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Serum YKL-40 Concentration and CHIT1 and MPO Activities in Women with Fibromyalgia: An Exploratory Case–Control Study

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Abstract

Background and Objectives: Evidence for peripheral inflammatory biomarkers in fibromyalgia (FM) remains heterogeneous. We compared serum YKL-40 concentration and chitotriosidase-1 (CHIT1) and myeloperoxidase (MPO) activities between women with FM and healthy controls. *Materials and Methods*: This single-center exploratory case–control study included 30 patients and 32 controls. YKL-40 was measured by enzyme-linked immunosorbent assay and CHIT1 and MPO by activity assays. Age-adjusted log-linear models estimated FM/control geometric mean ratios (GMRs) with robust 95% confidence intervals (CIs); Holm correction covered the three primary comparisons. *Results*: Mean age was 42.47 ± 7.33 years in patients and 26.88 ± 5.76 years in controls. Age-adjusted GMRs were 1.12 for YKL-40 (95% CI 0.62–1.99), 1.17 for CHIT1 (0.82–1.67), and 1.27 for MPO (0.98–1.65); Holm-adjusted p-values were 0.748, 0.748, and 0.205, respectively. Neither secondary age-adjusted comparisons nor exploratory clinical correlations met their multiplicity-adjusted thresholds. *Conclusions*: The primary comparisons did not show statistically significant group differences after multiplicity correction. Joint serum assessment adds quantitative evidence on serum YKL-40 concentration and enzyme activities, particularly CHIT1 activity, in women with FM.

Keywords: fibromyalgia; YKL-40; chitotriosidase; myeloperoxidase; inflammation; age adjustment

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1. Introduction

Fibromyalgia (FM) is a chronic pain condition characterized by widespread pain, fatigue, sleep disturbance, and cognitive symptoms [1]. The 2016 revision of the American College of Rheumatology (ACR) criteria incorporates the Widespread Pain Index (WPI), Symptom Severity Scale (SSS), generalized pain distribution, and symptom duration [2]. Clinical heterogeneity and the absence of an established diagnostic blood test continue to complicate biomarker research [3,4].

Peripheral immune alterations have been reported in FM, but circulating biomarker profiles are heterogeneous and lack established disease specificity [3,5]. Experimental transfer of patient immunoglobulin G induced sensory hypersensitivity in mice, supporting a peripheral immune contribution in that model [6]. The relevance of individual circulating analytes to human FM nevertheless requires direct investigation.

YKL-40 is a chitinase-like protein involved in inflammation and tissue remodeling [7]. Chitotriosidase-1 (CHIT1) is a catalytically active chitinase released by macrophages and

neutrophils, whereas myeloperoxidase (MPO) contributes to neutrophil oxidant production [8,9]. Previous FM studies assessed plasma YKL-40 within multianalyte profiles and MPO within oxidative-stress panels [10,11]; evidence concerning serum CHIT1 activity in FM remains limited. Joint measurement characterizes remodeling-associated protein concentration alongside phagocyte-related enzyme activities in the same participants. Protein concentration and catalytic activity, however, represent different biological measurements and cannot be interpreted interchangeably.

We compared serum YKL-40 concentration and CHIT1 and MPO activities between women with FM and healthy controls, with exploratory assessment of symptom associations and routine laboratory indices. Age was considered biologically relevant because population data show age-related variation in plasma YKL-40 [12]. The objective was to characterize complementary serum measurements in FM.

2. Materials and Methods

2.1. Study Design and Participants

This single-center case–control study used cross-sectional assessments at the Physical Therapy and Rehabilitation Clinic, Tınaztepe University Buca Medical Center, İzmir, Türkiye. Recruitment and blood sampling were reported for 7 March–25 August 2026. The protocol was approved by the İzmir Tınaztepe University Non-Interventional Clinical Research Ethics Committee (2026/26; 6 March 2026). Written informed consent was obtained before study-specific procedures, in accordance with the Declaration of Helsinki.

