

Variation of inflammatory cytokines levels after mud therapy in patients with ankylosing spondylitis

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- Ankylosing spondylitis (AS, from Greek *ankylos*, bent; *spondylos*, vertebrae) is the prototype for seronegative spondyloarthropathy and its name means “inflamed spine growing together”.

- It mainly affects joints in the spine and the sacroiliac joints in the pelvis, and can cause eventual fusion of the spine. Complete fusion results in a complete rigidity of the spine, a condition known as “bamboo spine”.



- Cytokines, particularly tumor necrosis factor-alpha (TNF- α) and transforming growth factor-beta (TGF- β), as well as IL-1 and IL-6 play an important role in the inflammatory process, leading to fibrosis and ossification at sites of enthesitis.

- Autoantibodies specific for AS have not been identified. Anti-neutrophil cytoplasmic antibodies ANCA are associated with AS, but don't correlate with disease severity. About 90% of the patients express the HLA-B27 genotype.

Objective

- The study aims to evaluate anti-inflammatory activity of saprogenic mud from Techirghiol lake (as cold mud ointment or warm mud application) in patients with AS and so to point out the important role of balneal therapy in slowing down the progression of the disease.

Material and method

- The study group included 23 patients (7 female and 16 male), all of them with AS (defined using ACR criteria). Most of the patients (16) underwent cold mud ointment, 4 patients got warm mud bath and 3 patients got mud packing.
- For the first ones the applied treatment consists in progressive heliotherapy, cold mud ointment on the lake shore and swimming into the salted water of Techirghiol lake, and additionally electrotherapy, kinetics, massage.

Material and method

- The patients were clinically evaluated and they were taken blood samples before treatment and after 12 days of mud application. As shown in actual similar studies, it can be seen a specific variation of serum levels of cytokines (IL-1 β , IL-6 and TNF- α).
- The values of these cytokines were determined at the Research Center for Microscopic Morphology and Immunology from Craiova University, using ELISA technique.

Material and method

- In this study we used t-Student test (considering a normal distribution of the values), in order to evaluate the working hypothesis concerning the variation of implied factors. Therefore, we are presenting relative variation of the values for each patient to show the modifications meanwhile the treatment.

$$\text{relative variation} = \frac{\text{finalvalue(aftercure)} - \text{initialvalue(beforecure)}}{\text{initialvalue(beforecure)}}$$

