, total cholesterol (Chol), high-density lipoproteins (HDL), low-density lipoproteins (LDL) and triglycerides (TG), transaminases were tested prior to and after treatment. The type of the drug was chosen by the dermatologist, with regard to disease severity, indications and the clinical or therapeutic history of the patients along with agreement with them. The patients started with two systemic medications given orally: 14 subjects methotrexate (MTX) 15 mg/week using folic acid supplementation (15 mg/week, 24 h after MTX intake) and 20 psoriatics received acitretin (ACY) at a dose of 0.5 mg/kg/day. The treatment lasted 12 weeks. All participants signed informed consent forms before the initiation. The study had the approval of the local Bioethical Committee (Protocol number R-I-002/429/2017) and was consistent with the principles of the Helsinki Declaration.

Serum collection

Fasting venous blood samples were collected under complete aseptic precaution from healthy subjects and patients before and after 12 weeks of treatment using vacutainer tubes with clot activator. Samples were centrifuged at 1000× g for 20 min and preserved at –80°C until assay for KLF4, which was measured using enzyme-immunoassay (ELISA) kit supplied by Quantikine® R&D Systems, Minneapolis, USA with sensitivity of 0.054 ng/ml. Optical density was read with a microtitre plate photometer at 450 nm. The concentrations were assessed by interpolation from calibration curves prepared with standard samples provided by the manufacturer. All the tests were taken by one person in standardized laboratory conditions.

Statistical analysis

Statistical analysis was performed using GraphPad 8 Prism and StatMate 2 software (GraphPad Software; La Jolla, California, USA). The normally distributed data were presented as mean and standard deviation, while the non-Gaussian data as median (full-range). Shapiro-Wilk W test was used to determine distribution. Continuous variables were analyzed using ANOVA, independent sample with Student t-test or non-parametric Mann-Whitney test. Categorical variables were analyzed using χ^2 test (chi-square test). The correlations were analyzed using Spearman's rank correlation analysis. Multiple regression analysis was performed using a stepwise model

with a forward elimination procedure to determine the combined influence of variables on particular parameters of the measured system. Multiple regression analysis was performed based on earlier outcomes of Spearman's rank correlation analysis with additional checking of the residuals normality. After statistical analysis all the results were subjected to checking of the statistical power of performed analysis. Presented data had at least 75% power to detect a difference between means of a high scientifically significance level (α) of 0.05 (two-tailed). A two-tailed $p < 0.05$ was considered as statistically significant, and non-significant (NS) when $p > 0.05$.

Results

Clinical, demographic and laboratory characteristics of patients and controls are listed in Table 1 and Supplementary materials. A total of 34 patients with active psoriasis vulgaris (12 women/22 men) and 15 age-, sex- and BMI-matched healthy subjects were recruited to the study. The median of basal PASI score was 16.3 (8.4–33.6) points and 10.2 (4.8–22.8) after treatment. In our study group, 23 psoriatics had a mild-to-moderate form of the disease and 11 were diagnosed with severe psoriasis. The family history of psoriasis among patients was positive in 14 (40%) cases. The median value of the serum KLF4 level in psoriatic patients was 1.09 ng/ml and the difference was not statistically significant in comparison to the controls – 1.36 ng/ml ($p > 0.05$) (Figure 1). However, after dividing the study group regarding the psoriasis intensity, KLF4 was significantly lower in patients with a severe form (PASI II) comparing to the controls ($p < 0.05$) (Figure 2). KLF4 did not correlate with the severity of psoriasis evaluated by total PASI ($p > 0.05$) (Table 2). According to demographic data, no significant relations between KLF4 and sex were found ($p > 0.05$), however in relation with age an upward trend was noted (Table 2). There were no significant relations between the protein and cardiometabolic indices such as liver enzymes activity or glucose or lipids levels ($p > 0.05$) (Table 2). However, a significant positive statistical correlation between KLF4 and BMI was noted ($p = 0.038$) (Table 2). Of the investigated basic inflammatory parameters, no statistical significance with KLF4 was noticed (Table 2). However, strong independent relations between particular pro-atherothrombotic parameters were found after multivariate linear regression analysis (Table 3).