Women aged 18–65 years with previously diagnosed FM were recruited consecutively from eligible clinic attendees. Controls were convenience volunteers from the same catchment area, screened by history and examination for FM, chronic widespread pain during the preceding three months, infection, inflammatory disease, pregnancy, and immune-modifying treatment. Exclusion criteria comprised inflammatory or autoimmune rheumatic disease, active infection, malignancy, hematologic disorders, pregnancy or lactation, systemic corticosteroid, immunosuppressive or biological treatment within six weeks, and other conditions considered to affect chitinase or MPO measurements. Consecutive patient recruitment and convenience control recruitment proceeded without individual age matching, resulting in a younger control group.

The reported screening flow included 42 patient candidates and 42 control candidates; 12 and 10, respectively, were excluded, leaving 30 patients and 32 controls. One primary exclusion reason was recorded for each excluded candidate. Figure 1 summarizes these recruitment counts and outcome-specific analysis populations.

2.2. Clinical Assessments

A Physical Medicine and Rehabilitation specialist (K.C.K.) assessed diagnostic eligibility using the 2016 ACR framework [2]. Previously diagnosed patients underwent a separate assessment of symptoms at blood sampling. The author-confirmed clinical information indicated that the two patients with SSS scores of 4 at blood sampling had met the WPI/SSS, generalized-pain, and symptom-duration requirements at diagnosis.

Height and weight were measured without shoes and in light clothing using a stadiometer and calibrated scale. Body mass index (BMI) was calculated as weight (kg)/height $(m)^2$ and was treated as a continuous variable.

WPI (0–19) and SSS (0–12) were clinician-administered in Turkish on the blood-sampling day using the 2016 framework, distinguishing preceding-week symptoms from six-month SSS items. Disease impact was assessed with the original Turkish Fibromyalgia Impact Questionnaire (FIQ; 0–100), using its ten-component weighting [13]. The Symptom Checklist-90-Revised (SCL-90-R) was reported as the Global Severity Index (GSI; 0–4),

the mean item response for the preceding week. WPI, FIQ, and SSS were available for 30 patients and GSI for 29. These assessments were not administered to controls.