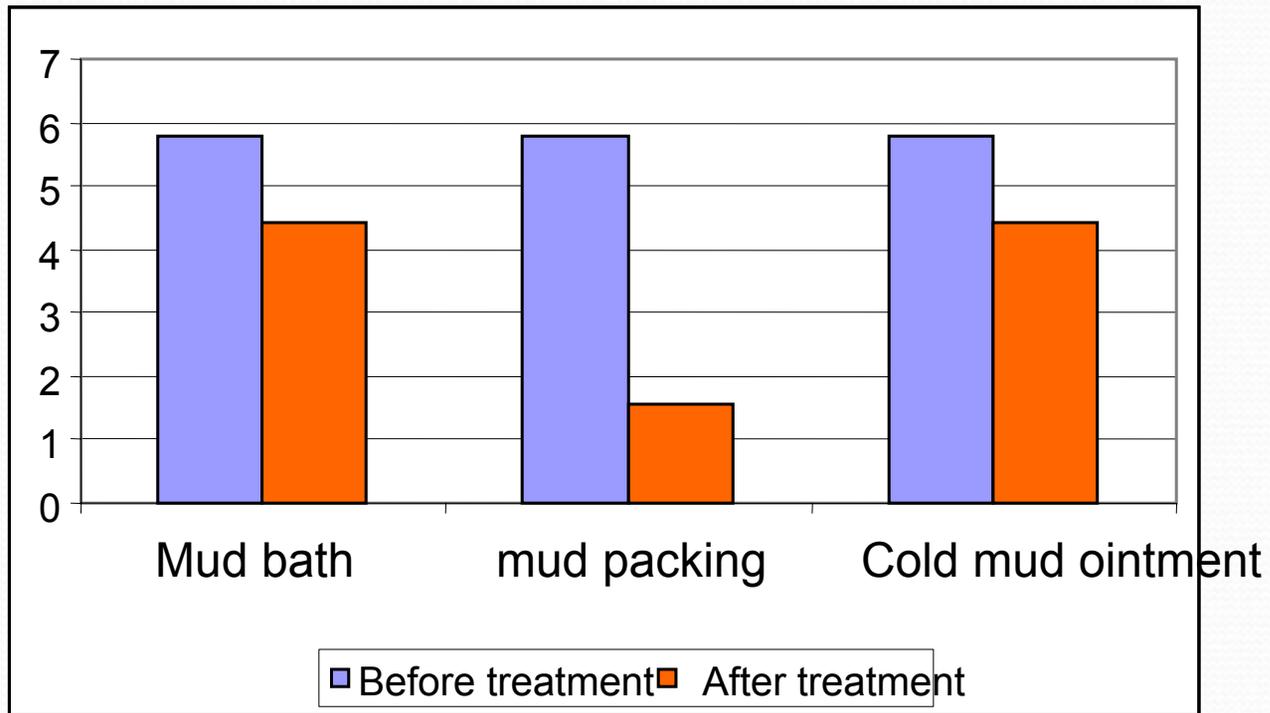
Results

The table below contains the values of average and standard deviation for all three of determined cytokines:

parametric	Med and dev std before treatment			Med and dev std after treatment		
	MB	CMO	MP	MB	CMO	MP
IL-1 β	2 \pm 1.21	4.93 \pm 6.97	3.2 \pm 0.84	3.066 \pm 2.45	3.12 \pm 1.92	2.205 \pm 1.13
IL-6	0.93 \pm 0.57	11.40 \pm 17.7	1.85 \pm 0.35	4.43 \pm 1.25	4.76 \pm 3.6	1.55 \pm 0.21
TNF- α	2.78 \pm 1.08	9.16 \pm 10.51	4.99 \pm 1.32	3.35 \pm 1.98	4.19 \pm 1.89	5.45 \pm 0.84

(MB=mud bath, CMO=cold mud ointment, MP=mud packing)

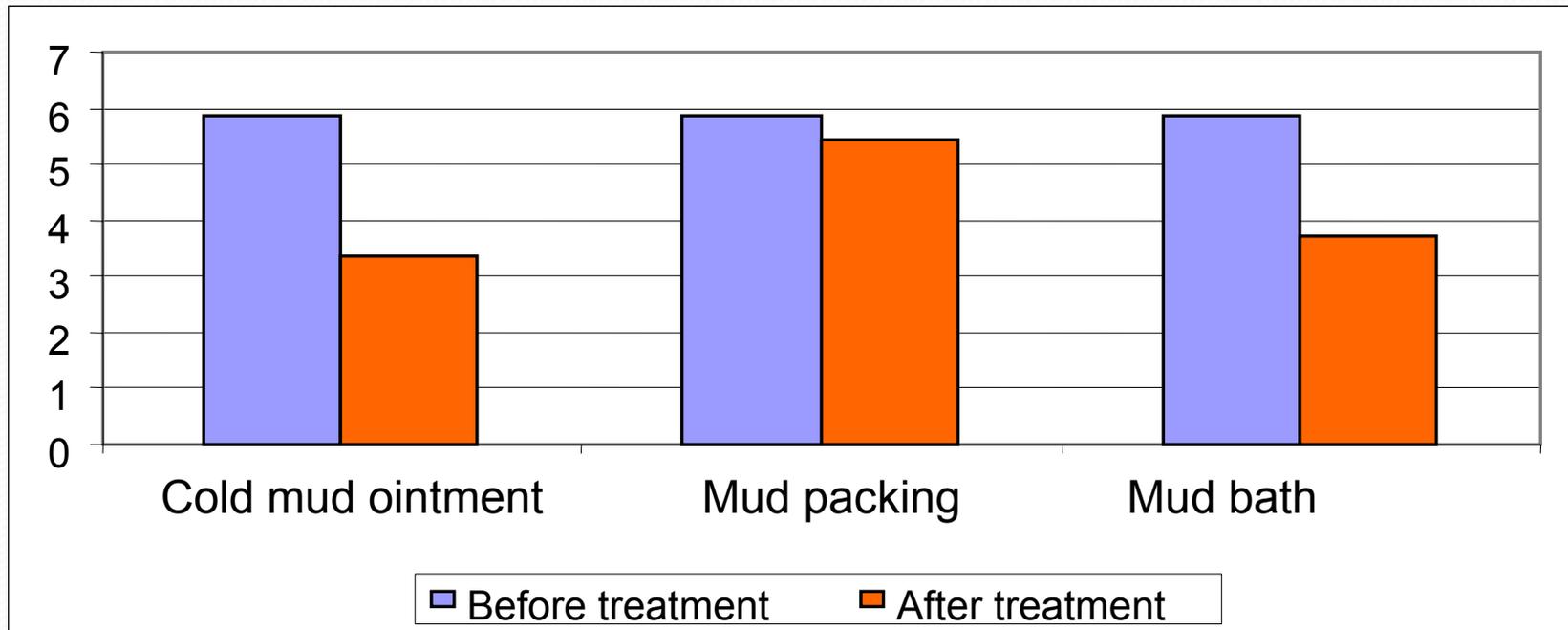
Variation of IL-6 depending of the type of mud application



