The Relationship Between Dental Caries and YKL-40 Levels in Saliva

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Abstract
Objective: YKL-40, a new biomarker of localized inflammation, is secreted by macrophages and regulates inflammation and immune responses. The aim of this study was to investigate YKL-40 levels in saliva and compare the level of this mediator in oral cavity.

Methods: 85 children (46 girls, 39 boys), aged 6-15 (mean±SD: 9.15±2.16) were included in this study. The children were divided into three groups: Group-I (control, n=25, DMFT/dmft=0), Group-II (n=30, exist of localized dental caries) and Group-III (n=30, exist of localized advanced dental caries). Gingival index (GI), plaque index (PI), DMFT/dmft, DMFS/dmfs, and the number of advanced dental caries according to the ICDAS II and PUFA/pufa index were recorded. Saliva was collected and YKL-40 concentrations were measured. One-way ANOVA with Tukey post hoc, Kruskal-Wallis, multiple regression analysis, and Sperman’s correlation tests were used for statistical analysis.

Results: The highest level of YKL-40 was obtained in group III, followed by groups II and I, respectively (p<0.01). In Group II, DMFT/dmft scores and the number of caries (DT/dt) were higher than in group III (p<0.01). In group III, there was a statistically significant correlation between YKL-40 levels in saliva and the number of advanced dental caries. In addition, there was no statistically significant difference in terms of age and gender (p>0.05).

Conclusion: Advanced dental caries, rather than DMFT/dmft score, may play an important role in the increasing levels of YKL-40 in saliva.

Background
In general, pathogenic oral bacteria are classified into two major groups; the first group is for dental caries and the second group is for periodontitis. Gram-positive bacteria (Streptococcus mutans -S. mutans) are major pathogens of dental caries, while gram-negative bacteria are involved in exaggerated extensive caries and periodontal diseases.1,2

Found in shallow caries or in the outer dentinal tubules of deep caries, gram-positive bacteria (Streptococcus and Lactobacillus spp., etc.) are widespread oral microflora. On the other hand, gram-negative bacteria (Fusobacterium, Porphyromonas and Prevotella spp.) are detected in deep caries and in the infected root canals and are related to pulpal and periradicular inflammation.1,3
The formation of a dental plaque biofilm is an important biological process associated with the attachment of oral bacteria, in particular *S. mutans*, on the tooth surface. The most common reason of the gingivitis is a mass of supragingival plaque along the teeth’s gingival margins where the plaque is formed the most. In some cases, it was observed that this plaque may cause gingivitis to bleed when the plaque flora has spirochetes and *Actinomyces viscosus*. Step by step, the flora, if not disturbed, turns to anaerobic gram-negative flora including black pigmented bacteroides and several types of spirochetes. The low oxidation-reduction potential of the aged-plaque and nutrients taken through the inflammatory exudate can be the reason of the increase in these anaerobic organisms.²

YKL–40 is a 40-kDa glycoprotein, which is produced by activated macrophages,⁴ neutrophils,⁵ and mast cells in the inflamed areas.⁶ The plasma levels of YKL–40 are elevated in the patients with acute inflammation (e.g. pneumonia, endotoxemia, and hepatitis) or chronic inflammation (e.g. rheumatoid arthritis, inflammatory bowel disease, asthma, chronic obstructive pulmonary disease, type I and II diabetes, and coronary artery disease) and in the patients with liver fibrosis and cancer.⁷⁻¹¹ The relationship between the proinflammatory cytokines and the oral pathogenic bacteria has been determined in previous studies.¹²,¹³ Some of these studies also determined the correlation between these proinflammatory markers and YKL–40 in body fluids.¹⁴,¹⁵

Of all the studies on YKL–40, only one of them was performed to investigate the possible role of YKL–40 in oral cavity.¹⁶ It was related to periodontal diseases and investigated in GCF and serum. Our hypothesis is that YKL–40 may be an important causative factor, related to the density of localized inflammation in the oral cavity by oral bacteria and may play a pathophysiological role in this entity. A new biomarker will help the clinician to determine the level of caries, gingival problems and the type of treatment. It is important to determine whether the caries are deep or shallow and whether there is inflammation in advanced caries with a pulpal involvement. For example, in children with dental anxiety or in individuals with special needs, which is indicated for dental treatment under general anesthesia or sedation, it will be beneficial for the dentist to acquire prior knowledge about the health status of the oral cavity from saliva samples.
Thus, the goal of this study was to investigate YKL-40 levels in saliva to be able to compare the level of this mediator in a healthy oral cavity and an unhealthy oral cavity, which had dental caries at different stages and poor oral hygiene.