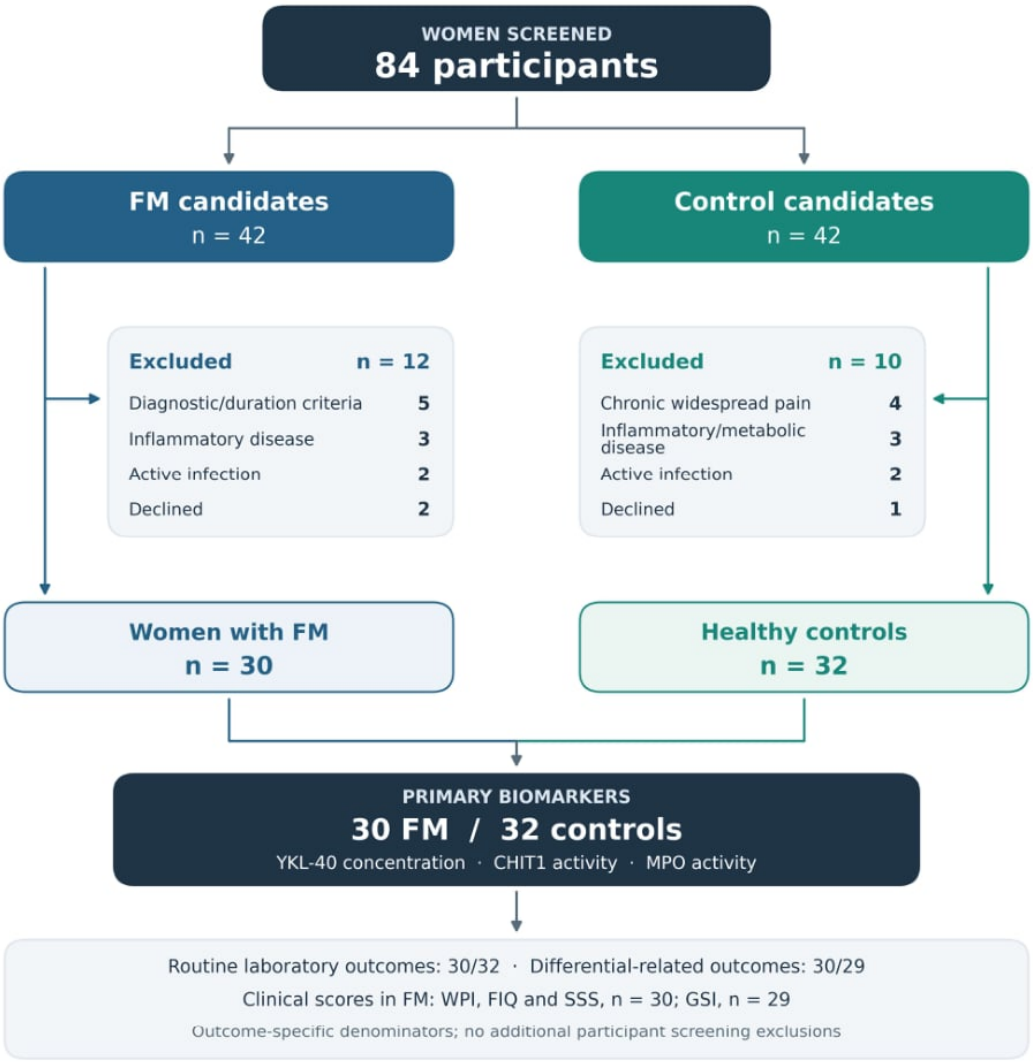


Figure 1. Reported participant recruitment and outcome-specific sample sizes. One primary exclusion reason was recorded per excluded candidate. FM, fibromyalgia; CHIT1, chitotriosidase-1; MPO, myeloperoxidase; GSI, Global Severity Index; WPI, Widespread Pain Index; FIQ, Fibromyalgia Impact Questionnaire; SSS, Symptom Severity Scale.

2.3. Laboratory Measurements

Venous blood for the three serum biomarker assays was collected between 08:00 and 10:00 after 8–12 h of fasting into tubes without anticoagulant. Samples clotted for 20 min at room temperature, were centrifuged at 3000× *g* for 20 min, and were stored in 0.5-mL serum aliquots at −80 °C. The laboratory reported assays on 1 September 2026 after 7–178 days of storage. Samples underwent one freeze–thaw cycle; repeat measurements used the same thawed aliquot. Both groups underwent the same processing.

Serum YKL-40 concentration (ng/mL) was measured by enzyme-linked immunosorbent assay (ELISA; Bioassay Technology Laboratory, Shanghai, China, E3587Hu) with four-parameter logistic calibration. Manufacturer specifications were a calibration range of 0.5–200 ng/mL, analytical sensitivity of 0.21 ng/mL, and intra-assay and inter-assay coefficients of variation (CVs) of <8% and <10%, respectively. These specifications describe kit performance rather than precision measured in the present study.

CHIT1 activity (nmol/mL/h) was measured using the fluorometric method of Hollak et al. [14], with the one-hour procedure of Gunes et al. adapted to serum and a Varioskan reader (Thermo Fisher Scientific Inc., Waltham, MA, USA) [15]. The reaction used 10 µL of serum and 100 µL of 0.09 mmol/L 4-methylumbelliferyl-linked chitotriose substrate in 0.25 mol/L sodium acetate buffer (pH 5.5) at 37 °C for 1 h and was stopped with 200 µL of 0.1 mol/L ethylenediamine. Released 4-methylumbelliferone was measured at 365 nm excitation against a product calibration curve. Activity was calculated as product amount (nmol) / (serum volume [mL] × incubation time [h]), with a dilution factor of 1.

MPO activity was measured using an o-dianisidine kinetic assay adapted from Grisham et al. [16]. Absorbance was recorded at 492 nm every 15 s for 3 min; blank-corrected slopes were converted using an activity-standard calibration curve with a dilution factor of 1. Results are expressed in laboratory-assigned U/L.

Samples were assayed in duplicate, with calibrators and blanks. Coded patient and control samples were mixed across plates, and the operator was blinded to group.