- t test initial/final mud bath 1.74
- P(t<0.05) initial/final mud bath 0.05
- t test initial/final mud packing 2.52
- P(t<0.05) initial/final mud packing 0.01
- t test initial/final cold mud ointment 1.71
- P(t<0.05) initial/final cold mud ointment 0.05

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- From the data analysis it can be seen a statistically significant decrease in serum level of IL-6 after mud treatment. General mud packing determines the greatest decrease of IL-6 serum level. General mud bath and cold mud ointment determine smaller decreases, comparable one each other, both at the limit of statistic significance: $P (t = 0.05)$.

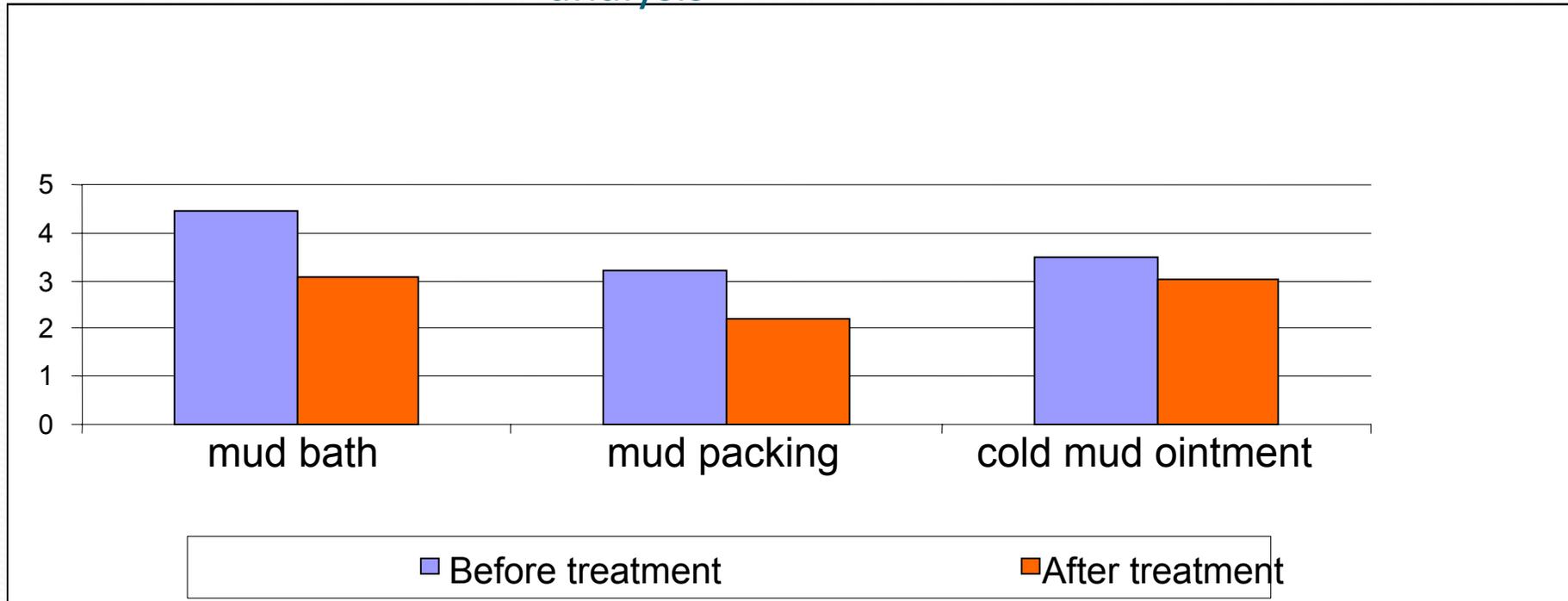
Variation of TNF- α depending of the type of mud application