After dividing the study group according to BMI, a downward trend of KLF4 in normal-weight psoriatics with almost half the value as compared to controls and the increasing trend with body weight, especially visible in the group of obese ones comparing to the controls was noted, but they did not reach statistical significance (Figure 3, Supplementary materials). Regarding selected relations inside the BMI subgroups of patients we found that KLF4 negatively correlated with psoriasis severity

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Table 1. Basal characteristics of the patients group before and after total treatment and after both drugs separately

| Characteristics	Before treatment	After treatment	After acitretin	After methotrexate
WBC [× 10^3/ml]	7.59 ±2.14^	7.01 ±1.82^	7.59 ±2.08^	6.32 ±1.01**^
RBC [× 10^3/ml]	4.65 ±0.53^	4.67 ±0.54^	4.68 ±0.48^	4.72 ±0.58^
PLT [× 10^3/ml]	228.8 ±66.26^	232.4 ±57.92^	233.9 ±57.25^	232.5 ±62.53^
Chol [mg/dl]	169.6 ±28.3^	179.9 ±32.14^	180.5 ±27.61^	180.2 ±39.52^
HDL [mg/dl]	46.71 ±11.93^	47.66 ±19.3^	50.2 ±21.11^	42 ±14.62^
LDL [mg/dl]	103.6 ±23.27^	106.2 ±26.5^	104.6 ±26.82^	110.5 ±26.62^
TG [mg/dl]	125.4 ±47.69^	148.5 ±71.76^ ($p = 0.08$)~	134.7 ±63.85^	173.6 ±78.3*^
Glucose [mg/dl]	84 (66–163)	88 (69–250)	88.5 (72–250)	88 (69–110)
ALT [U/l]	17.5 (8–72)	18 (7–113)	17 (9–113)	18.5 (7–87)
AST [U/l]	19 (12–59)	18 (11–114)	20.5 (14–114)	17 (11–77)
CRP [mg/l]	3.05 (1–58.68)	1.9 (1–15.2)*	1.89 (1–15.2)*	2.05 (1–5.1)*
PASI	16.3 (8.4–33.6)	10.2 (4.8–22.8)**	10.15 (4.8–21.9)*	10.25 (6.3–15.3)

*/**The existence of a statistically significant difference between values after and before treatment with p-value < 0.05/< 0.001, respectively. ~The existence of the trend, ^ ± 1 SD, non-Gaussian data as median (full-range) in brackets. PASI – psoriasis area and severity index, RBC – red blood cells, PLT – platelets, WBC – white blood cells, TGs – triglycerides, HDL – high-density lipoproteins, LDL – low-density lipoproteins, CRP – C-reactive protein, ALT – alanine transaminase, AST – asparagine transaminase.*

in normal-weight ones, but not significantly ($p = 0.078$) (Supplementary materials). A negative trend between the protein and platelet count in obese psoriatics was observed ($p = 0.082$) (Supplementary materials).

After 12 weeks of systemic therapy skin lesions have significantly improved ($p < 0.001$) (Table 1). After dividing the patients taking the particular drug, a better, significant improvement after acitretin ($p < 0.05$) than methotrexate was noted ($p > 0.05$) (Table 1). Notewor-