Routine measurements included C-reactive protein (CRP; mg/L), white blood cell count (WBC; $\times 10^9$/L), neutrophil, lymphocyte, and monocyte percentages, hemoglobin (g/dL), hematocrit (%), and erythrocyte sedimentation rate (ESR; mm/h).

Absolute neutrophil, lymphocyte, and monocyte counts (ANC, ALC, and AMC; $\times 10^9$/L) were calculated as WBC × the corresponding percentage / 100. The neutrophil-to-lymphocyte ratio (NLR) and monocyte-to-lymphocyte ratio (MLR) were dimensionless. The systemic inflammation response index (SIRI; $\times 10^9$/L) was calculated as ANC × AMC / ALC.

All YKL-40, CHIT1, and MPO values were positive; all 62 participants contributed to the primary biomarker comparisons. Sample sizes for each analysis are reported in Supplementary Table S6.

2.4. Statistical Analysis

The original analyses used IBM SPSS Statistics for Windows, Version 27.0 (IBM Corp., Armonk, NY, USA). Data are summarized as mean ± standard deviation, median [first–third quartiles], or *n* (%). Groups were compared using Welch's *t*-test, Mann–Whitney U test, or Fisher's exact test. Age-adjusted log-linear models with type 3 heteroskedasticity-consistent (HC3) standard errors estimated FM/control geometric mean ratios (GMRs) with t-based 95% confidence intervals (CIs). Spearman correlation assessed clinical associations. Sensitivity analyses considered the common age range, quadratic age, BMI and smoking, age-overlap weighting [17], and selected-record exclusions. All 62 participants contributed to primary comparisons; differential-related outcomes used 59 eligible records, with component totals >102% omitted and 100.1% retained as rounding. Available observations were analyzed without imputation. Percentile-bootstrap CIs used 10,000 resamples for unadjusted effects, correlations, and primary adjusted estimates, and 2000 attempts for weighted estimates, refitting weights in each resample. Holm correction covered three biomarkers within each model; separate Benjamini–Hochberg families covered 14 adjusted secondary outcomes, 14 unadjusted secondary comparisons, and 68 correlations. Sample size reflected recruitment feasibility. The analytical hierarchy was specified during revision, and all analyses are exploratory. Two-sided adjusted *p*-values or q-values < 0.05 defined the reporting threshold.

2.5. Use of Generative Artificial Intelligence

ChatGPT (OpenAI, San Francisco, CA, USA, https://chatgpt.com; accessed 16 November 2026) assisted with language editing, formatting, figure preparation, statistical code development and review, document consistency checks, and preparation of revision docu-

ments. The authors retain responsibility for the analytical methods, verification, interpretation, and manuscript content.

3. Results

3.1. Participant Characteristics

The analysis included 30 women with FM and 32 controls (Figure 1; Table 1). Patients were older by 15.59 years (95% CI 12.22–18.96): mean age was 42.47 ± 7.33 versus 26.88 ± 5.76 years. BMI averaged 24.36 ± 2.52 versus 23.64 ± 2.95 kg/m^2, and smoking was reported by 9 patients (30.0%) and 10 controls (31.3%).

Table 1. Demographic characteristics of the study groups.

Variable	FM (n = 30)	Controls (n = 32)	Effect Estimate	95% CI	p-Value
Age (years)	42.47 ± 7.33	26.88 ± 5.76	MD 15.59 g = 2.35	12.22–18.96	<0.001
Height (cm)	161.00 ± 5.71	163.31 ± 4.65	MD −2.31 g = −0.44	−4.97–0.35	0.087
Weight (kg)	63.13 ± 7.40	62.97 ± 7.55	MD 0.16 g = 0.02	−3.63–3.96	0.931
BMI (kg/m^2)	24.36 ± 2.52	23.64 ± 2.95	MD 0.72 g = 0.26	−0.67–2.11	0.306
Smoking, n (%)	9 (30.0)	10 (31.3)	OR 0.94	0.32–2.78	1.000

Values are mean ± SD or n (%). Mean differences (MDs) and Hedges' g are oriented FM minus control; the smoking odds ratio (OR) compares FM with controls. The 95% CI column refers to the MD or OR, not to Hedges' g. Continuous comparisons used Welch's t-test, and smoking used Fisher's exact test. BMI was calculated from height and weight; p-values are unadjusted descriptive comparisons. BMI, body mass index; CI, confidence interval; FM, fibromyalgia; SD, standard deviation.