- t test initial/final mud bath
 - P(t<0.05) initial/final mud bath
 - t test initial/final mud packing
 - P(t<0.05) initial/final mud packing
 - t test initial/final cold mud ointment
 - P(t<0.05) initial/final cold mud ointment
- | | | |
|--|------|------|
| | 3.58 | 3.84 |
| | 1.62 | 0.99 |
| | 3.58 | 4.60 |
| | 1.62 | 2.77 |
| | | 0.05 |

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- Analyzing the data, a decrease in serum levels of TNF- α can be seen for all types of mud application, but only cold mud ointment determines a statistically significant decrease.
 - The equal values of average for TNF- α serum levels after mud bath and mud packing suggest the presence of an intrinsic factor, which interfere with TNF- α .

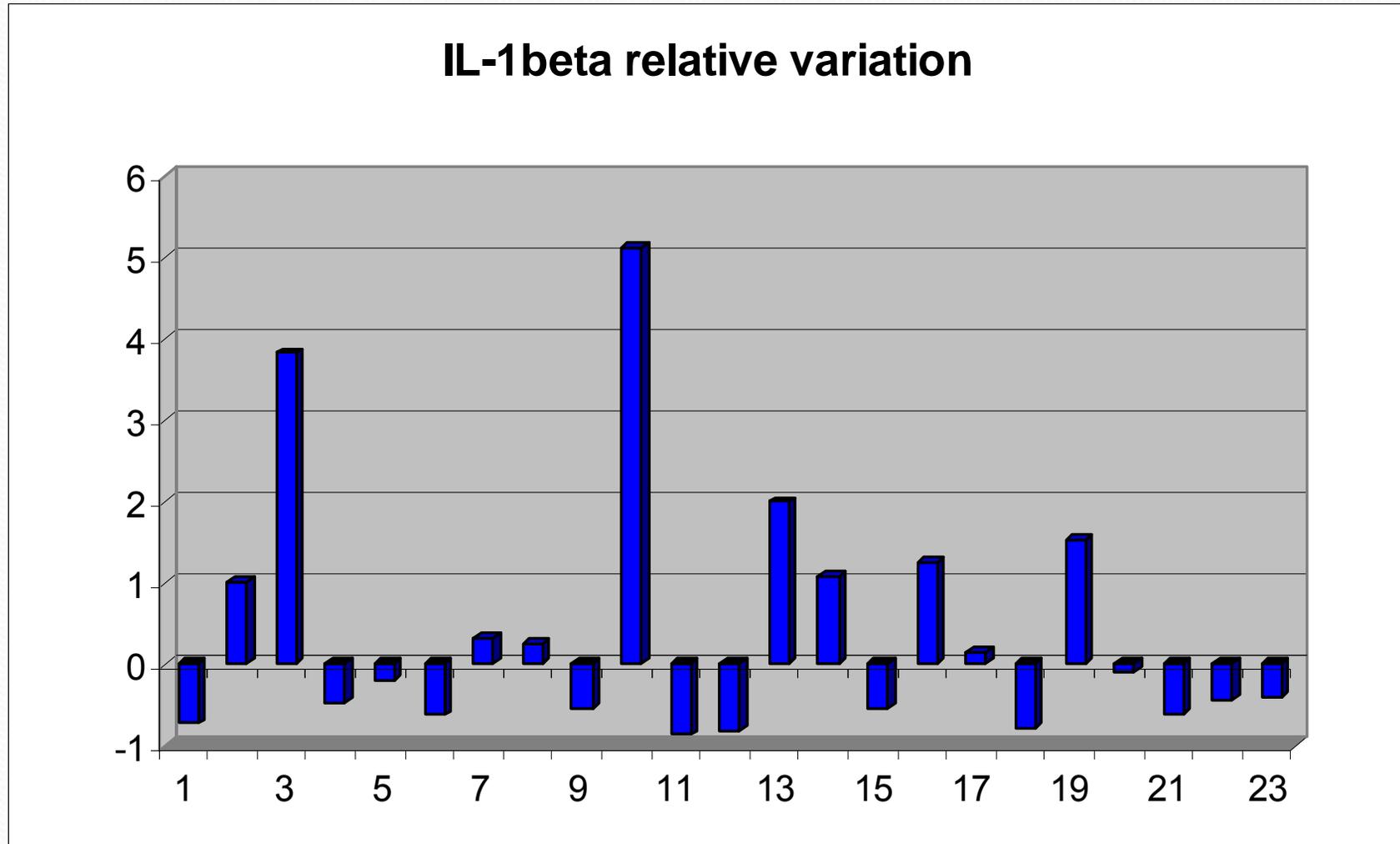
Variation of IL-1 β after mud therapy and statistic significance analysis