thy is that a significant increase in the TG level occurred after MTX and not acitretin (Table 1). Beside clinical effects of the therapy also limitation of inflammation expressed in a significant decrease in serum inflammatory parameters levels as CRP and white blood cells (WBC) (only after MTX) was noted (Table 1). The median value of KLF4 level in patients after total treatment did not statistically change in comparison to the initial one and of the controls (both $p > 0.05$) (Figure 1). Similarly, con-
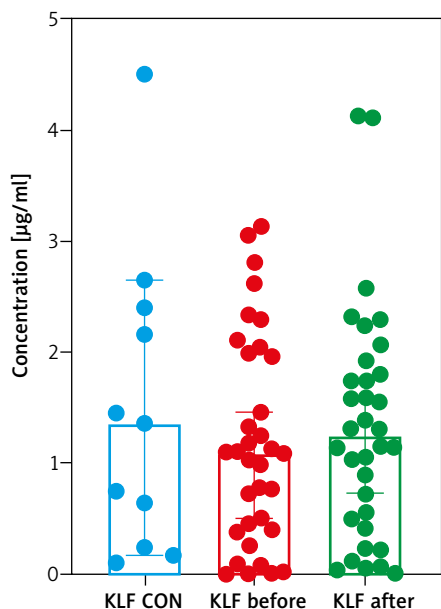
Figure 1. The levels of KLF4 in psoriatics before and after total treatment compared to controls
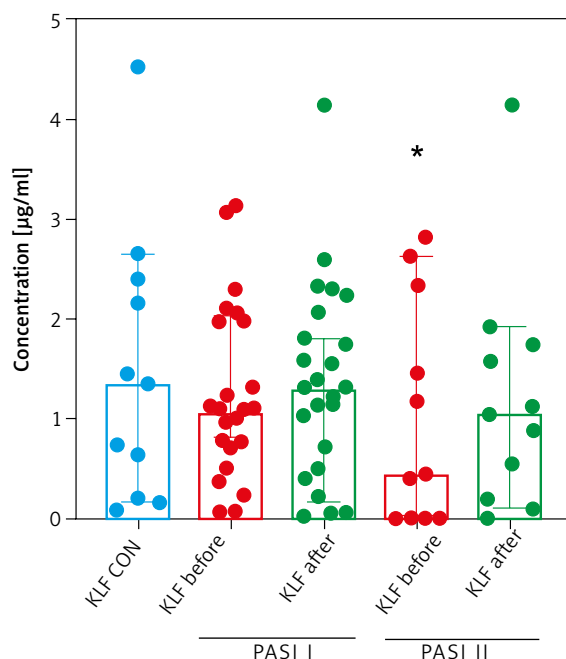
Figure 2. Comparison between KLF4 concentrations depending on the psoriasis area and severity index (PASI) before and after treatment and controls

Table 2. Correlations between baseline parameters and KLF4 in sera of the study group before and after total treatment and both drugs separately

Parameter	R (p-value)			
	Before treatment	After treatment	After acitretin	After methotrexate
PASI	−0.203 (NS)	−0.105 (NS)	−0.454 (0.048)*	0.533 (0.042)*
BMI	0.382 (0.038)*	N/A	N/A	N/A
CRP	−0.101 (NS)	−0.545 (0.0011)*	−0.517 (0.022)*	−0.566 (0.037)*
WBC	0.049 (NS)	−0.248 (NS)	−0.143 (NS)	−0.622 (0.019)*
RBC	0.221 (NS)	−0.248 (NS)	−0.05 (NS)	0.201 (NS)
PLT	−0.243 (NS)	−0.482 (0.0074)*	−0.299 (NS)	−0.572 (0.036)*
Chol	0.133 (NS)	0.027 (NS)	−0.137 (NS)	0.134 (NS)
HDL	−0.197 (NS)	0.001 (NS)	−0.012 (NS)	−0.154 (NS)
LDL	0.138 (NS)	−0.087 (NS)	−0.178 (NS)	−0.06 (NS)
TG	0.145 (NS)	0.312 (0.072)⁻	0.173 (NS)	0.522 (0.022)*
Glucose	0.121(NS)	0.246 (NS)	0.092 (NS)	0.266 (NS)
ALT	−0.027 (NS)	−0.051 (NS)	−0.23 (NS)	0.057 (NS)
AST	0.024 (NS)	−0.270 (0.098)⁻	−0.062 (NS)	−0.531 (0.022)*
Age	0.291 (0.084)⁻	−	−	−
Sex	0.072 (NS)	−	−	−

Statistical significance with p-values < 0.05, ⁻the existence of the trend.