Methods
Patient selection
This study was performed in the Faculty of Dentistry, Department of Pedodontics, at İnönü University. According to the previous power analysis clarifying the changes of YKL-40 in saliva, the estimated number of participants was 15 per group, with an alpha level of 0.05 and a power of 0.80. Samples were analyzed at the Biochemistry Department.

This study was conducted in accordance with the Declaration of Helsinki after obtaining ethical approval. It was approved by a Clinical Research Ethics Committee in Turkey: İnönü University School of Medicine (ethical number: 2017/67). Written informed consent was obtained from the parents before the examination.

A total of 85 subjects, aged 6-15 (9.15 ± 2.16 years; 39 male, 46 female) participated in this study. The participants were divided into three groups: Group I (control, n = 25, mean age = 7.72 ± 1.34), Group II (n = 30, with dental caries, mean age = 9.40 ± 2.13) and Group III (n = 30, with advanced dental caries, mean age = 10.10 ± 2.19). The children were excluded from the study if they had any systemic and/or periodontal diseases, and if they had taken antibiotics or anti-inflammatory drugs in the last 30 days.

Calibration of the examiners
All clinical examinations were performed by an experienced clinician (GD). On the other hand, biochemical analysis was performed by another experienced researcher (EL).

The examiners were calibrated prior to the study (intra-examiner kappa>0.8 for both of them)

One of the examiners (GD) was calibrated to measure clinical parameters (DMFT/dmft, ICDAS II, PUFA/pufa, GI, PI, saliva collection) and radiological parameters (periapical x-ray evaluation), and the other (EL) was calibrated for YKL-40 extraction from saliva samples and the measurement of its amount (ng/mL).

Clinical measurements
The gingival conditions of the patients were evaluated by Silness-Loe plaque index (PI)\textsuperscript{17} and Loe gingival index (GI)\textsuperscript{18}. Decayed-missing-filled teeth (DMFT/dmft) and decayed-missing-filled teeth surfaces (DMFS/dmfs) were recorded. The extraction of the primary teeth due to the physiological root resorption was not recorded as a missing tooth. The teeth were scored using International Caries Detection and Assessment System (ICDAS) II\textsuperscript{19} and PUFA/pufa index (Exposed pulp, Ulceration, Fistula, Abscess) (Table I)\textsuperscript{20}. The diagnosis was based on two factors: clinical and radiographic features.

Group I; control. ICDAS II code = 0, DMFT/dmft = 0

Group II; shallow caries; caries in dentin and cavitation was not exaggerated. ICDAS II code = 1–4

Group III; deep caries; caries lesion was close to the dentin-pulp interface, the dentin thickness was less than 1mm, with or without pulpal exposure. ICDAS II code = 5–6. Group III had at least one tooth with ICDAS II code–5 or 6. The caries lesion extending into the pulp tissue were classified according to the PUFA/pufa index.

Radiological examination
The radiographic examination was made to confirm caries lesion depth, an endodontic situation of the teeth with pulpal involvement of caries lesions especially in groups II and III.

Saliva collection
All the saliva samples were obtained in the morning and the participants were asked to avoid eating or drinking 1 h before the collection of samples. All the unstimulated saliva samples were collected by the spitting method and transferred into a 2-ml polypropylene tube. All the saliva samples were homogenized on a Vortex mixer (1 min) and centrifuged (10,000 \( \times \) g, 10 min) to remove cellular debris. The resultant supernatants of the samples were stored at \(-80 \, ^\circ\)C for further analyses.

YKL–40 assay
The level of YKL–40 in saliva was measured by ELISA (R&D Systems, Minneapolis, MN) and the analysis was performed according to the manufacturer’s instructions using human recombinant standards in Biochemistry Laboratory. All samples were run in duplicate and the results were averaged for the analysis. The results were reported in pg/mL. The detection limit was 3.5 pg/mL for YKL–40. The samples with YKL–40 levels below the limits of the assay’s detectability were scored 0.
The results recorded in pg/mL were converted to ng/mL.