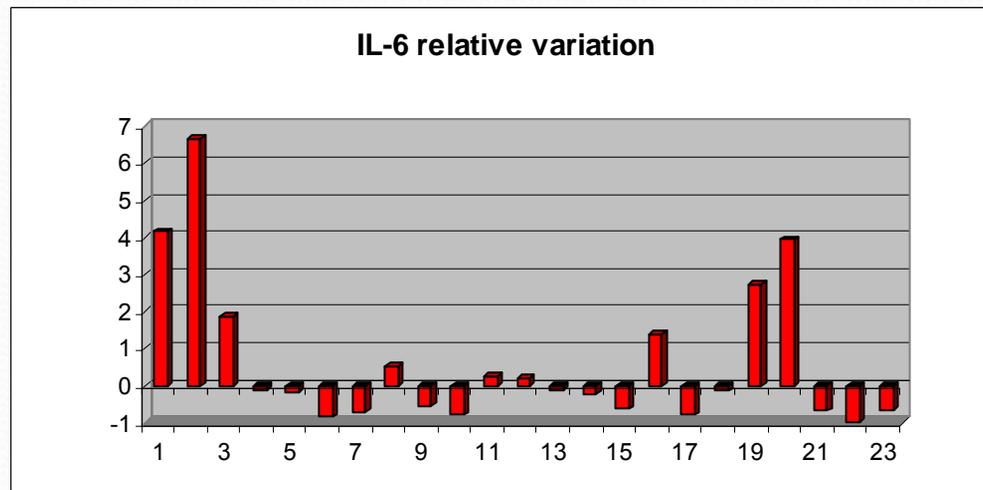
- t test inițial/final mud bath 0,73
- P(t<0.05) inițial/final mud bath 0,25
- t test inițial/final mud packing 1.52
- P(t<0.05) inițial/final mud packing 0.08
- t test inițial/final cold mud ointment 1.01
- P(t<0.05) inițial/final cold mud ointment 0.16

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- As it can be seen from the chart above, there is also a decrease for IL-1 β values in all three types of mud application, but none of them statistically significant [P (t>0.05)].
 - IL-6 and TNF- α are cytokines that initiate and maintain inflammation, that's why the decrease of their serum levels point out the anti-inflammatory effect of mud application.