Table 3. Parameters that independently predict the levels of KLF4 – main effects **(A)** and two-way interactions **(B)**

A

Parameter	|t| Value	P-value	P-value summary
TG	3.651	0.0218	*
HDL	3.81	0.0189	*
WBC	3.732	0.0203	*
PLT	5.032	0.0073	**
Glucose	2.906	0.0439	*

B

Parameter I	Parameter II	|t| Value	P-value	P-value summary
Chol	TG	3.016	0.0393	*
Chol	HDL	3.684	0.0211	*
TG	WBC	4.081	0.0151	*
TG	PLT	4.15	0.0143	*
HDL	PLT	5.287	0.0061	**
HDL	Glucose	4.686	0.0094	**
WBC	Glucose	3.739	0.0201	*
PLT	Glucose	2.983	0.0406	*

*Statistical significance, **strong statistical significance. Variables: KLF4, lipid metabolism parameters, morphological parameters, glucose. Goodness of Fit: Multiple R = 0.982; R^2 = 0.9643; Adjusted R^2 = 0.7141.*

sidering the impact of the particular drug, no significant changes in the KLF4 level were noted, beside a subtle upward tendency after both acitretin and methotrexate (both $p > 0.05$) (Figure 4). Regarding subgroups of patients, no significant changes in the KLF4 level were found after therapy in particular BMI subgroups, but in psoriatics with a severe form the initial statistically lower KLF4 level lost its significance, raised over twice, but still was insignificant comparing with the controls (Figure 3, Supplementary materials).

Assessing the relations after total treatment with laboratory parameters, strong negative correlations
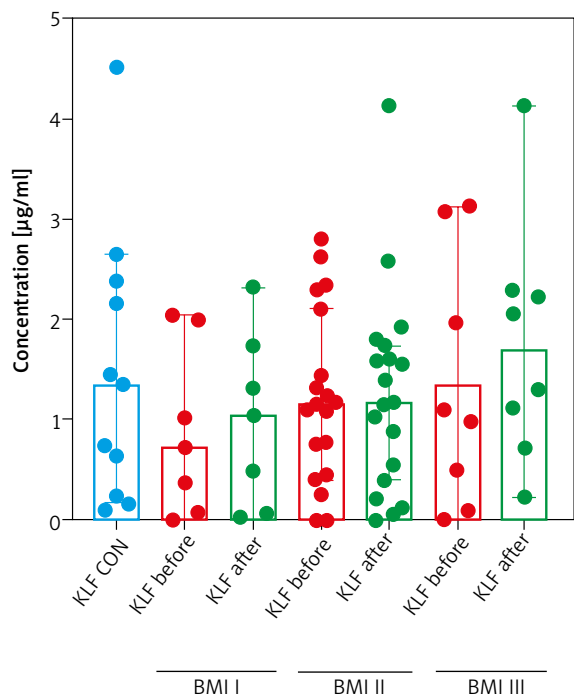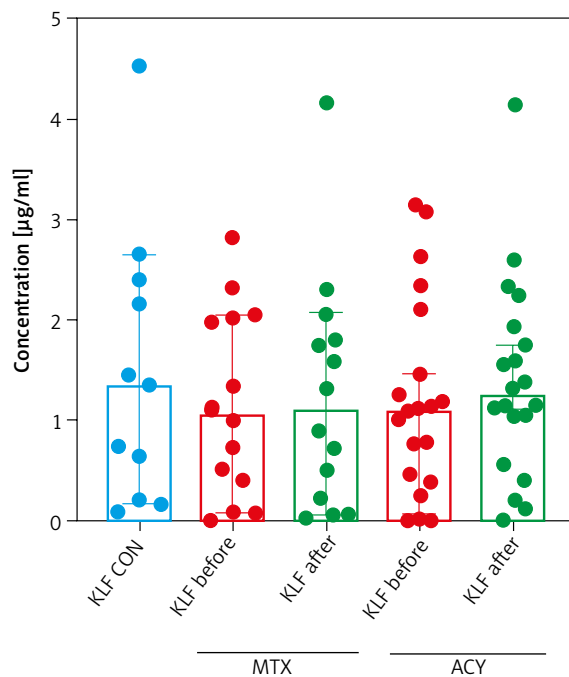
Figure 3. Comparison between KLF4 concentrations depending on BMI before and after treatment and controls