3.2. Primary Biomarker Comparisons

The primary age-adjusted estimates were YKL-40 GMR 1.12 (95% CI 0.62–1.99; Holm $p = 0.748$), CHIT1 GMR 1.17 (0.82–1.67; Holm $p = 0.748$), and MPO GMR 1.27 (0.98–1.65; Holm $p = 0.205$). None met the multiplicity-adjusted threshold. All three comparisons included 30 patients and 32 controls (Table 2).

Table 2. Unadjusted and age-adjusted comparisons of YKL-40 concentration and CHIT1 and MPO activities.

(a) Biomarker	n FM/C	FM Median [Q1–Q3]	Control Median [Q1–Q3]	HL (95% CI)	r	Raw p	Holm p
YKL-40 concentration (ng/mL)	30/32	19.35 [16.62–24.25]	19.40 [12.71–22.62]	1.70 (−2.48–5.60)	0.12	0.410	0.410
CHIT1 activity (nmol/mL/h)	30/32	17.71 [12.80–27.10]	15.13 [8.74–18.40]	4.31 (0.23–9.18)	0.30	0.043	0.130
MPO activity (U/L)	30/32	5.58 [4.70–7.90]	5.13 [4.37–6.67]	0.63 (−0.16–1.64)	0.24	0.099	0.199
(b) Biomarker	**n FM/C**	**Age-Adjusted GMR (95% CI)**				**Raw p**	**Holm p**
YKL-40 concentration	30/32	1.12 (0.62–1.99)				0.709	0.748
CHIT1 activity	30/32	1.17 (0.82–1.67)				0.374	0.748
MPO activity	30/32	1.27 (0.98–1.65)				0.068	0.205

Panel (a): medians [Q1–Q3], Hodges–Lehmann (HL) shifts with 10,000-resample percentile-bootstrap 95% CIs, rank-biserial r and Mann–Whitney p-values. Positive effects indicate higher values in FM. Panel (b): age-adjusted FM/control GMRs with HC3 robust t-based 95% CIs. All three comparisons include 30 patients and 32 controls (residual df 59). Holm correction covers three comparisons separately within each panel. GMRs and r are dimensionless; medians, quartiles, and HL estimates use the stated assay units. CIs are pointwise. CHIT1, chitotriosidase-1; CI, confidence interval; FM, fibromyalgia; GMR, geometric mean ratio; MPO, myeloperoxidase.

3.3. Secondary Laboratory Outcomes

None of the 14 age-adjusted secondary outcomes met the BH-FDR threshold (Table 3). ANC and ALC had raw *p*-values of 0.046 and 0.045, respectively, and q = 0.319 for both. Differential-related analyses included 30 patients and 29 controls.

Table 3. Age-adjusted secondary laboratory and inflammatory outcomes.

Outcome (Unit)	*n* FM/C	Age-Adjusted GMR (95% CI)	Raw *p*	BH q
CRP (mg/L)	30/32	0.95 (0.31–2.87)	0.926	0.963
WBC ($\times 10^9$/L)	30/32	1.28 (0.97–1.69)	0.075	0.326
Neutrophils (%)	30/29	1.08 (0.99–1.17)	0.093	0.326
Lymphocytes (%)	30/29	1.02 (0.91–1.15)	0.732	0.854
Monocytes (%)	30/29	0.96 (0.82–1.13)	0.630	0.802
Hemoglobin (g/dL)	30/32	1.00 (0.93–1.07)	0.963	0.963
Hematocrit (%)	30/32	0.97 (0.92–1.03)	0.328	0.657
ESR (mm/h)	30/32	1.18 (0.77–1.79)	0.442	0.774
ANC ($\times 10^9$/L)	30/29	1.39 (1.01–1.91)	0.046	0.319
ALC ($\times 10^9$/L)	30/29	1.32 (1.01–1.72)	0.045	0.319
AMC ($\times 10^9$/L)	30/29	1.24 (0.89–1.72)	0.192	0.507
NLR (dimensionless)	30/29	1.05 (0.86–1.28)	0.599	0.802
MLR (dimensionless)	30/29	0.94 (0.77–1.16)	0.565	0.802
SIRI ($\times 10^9$/L)	30/29	1.31 (0.85–2.01)	0.217	0.507