Relative variation of serum IL-1 β after mud application

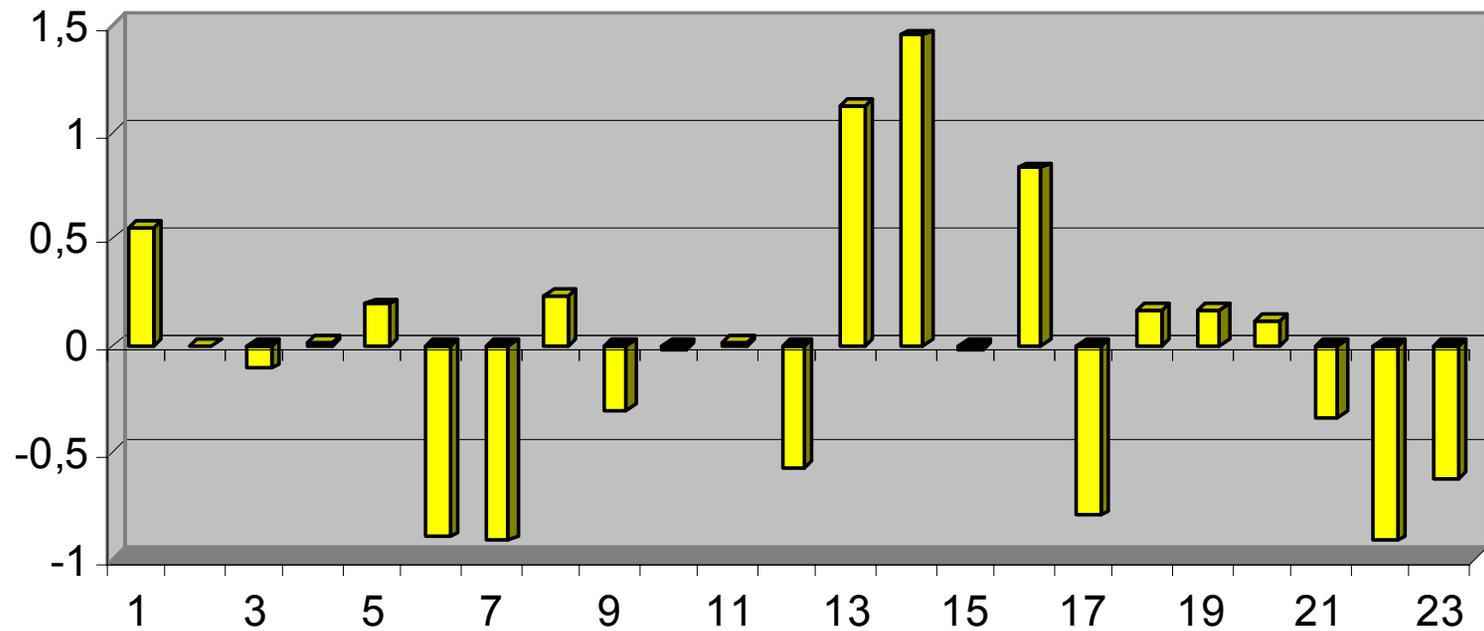


Relative variation of serum IL-6 after mud therapy



Relative variation of serum TNF- α after mud therapy

TNF- α relative variation



Discussions

- After cold mud ointment we observe an important decrease of IL-6 and TNF- α serum levels in the studied group, which can be correlated with clinical improvement shown by the patients .
- The other cytokine (IL-1 β) has slightly decreased, but not statistically significant. However, it can be made an interesting observation, that in patients who had peak values (higher than normal) at the beginning we observed an important decrease of IL-6 and TNF- α , that means a slower progression of the disease and a better prognosis.

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- Although we chose the patients who formed the group respecting excluding criteria (no other additional inflammatory pathology), we found a very large distribution of relative variation for all three cytokynes in the studied group.
 - We propose to ourselves to extend the study this summer in order to obtain useful data for the complex treatment of AS.

Conclusions

- In patients with AS, cold mud ointment is decreasing significantly serum levels of IL-6 and TNF- α , cytokines that normally stimulate inflammatory processes, thus improving the symptoms and signs and the evolution of disease.
- We recommend this natural therapy in patients with AS in a stable stage of the disease, in order to minimise the NSAID's doses and to prevent as long as possible disabilities.

References

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- Galzigna L., Ceschi-Berrini C., Moschin E., Tolomio C.: *Thermal mud-pack as an anti-inflammatory treatment*. Clinical Trial. PMID:9856289 Fortschr Med 1989 Aug 10; 107(23):24-5 Biomed Pharmacother 1998;52(9):408-9.
- Surdu O.- *Studiu histologic comparativ al actiunii namolului sapropelic de Techirghiol asupra tegumentului*. Teza de doctorat, 2007.
- ******biostatistica.....**

Thank you for your attention!