Figure 4. Comparison between KLF4 levels before and after treatment regarding patients undergoing therapy with a particular drug comparing to the controls

between the KLF4 level and CRP ($p = 0.0011$) or platelet count ($p = 0.0074$) appeared (Table 3). An upward trend between the protein and triglycerides level ($p = 0.072$) and downward trend with aspartate aminotransferase (AST) activity ($p = 0.098$) was noted (Table 3). After therapy with acitretin a negative significant correlation of the KLF4 level with PASI ($p = 0.048$) and CRP ($p = 0.022$) appeared (Table 3). After treatment with methotrexate we observed some positive relations between KLF4 and PASI ($p = 0.042$) or triglycerides ($p = 0.022$) (Table 3). Furthermore, negative significant correlations with CRP ($p = 0.037$), WBC ($p = 0.019$), platelets (PLT) ($p = 0.036$) and AST activity ($p = 0.022$) appeared (Table 3).

Discussion

Psoriasis is a systemic disease closely related with CMDs mainly through metabolically-driven inflammation. Recent papers published by our team and others showed that psoriatics still require discoveries of new markers of the disease as well as underlying conditions related to the psoriatic progression [1, 20]. KLFs have been an area of intense research lately and thus they constitute a huge excitement for their potential role in medicine, in multiple diseases, not only CVD, but also psoriasis. It has been widely confirmed that KLF4 is a crucial anti-thrombotic and anti-inflammatory factor [7, 8, 21].

To the best of our knowledge, this is the first study evaluating serum KLF4 concentrations in patients with psoriasis and what raises its value, the relationship with

systemic treatment. Obviously, conducting the discussion on our outcomes is challenging and hence we will also rely on the results of diseases other than psoriasis, but still related. We demonstrated that the serum KLF4 level did not significantly differ in psoriatics compared with the healthy volunteers. No relation between the factor and disease severity was noted. As cited previously, Kim *et al.* demonstrated overexpression of KLF4 in the lesional skin [10]. On the contrary, Madonna *et al.* reported significantly reduced KLF4 levels in psoriatic keratinocytes [12]. The methods used differed, therefore those data cannot be equally discussed. Similarly, with our ones, for the first time serum of psoriatics was evaluated. Although, in the absence of similar data, we cannot draw clear conclusions, the formerly published studies together with own presented point to an uncertain role of KLF4 in psoriasis and perhaps its comorbidities. Presumably, in serum of psoriatics some other confounding mechanisms take part followed by some methodological nuances, small sample sized study group or other ethnical, environmental or lifestyle influences. We found that the KLF4 level positively correlated with BMI. Additionally, we noted a downward trend in normal-weight patients and an upward one in obese psoriatics. These results did not reach statistical significance, presumably in case of larger BMI-subgroups it could be achieved. These data reflect a close relation between KLF4 and adiposity, which is crucial in psoriasis, or could be a compensatory mechanism in response to the metabolically-driven inflammation or perhaps be due to some kind of resistance to

KLF4 induced by obesity. Wang *et al.* conducted a study on omental adipocytes culture, rat models but also on humans [22]. In obese persons the mRNA expression levels of KLF4 were significantly lower than in the normal-weight subjects and they significantly and negatively correlated with BMI, TG, and TNF-α [22]. These results are contradictory to our own ones, however they evaluated the mRNA expression of KLF4 not serum level and the study groups were totally different. Definitely further larger studies are needed to elucidate these divergent mechanisms.

We did not find any relations between the serum KLF4 level and metabolic or inflammatory indicators. However, we found that in multivariate linear regression analysis particular cardiometabolic indices were strongly and independently related with the serum KLF4 level. This undermines that KLF4 might be certainly linked with pro-atherothrombotic risk assessment in psoriasis. As for demographic and clinical data, our results did not show any relation between KLF4 and gender of the subjects studied, however an upward trend with age was observed.