Models include group and age and report dimensionless FM/control GMRs with HC3 robust t-based pointwise 95% CIs. Sample sizes are 30/32 (residual df 59) for CRP, WBC, hemoglobin, hematocrit and ESR, and 30/29 (df 56) for differential percentages and derived indices. BH-FDR correction covers all 14 outcomes. CRP, C-reactive protein; WBC, white blood cell count; ESR, erythrocyte sedimentation rate; ANC/ALC/AMC, absolute neutrophil/lymphocyte/monocyte count; NLR/MLR, neutrophil/monocyte-to-lymphocyte ratio; SIRI, systemic inflammation response index; CI, confidence interval; FM, fibromyalgia; GMR, geometric mean ratio.

3.4. Clinical Characteristics and Exploratory Correlations

Table 4 summarizes WPI, SSS, and FIQ in 30 patients and SCL-90-R GSI in 29 patients. None of the 68 clinical correlations met the BH-FDR threshold. The largest absolute coefficient was between CHIT1 activity and WPI ($\rho = 0.36$; bootstrap 95% CI −0.02–0.68; q = 0.999).

Table 4. Clinical scale distributions in women with fibromyalgia.

Clinical Scale	*n*	Mean ± SD	Median [Q1–Q3]	Range
WPI	30	12.13 ± 2.37	12.00 [10.25–14.00]	8.00–17.00
SSS	30	6.80 ± 1.65	6.50 [6.00–8.00]	4.00–11.00
FIQ	30	63.63 ± 9.81	63.50 [56.50–71.50]	46.00–82.00
SCL-90-R GSI	29	1.41 ± 0.49	1.44 [1.14–1.68]	0.65–2.80

Values summarize available patient observations; these scales were not administered to controls. FIQ is the original Turkish Fibromyalgia Impact Questionnaire (0–100); SCL-90-R GSI is the Global Severity Index (0–4); SSS, Symptom Severity Scale (0–12); WPI, Widespread Pain Index (0–19); SD, standard deviation.

3.5. Age Overlap and Sensitivity Results

The standardized mean difference (SMD) in age was 2.366. Restriction to 29–46 years retained 21 patients and nine controls for each biomarker, with a residual age SMD of 0.843. Overlap weighting balanced mean age at 34.96 years in both groups, with effective sample sizes of 11.8 patients and 10.1 controls. Figure 2 displays the age distributions and weights. Figure 3 shows biomarker measurements against age.