Statistical analysis
Data analysis was performed using the statistical package SPSS 21 (SPSS Inc., Chicago Illinois, USA). The results were expressed as means ± standard deviations. The data were firstly analyzed for the normal distribution using Shapiro-Wilk test. The parameters between the groups were compared with one another using One-way ANOVA, Tukey post hoc and Kruskal-Wallis tests. Multiple regression analysis was used to explain YKL-40 level. Sperman rank correlation test was used to verify the correlations between the parameters.

Results
DMFT/dmft, DMFS/dmfs, ICDAS II, PUFA/pufa, GI, and PI scores were used in this study. For each participant, we recorded the followings: YKL-40 levels in saliva, the number of decayed, missing, and filled teeth, according to the ICDAS II, and advanced dental caries, according to the ICDAS II and PUFA/pufa.

There were statistically significant differences among the groups for YKL-40, GI, PI, DMFT/dmft, DMFS/dmfs, the number of shallow caries and the advanced caries (p<0.01) (Table II).

In group II, 276 teeth in 30 patients had shallow caries.
In group III, only 4 patients’ oral cavity had dental caries lesions, extending close to the dentin-pulp interface. There were dental caries lesions, which were expected to extend into the coronal pulp in 12 patients. The remaining 14 patients had caries lesions, extending to the total pulp, which were found to have high YKL-40 levels in saliva (Table III). 85 teeth in 30 patients had advanced caries and 45 teeth in these patients had shallow caries. There was a statistically significant correlation between YKL-40 and the number of advanced dental caries (Table IV). A positive correlation between YKL-40 and GI was found in all groups (Table IV). In addition, there was no statistically significant difference in terms of age and gender (p>0.05).

In group III, GI score, shallow caries, and advanced caries describe 21%, 13%, and 51% of the YKL-40 levels in saliva, respectively. 40% of the YKL-40 levels in saliva are described by GI, while 38% of them are described by shallow caries in group II. GI scores describe 30% of them in group I (Table IV).
GI score, shallow caries, and advanced caries explain 76% of the YKL–40 level in saliva. The best explanation of the saliva YKL–40 was made with advanced caries ($\beta = 0.663$) (Table V).

**Discussion**

In this study, we found that YKL–40 levels of saliva were significantly higher in an unhealthy oral cavity with caries-deep&shallow caries than in a healthy oral cavity, and these levels were correlated with dental caries and oral hygiene. Dental caries and periodontal diseases are the most common chronic diseases. Of the more than 300 species of bacteria in the oral cavity, only some of them, known as *S. mutans*, are caries-causing (cariogenic) organisms. *S. mutans* is the first bacteria growing in dental plaques. Dental plaque may cause tooth decay, periodontal diseases, or the both at the same time.

There are studies showing that *S. mutans* stimulates the expression of cytokines, TNF-α, IL-1β, IFN-γ. On the other hand, it was reported that IFN-γ led to an increase in the production of YKL–40. Based on the results of these studies, it can be conjectured that there is a correlation between YKL–40 and *S. mutans*. In our study, a correlation was found between YKL–40 in saliva and dental caries and it is known that *S. mutans* is the source of caries.

Keles et al. found that serum and GCF YKL–40 levels were also significantly higher in the patients with gingivitis and chronic periodontitis than those of control groups. The highest level of YKL–40 was obtained in the group with periodontitis, followed by the group with gingivitis and healthy group, respectively. YKL–40 is considered being associated with severity of periodontal diseases. In our study, which is similar to Keles et al.’s, YKL–40 levels gradually increased from healthy group to the group with shallow caries and then to the group with deep caries that was correlated with GI. This correlation can also be related to the situation that chewing cannot be performed well in oral cavity with deep caries due to both anatomical disruption of teeth and dental pain. The reason is that when an individual does not brush his/her teeth, the dental plaque accumulation is higher in the quadrant where no chewing is performed.