What is meaningful, serum KLF4 concentration was significantly higher in most diseased patients. These outcomes suggest a potential role of KLF4 in the interplay between the intensity of the inflammatory process and the increased risk of CMDs in psoriatics with the greatest skin involvement. Notably, KLF4 might be a relevant biomarker of the proatherogenic risk in severe psoriasis, which needs to be further confirmed. Assuming, in milder psoriasis some kind of physiological homeostasis seems to be maintained. Within the intensifying inflammation in more severe cases it is interrupted and possibly a cascade of pro-inflammatory cytokines or oxidative stress is being overexpressed and up-regulates the serum KLF4 level. It has been confirmed that overexpression of that factor in endothelial cells enhances anti-thrombotic genes, while decreased KLF4 results in the increase in TNF-α-induced expression of vascular cell adhesion molecule-1 (VCAM-1) or E-selectin and thus promotes vascular inflammation [6, 21]. Therefore, gathering this knowledge and our outcomes it would be worth looking for new therapeutic methods that could raise KLF4 in psoriasis. There are reports on drugs that regulate KLF expression. It has been shown that rapamycin, simvastatin, lovastatin, mevastatin and resveratrol induce the KLF2 and KLF4 expression in endothelial cells via different pathways leading to vasoprotection and contributing to prevention of CVD [19, 23, 24]. The positive effect of such a commonly administered drug as simvastatin has been widely documented to date in psoriatics. Recently, Trong *et al.* has demonstrated that adding oral simvastatin to antipsoriatic therapy might be beneficial in controlling hyperlipidemia and decreasing the severity of the disease [25]. Going further, an improvement in psoriatic lesions has been observed in

diabetic patients treated orally with troglitazone and pioglitazone which are PPAR-γ agonists [26, 27]. Lately, Wang *et al.* has demonstrated that pioglitazone down-regulated the gene transcription of apelin by stimulating transcription of KLF4 in the apelin promoter what leads to prevent diabetic macroangiopathy in type 2 DM rat models [28]. As mentioned above, KLFs, particularly KLF4, stimulate PPAR-γ receptors, thus this interplay additionally highlights that their agonists could be helpful in reducing CMDs in psoriasis. Valuable research was conducted by Garvey *et al.* [29]. They demonstrated a remarkable remodeling of cyclosporine-A (CSA) treated arteries as well as up-regulation of KLF4 immunostaining in vascular smooth muscle cells and endothelial cells [29]. These outcomes encourage to extend the research with use of CSA, as one of well-established antipsoriatic drugs, on the KLF level and inhibition of vascular inflammation in psoriatics.

To date, there have been no investigations on the effect of systemic antipsoriatic treatment on the serum KLF4 level. The only one convergent study we have found was of Kim *et al.* [10]. They noted after two months of treatment, a significant increase in the intensity of KLF4 mRNA expression, however the relative one was insignificant comparing to the controls [10]. We are the first ones who demonstrated no significant influence of therapy with methotrexate or acitretin, in total and separately, on serum KLF4 concentration in psoriatics compared with the healthy subjects. However, primary significantly elevated KLF4 in severe patients decreased over twice after systemic therapy, losing its significance vs. controls. We might assume that those classic drugs are insufficient for up-regulating the KLF4 level and its great anti-inflammatory and anti-atherogenic properties, what would be desirable. Furthermore, it does not contradict the CVD predictive value of the factor or antipsoriatic therapy, however there must be other underlying and modulating mechanisms to be elucidated.

We proved that the KLF4 level cannot be a useful marker of effectiveness of psoriasis treatment. Interestingly, after total therapy some correlations have appeared. The serum KLF4 level was negatively related with CRP, PLT and WBC in the MTX-subgroup. These data are puzzling, it could be assumed that KLF4 is a less sensitive inflammatory marker in psoriasis than CRP or perhaps KLF4 is insufficiently up-regulated. Further intriguing results were a significant TG increase after methotrexate and not acitretin, considered more lipids disturbing, which appeared also to be negatively related with KLF4. Hypothesizing, the factor could be a predictor of hypertriglyceridemia in psoriatics undergoing MTX or this drug should be considered as a modulator of pathways dependent on KLF4 in psoriasis, however this requires further research.