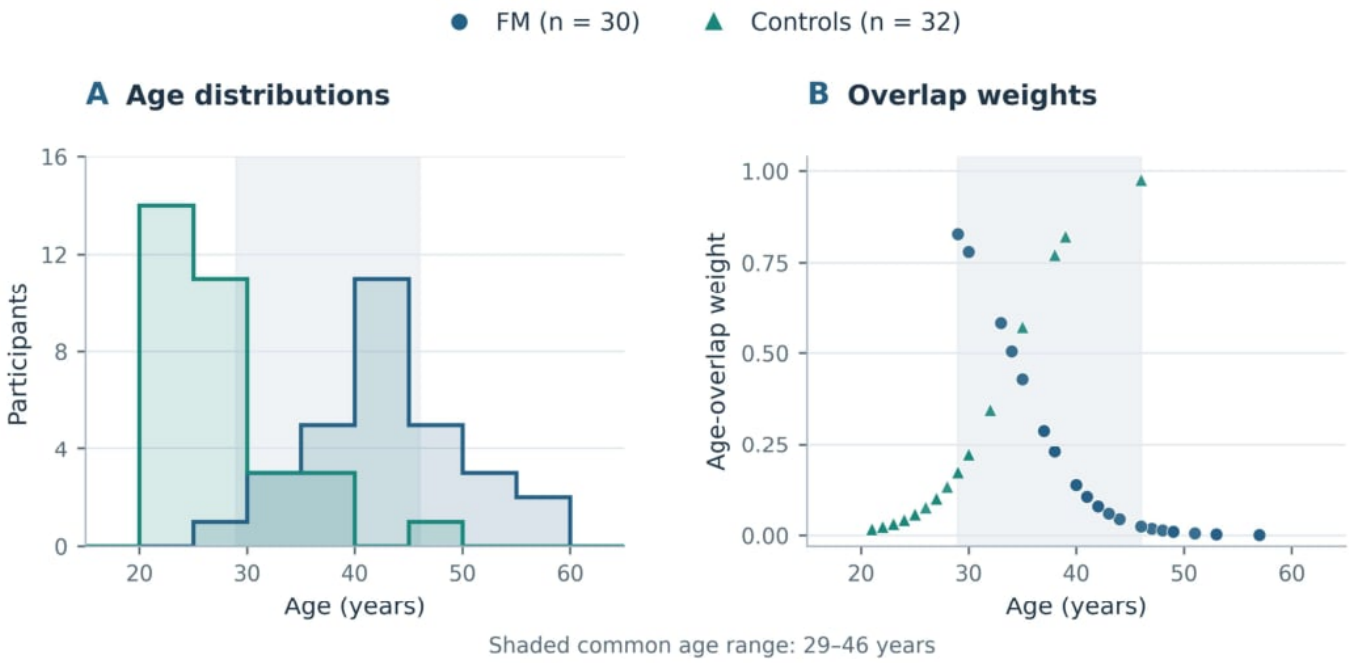


Figure 2. Age distributions (**A**) and age-overlap weights (**B**). Blue circles identify women with FM and green triangles identify controls; the histograms use the same group colors. Shading marks the common age range (29–46 years). Weights are from the age-only group-membership model for 62 participants; effective sample sizes are 11.8 patients and 10.1 controls. FM, fibromyalgia.