In this study, it was found out that the deep dentinal caries led to an increase in the level of YKL–40 in
saliva but did not cause an increase in the number of dental caries. This results shows that intense inflammatory properties in oral cavity are more affected by the size of caries than by the number of caries. Gram-positive and negative bacteria can be detected in dental caries. There are different responses of odontoblasts to gram-positive and gram-negative bacteria. The studies on the relationship between dental caries and cytokines reported that dental caries could lead to inflammation of the dental pulp, resulting in aggregation of inflammatory cells that in turn release inflammatory cytokines.\textsuperscript{12,13}

In this study, YKL–40 was considered being associated with severity of caries and gingivitis. All of the shallow and advanced dental caries and gingivitis indicate YKL–40 level in saliva, but the descriptive capacity of advanced dental caries was higher than that of GI and shallow dental caries. In addition, YKL–40 can be used as a biomarker of inflammation in advanced caries with a pulpal involvement. As the level of inflammation, measured by using PUFA/pufa in dental caries increases, the YKL–40 level in saliva increases too.

Data in this field indicate that inflammatory process is responsible for enhancing YKL–40 production\textsuperscript{14,15} Many inflammation markers have been correlated with YKL–40. This study showed that YKL–40, the indicator of many disease, could also be an indicator of dental caries and gingivitis.

Limitations
1. No previous studies in this region were available for comparison.
2. Serum levels could not be evaluated because it was difficult to take blood from the children.
3. The mean age of the participants in the control group was smaller than in test groups. The number of individuals without dental caries is quite few in Turkey. We detected the individuals in the control group during our dental screening in kindergartens for this study. The individuals in the test groups were mostly in mixed dentition period, and the period of teeth eruption may have affected the YKL–40 levels somewhat, which had to be ignored, though. This, however, can be evaluated
in future studies.

4. This research is a cross-sectional study. It cannot allow causative relationship to be established.

Conclusions
It may be suggested that dental caries, gingivitis, and an infected pulp could increase YKL-40 release in saliva. Additional studies should be conducted to explain the role of YKL-40 in hard and soft tissue pathogenesis in oral cavity, and longitudinal prospective studies with a larger population are needed to confirm the findings of the present study. The authors of this study suggest that further studies will determine the relationship between the amount of bacteria causing dental caries and periodontal diseases and YKL-40 level in saliva.

Abbreviations
GI: Gingival index
PI: Plaque index
DMFT/dmft: Decayed-missing-filled teeth
DMFS/dmfs: Decayed-missing-filled teeth surfaces
ICDAS: International Caries Detection and Assessment System
PUFA/pufa: Exposed pulp, Ulceration, Fistula, Absess
S. mutans: Streptococcus mutans
pg/mL: Picogram/milliliter
ng/mL: Nanogram/milliliter
min: minute

Declarations
Ethics approval and consent to participate
Ethical approval was obtained from Clinical Research Ethics Committee in Turkey: Inönü University School of Medicine (ethic number: 2017/67). This study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from the parents before the examination.

Consent for publication
Authors provide formal written Consent to Publish before publication. The Author grants the Publisher the sole and exclusive license of the full copyright in the Contribution, which license the Publisher hereby accepts.

Availability of data and material
Not applicable

Competing interests
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Authors’ contributions
GD conceived the idea for the research, wrote the initial framework, performed the statistical analysis, and drafted the manuscript as the principal author. EL participated in the design of the study, performed the biochemical tests in the laboratory, and revised the manuscript.

Both authors read and approved the final manuscript.

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Tables

Table I. ICDAS II code
| ICDAS II code | Description |
|----------------|-------------|
| 0              | Sound tooth surface, no evidence of caries after prolonged air drying (5 seconds) |
| 1              | First visual change in enamel: opacity or discolouration (white or brown) is visible at the entrance to the pit or fissure after prolonged air drying, which is not or hardly seen on a wet surface |
| 2              | Distinct visual change in enamel: Opacity or discoloration distinctly visible at the entrance to the pit and fissure when wet, the lesion must still be visible when dry |
| 3              | Localized enamel breakdown due to caries with dentine or underlying shadow: opacity or discoloration wider than the natural fissure/fossa when wet and after prolonged air drying |
| 4              | Underlying dark shadow from dentin, +/− Localized enamel breakdown |
| 5              | Distinct cavity with visible dentine: visual evidence of demineralisation and dentine exposed |
| 6              | Extensive distinct cavity with visible dentine and more than half of the surface involved |

| PUFA / puFA index | Description |
|-------------------|-------------|
| P/p               | Pulpal involvement |
| U/u               | Ulceration |
| F/f               | Fistula |
| A/a               | Abscess |