The limitations of our study are a small number of patients and controls enrolled, although the groups were

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relatively uniform in terms of biochemical and clinical data. Additional sub-dividing of an already small study group reduces the reliability of analyses obtained. On the other hand, trends observed in BMI-subgroups point to a potential significance in our future larger research. Thus, our outcomes should be considered as preliminary data. However, we believe that presented results will stimulate further effort to fully elucidate the role of KLFs and particularly KLF4 in psoriasis, the interplay with its comorbidities and treatment together with the discovery of novel therapeutic targets.

Conclusions

Considering the limitations of the study, the lack of data from the literature to refer to, the results obtained cannot be conclusive and final. However, they are undoubtedly valuable and point the way forward to clarify the precise role of KLF4 in psoriasis and its interactions with parameters involved in CMD occurrence. For the first time this study evaluated serum KLF4 levels in psoriatics which were not significantly different comparing to the healthy controls. However, this does not exclude KLF4 as an indicator of comorbidities in those patients. Knowing anti-inflammatory and anti-thrombotic properties of KLF4 and the results obtained, it can be presumed that the factor may serve as an independent marker of the risk of vascular disorders development especially in patients with severe psoriasis or obesity, which are characterized by increased KLF4 concentration. Considering a positive correlation with BMI, KLF4 might be also related with adiposity in psoriasis and thus with systemic metabolically-driven inflammation. Although no significant changes occurred after therapy, the interplay between KLF4 and particularly methotrexate needs in-depth research. The results obtained, promising data on other commonly used drugs up-regulating KLF4 followed by further more intense understanding of KLF impact on various processes should lead to attempts to develop new targeted therapies, also in psoriasis.

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Conflict of interest

The authors declare no conflict of interest.

References

1. Tampa M, Maria-Isabela Sarbu MI, Madalina-Irina Mitran MI, et al. The pathophysiological mechanisms and the quest for biomarkers in psoriasis, a stress-related skin disease. Dis Markers 2018; 2018: 5823684.
2. Horreau C, Pouplard C, Brenaut E, et al. Cardiovascular morbidity and mortality in psoriasis and psoriatic arthritis: a systematic literature review. J Eur Acad Dermatol Venerol 2013; 27 (Suppl. 3): 12-29.
3. Hu SC, Lan CE. Psoriasis and cardiovascular comorbidities: focusing on severe vascular events, cardiovascular risk factors and implications for treatment. Int J Mol Sci 2017; 18: pii: E2211.
4. Samarasekera EJ, Neilson JM, Warren RB, et al. Incidence of cardiovasular disease in individuals with psoriasis: a systemic review and meta-analysis. J Invest Dermatol 2013; 133: 2340-6.
5. Pearson R, Fleetwood J, Eaton S, et al. Kruppel-like transcription factors: a functional family. Int J Biochem Cell Biol 2008; 40: 1996-2001.
6. McConnell BB, Yang VW. Mammalian Kruppel-like factors in health and diseases. Physiol Rev 2010; 90: 1337-81.
7. Pollak NM, Hoffman M, Goldberg IJ, Drosatos K. Krüppel-like factors: crippling and uncrippling metabolic pathways. JACC Basic Transl Sci 2018; 3: 132-56.
8. Sweet DR, Fan L, Hsieh PN, Jain MK. Kruppel-like factors in vascular inflammation: mechanistic insights and therapeutic potential. Front Cardiovasc Med 2018; 5: 6.
9. Yet SF, McA'Nulty NM, Folta SC, et al. Kruppel like Zinc finger protein is expressed in vascular endothelial cells and contains transcriptional activation and repression domains. J Biol Chem 1998; 273: 1026-31.
10. Kim KJ, Park S, Park YH, et al. The expression and role of krüppel-like factor 4 in psoriasis. Ann Dermatol 2014; 26: 675-80.
11. Segre JA, Bauer C, Fuchs E. Klf4 is a transcription factor required for establishing the barrier function of the skin. Nat Genet 1999; 22: 356-60.
12. Madonna S, Scarponi C, Sestito R, et al. The IFN-gamma-dependent suppressor of cyto-kine signaling 1 promoter activity is positively regulated by IFN regulatory factor-1 and Sp1 but repressed by growth factor independence-1b and Krüppel-like factor-4, and it is dysregulated in psoriatic keratinocytes. J Immunol 2010; 185: 2467-81.
13. Tsoi LC, Spain SL, Knight Jo, et al. Identification of fifteen new psoriasis susceptibility loci highlights the role of innate immunity. Nat Genet 2012; 44: 1341-8.
14. Ali G, Elsayed AK, Nandakumar M, et al. Keratinocytes derived from patient-specific induced pluripotent stem cells recapitulate the genetic signature of psoriasis disease. Stem Cells Dev 2020; 29: 383-400.
15. Liao X, Sharma N, Kapadia F, et al. Krüppel-like factor 4 regulates macrophage polarization. J Clin Invest 2011; 121: 2736-49.
16. Czepluch FS, Vogler M, Kuschicke H, et al. Circulating endothelial cells expressing the angiogenic transcription factor Krüppel-like factor 4 are decreased in patients with coronary artery disease. Microcirculation 2015; 22: 700-10.
17. Liu H, Lyu YN, Li D, et al. Association of circulating growth differentiation factor-15, Krüppel-like factor 4 and growth arrest-specific 6 with coronary artery disease. Clin Chim Acta 2019; 495: 630-6.
18. Alaiti MA, Orasanu G, Tugal D, et al. Kruppel-like factors and vascular inflammation: implications for atherosclerosis. Curr Atheroscler Rep 2012; 14: 438-49.
19. Fan Y, Lu H, Liang W, et al. Krüppel-like factors and vascular wall homeostasis. J Mol Cell Biol 2017; 9: 352-63.
20. Krahel JA, Baran A, Kamiński TW, et al. Methotrexate decreases the level of PCSK9-a novel indicator of the risk of proatherogenic lipid profile in psoriasis. The preliminary data. J Clin Med 2020; 9. pii: E910.
21. Hamik A, Lin Z, Kumar A, et al. Kruppel-like factor 4 regulates endothelial inflammation. J Biol Chem 2007; 282: 13769-79.