Table II. The mean±SD of YKL-40 levels in saliva and clinic parameters of groups.
|                                      | Group I     | Group II    | Group III   |
|--------------------------------------|-------------|-------------|-------------|
| YKL-40 (ng/mL)                       | 25.76 ± 9.29| 52.42 ± 19.18| 83.91 ± 34.54|
| DMFT/dmft                            | 0.00 ± 0.00 | 18.03 ± 3.91| 8.80 ± 2.57 |
| DMFS/dmfs                            | 0.00 ± 0.00 | 53.40 ± 10.33| 24.23 ± 8.11|
| Filling teeth (n)                    | 0.00 ± 0.00 | 4.10 ± 4.37<sup>a</sup> | 2.83 ± 1.80<sup>a</sup> |
| Missing teeth (n)                    | 0.00 ± 0.00 | 4.73 ± 2.33  | 1.63 ± 1.50  |
| Caries teeth (n)                     | 0.00 ± 0.00 | 9.20 ± 3.56  | 4.33 ± 2.00  |
| Advanced caries teeth (n)            | 0.00 ± 0.00 | 0.00 ± 0.00  | 2.83 ± 1.60  |
| GI                                   | 0.27 ± 0.23 | 0.82 ± 0.34  | 1.11 ± 0.41  |
| PI                                   | 0.78 ± 0.28 | 1.07 ± 0.41<sup>a</sup> | 1.02 ± 0.22<sup>a</sup> |

*<p><sup>a</sup>p<0.01. The same letters in the same row do not indicate significant differences (p>0.05)</p>

Table III. The distribution of the advanced caries teeth (n) according to the PUFA/pufa index and the mean values of YKL-40 in group III
The number of patients
(n) | Advanced caries teeth (n) | PUFA/PUFA | YKL-40 Mean ± Std. Deviation
---|---|---|---
1 | 1 | A | 132.88
4 | 1 | N | 38.06 ± 3.11
1 | 4 | NNFF | 130.12
1 | 5 | NNNPP | 106.31
1 | 4 | NNPP | 113.42
1 | 5 | NNPPF | 121.21
2 | 5 | NNPPP | 113.76 ± 10.54
1 | 6 | NNPPPF | 115.93
1 | 4 | NPFA | 130.12
1 | 4 | NPPP | 88.81
3 | 4 | NPPU | 97.01 ± 14.21
3 | 1 | P | 59.40 ± 4.18
1 | 3 | PFA | 132.88
2 | 2 | NP | 46.87 ± 0.00
1 | 2 | NNP | 40.57
1 | 2 | PP | 76.21
1 | 4 | PPPF | 130.12
3 | 2 | PU | 64.48 ± 10.93
1 | 1 | U | 62.68
30 | 85 teeth | | 83.91 ± 34.54

**N**: None- pulp involvement

**Table IV. The correlation between the parameters**

| Groups | DMFT | DMFS | Filling | Caries Missing | Advanced Caries | GI | PI |
|---|---|---|---|---|---|---|---|
| 1 | YKL-40 | . | . | . | . | r; 0.501* p; 0.011 | r; -0.133 p; 0.525 |
| GI | . | . | . | . | . | . | r; -0.274 |
|        | YKL-40 | DMFT/dmft | DMFS/dmfs | Filling | Shallow caries | Missing | GI | YKL-40 | DMFT/dmft | DMFS/dmfs | Filling | Shallow caries | Missing | GI |
|--------|--------|-----------|-----------|---------|---------------|---------|----|--------|-----------|-----------|---------|---------------|---------|----|
| IV     | r; 0.502** p; 0.005 | r; 0.671** p; 0.000 | r; -0.231 p; 0.220 | r; 0.642** p; 0.000 | r; 0.280 p; 0.135 | . | r; 0.638** p; 0.000 | r; 0.485** p; 0.007 | . | . | . | . | . |
|        | r; 0.713** p; 0.000 | r; 0.326 p; 0.079 | r; 0.401* p; 0.028 | r; 0.280 p; 0.134 | . | r; 0.547** p; 0.002 | r; 0.349 p; 0.059 | . | . | . | . | . | . |
|        | r; -0.146 p; 0.441 | r; 0.481** p; 0.007 | r; 0.578** p; 0.001 | . | r; 0.706** p; 0.000 | r; 0.589** p; 0.001 | . | r; -0.159 p; 0.401 | r; -0.264 p; 0.158 | . | . | . | . | . |
|        | r; -0.496* p; 0.005 | r; -0.402* p; 0.028 | . | r; 0.142 p; 0.453 | . | r; 0.631** p; 0.000 | r; 0.632** p; 0.000 | . | . | . | . | . | . | . |
|        | r; 0.114 p; 0.550 | r; 0.037 p; 0.845 | r; 0.034 p; 0.860 | r; 0.267 p; 0.153 | r; -0.327 p; 0.078 | . | r; 0.653** p; 0.000 | r; 0.452* p; 0.012 | r; 0.386* p; 0.035 | . | . | . | . | . |
|        | r; 0.766** p; 0.000 | r; 0.544** p; 0.002 | r; 0.496** p; 0.005 | r; 0.195 p; 0.302 | r; -0.139 p; 0.463 | r; -0.286 p; 0.126 | r; 0.038 p; 0.842 | . | . | . | . | . | . | . |
|        | r; 0.167 p; 0.378 | r; 0.274 p; 0.143 | r; 0.598** p; 0.000 | r; -0.094 p; 0.622 | . | r; -0.271 p; 0.147 | r; 0.151 p; 0.425 | . | . | . | . | . | . | . |
|        | r; -0.079 p; 0.679 | r; -0.303 p; 0.103 | r; -0.303 p; 0.103 | r; -0.046 p; 0.810 | r; -0.075 p; 0.693 | r; 0.131 p; 0.491 | . | . | . | . | . | . | . |
|        | r; -0.271 p; 0.148 | r; 0.228 p; 0.227 | r; 0.228 p; 0.227 | r; -0.099 p; 0.603 | . | r; -0.273 p; 0.145 | r; 0.117 p; 0.540 | . | . | . | . | . | . | . |
Table V. Explanation of salivary YKL-40 with linear and multiple regression analyses

| Group | YKL-40 | B  | SE B | β   | t   | YKL-40 | B  | SE B | β   | t   | YKL-40 | B  | SE B | β   | t   |
|-------|--------|----|------|-----|-----|--------|----|------|-----|-----|--------|----|------|-----|-----|
| I     | Cons   | 19.7| 2.48 |    | 7.95| ***    |    |      |     |     |        |    |      |     |     |
|       | Predict| 22.6| 7.14 | 0.55| 3.17| **     |    |      |     |     |        |    |      |     |     |
|       | R      | 0.552|     |     |     |        |    |      |     |     |        |    |      |     |     |
| II    | Cons   | 23.3| 7.33 | 3.19| 8.77| **     |    |      |     |     |        |    |      |     |     |
|       | Predict| 35.5| 8.31 | 0.63| 4.15| ***    |    |      |     |     |        |    |      |     |     |
|       | R      | 0.629|     |     |     |        |    |      |     |     |        |    |      |     |     |
| III   | Cons   | 40.3| 16.9 | 2.39| 2.74| *      |    |      |     |     |        |    |      |     |     |
|       | Predict| 39.1| 14.2 | 0.46| 2.05| *      |    |      |     |     |        |    |      |     |     |
|       | R      | 0.459|     |     |     |        |    |      |     |     |        |    |      |     |     |

Multiple regression analysis for all participants

| YKL-40 | B  | SE B | β   | t   | p val |
|--------|----|------|-----|-----|-------|
| (Constant) | 20.531| 3.451|    | 5.949| <0.001|
| Advanced caries | 13.402| 1.527| 0.663| 8.777| <0.001|
| (n=85) |     |      |     |     |       |
| Shallow caries | 2.428| 0.516| 0.321| 4.702| <0.001|
| (n=321) |     |      |     |     |       |
| GI     | 13.372| 5.964| 0.192| 2.242| 0.02  |

Model

| R   | R²   | Adjusted R² | SEE   |
|-----|------|-------------|-------|
| 0.877| 0.769| 0.761| 16.36501|

Figures
Figure 1

YKL-40 levels (ng/ml) in groups

Figure 2

GI and PI levels in groups