22. Wang C, Zhang M, Wu J, et al. The effect and mechanism of TLR9/KLF4 in FFA-induced adipocyte inflammation. Mediators Inflamm 2018; 2018: 6313484.

23. Villareal G, Zhang Y, Larman HB, et al. Defining the regulation of KLF4 expression and its downstream transcriptional targets in vascular endothelial cells. Biochem Biophys Res Commun 2010; 391: 984-9.

24. Wang Y, Zhao B, Zhang Y, et al. Krüppel-like factor 4 is induced by rapamycin and mediates the anti-proliferative effect of rapamycin in rat carotid arteries after balloon injury. Br J Pharmacol 2012; 165: 2378-88.

25. Trong HN, Tat TN, Anh TTN, et al. Efficacy of adding oral simvastatin to topical therapy for treatment of psoriasis: the Vietnamese experience. Open Access Maced J Med Sci 2019; 7: 237-42.

26. Ellis C, Varani J, Fisher GJ, et al. Troglitazone improves psoriasis and normalizes models of proliferative skin disease: ligands for peroxisome proliferator-activated receptor-gamma inhibit keratinocyte proliferation. Arch Dermatol 2000; 136: 609-16.

27. Bongartz T, Coras B, Vogt T, et al. Treatment of active psoriatic arthritis with the PPAR gamma ligand pioglitazone: an open-label pilot study. Rheumatology 2005; 44: 126-9.

28. Wang Y, Zhang R, Shen H, et al. Pioglitazone protects blood vessels through inhibition of the apelin signaling pathway by promoting KLF4 expression in rat models of T2DM. Biosci Rep 2019; 39: BSR20190317.

29. Garvey SM, Sinden DS, Schoppee Bortz PD, Wamhoff BR. Cyclosporine up-regulates Krüppel-Like Factor-4 (KLF4) in vascular smooth muscle cells and drives phenotypic modulation in vivo. J Pharmacol Exp Ther 2010; 333: 34-42.