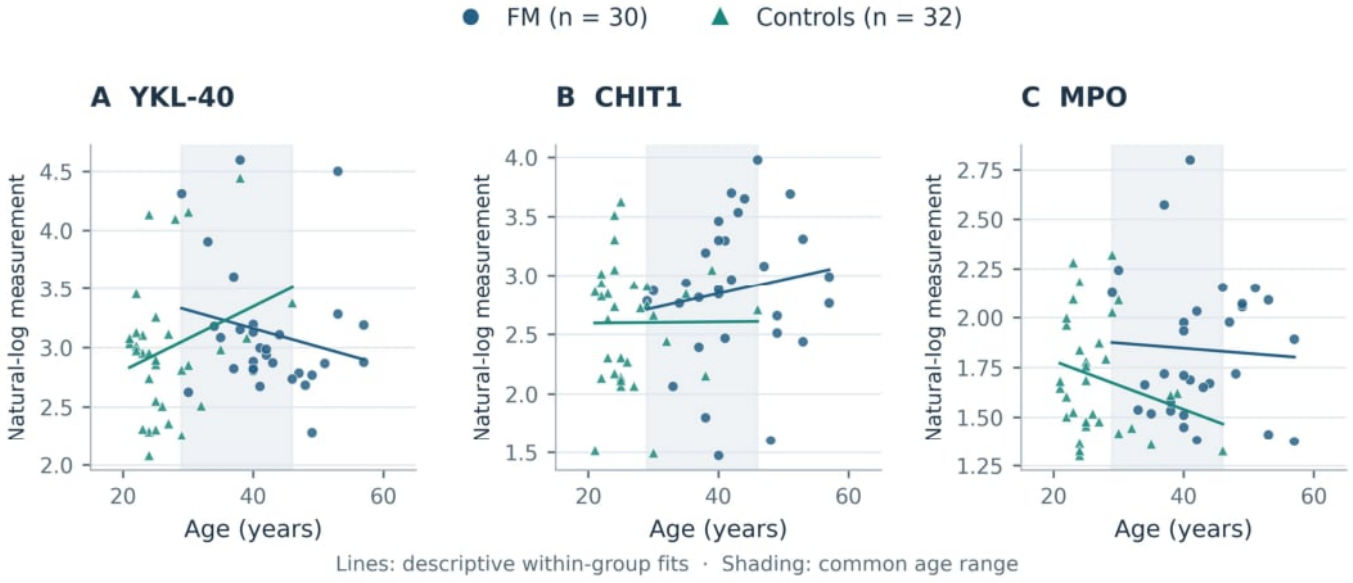


Figure 3. Natural-log biomarker values versus age: YKL-40 (**A**), CHIT1 (**B**), and MPO (**C**). Each panel includes 30 patients (blue circles) and 32 controls (green triangles). Lines are descriptive group-specific linear fits; shading marks the common age range (29–46 years). Original measurement units: YKL-40, ng/mL; CHIT1, nmol/mL/h; MPO, U/L.

In the full-sample model adjusted for age, calculated BMI, and smoking, the MPO GMR was 1.27 (95% CI 0.97–1.68; Holm $p = 0.246$). The earlier eight-record exclusion approach retained 29 patients and 25 controls and yielded an MPO GMR of 1.41 (1.08–1.84; within-model Holm $p = 0.037$). This selected-sample result remained exploratory. Common-age-range, quadratic-age, and overlap-weighted comparisons did not meet the within-model Holm threshold for any primary biomarker (Table 5). Primary age-adjusted bootstrap CIs

included one for all three biomarkers. The exploratory overlap-weighted MPO bootstrap CI was 1.06–1.63; this pointwise interval was not adjusted for multiple comparisons.

Table 5. Sensitivity analyses of YKL-40 concentration and CHIT1 and MPO activities.

Sensitivity Model	Biomarker	*n* FM/C	GMR (95% CI)	Raw *p*	Holm *p*
Age 29–46 years	YKL-40 concentration	21/9	1.06 (0.52–2.13)	0.874	0.959
Age 29–46 years	CHIT1 activity	21/9	1.18 (0.74–1.86)	0.479	0.959
Age 29–46 years	MPO activity	21/9	1.25 (0.96–1.63)	0.100	0.300
Age-overlap weighting	YKL-40 concentration	30/32	1.10 (0.57–2.09)	0.779	1.000
Age-overlap weighting	CHIT1 activity	30/32	1.12 (0.80–1.57)	0.508	1.000
Age-overlap weighting	MPO activity	30/32	1.33 (1.02–1.73)	0.036	0.108
Quadratic age	YKL-40 concentration	30/32	1.06 (0.55–2.05)	0.854	0.854
Quadratic age	CHIT1 activity	30/32	1.19 (0.82–1.72)	0.353	0.707
Quadratic age	MPO activity	30/32	1.30 (1.01–1.67)	0.042	0.126
Age, calculated BMI, smoking	YKL-40 concentration	30/32	1.11 (0.60–2.06)	0.737	0.793
Age, calculated BMI, smoking	CHIT1 activity	30/32	1.17 (0.81–1.70)	0.397	0.793
Age, calculated BMI, smoking	MPO activity	30/32	1.27 (0.97–1.68)	0.082	0.246
Age, source BMI, smoking	YKL-40 concentration	30/32	1.11 (0.59–2.07)	0.739	0.791
Age, source BMI, smoking	CHIT1 activity	30/32	1.17 (0.81–1.69)	0.396	0.791
Age, source BMI, smoking	MPO activity	30/32	1.27 (0.96–1.69)	0.092	0.275
Exclude study-visit SSS < 5	YKL-40 concentration	28/32	1.18 (0.65–2.15)	0.589	0.696
Exclude study-visit SSS < 5	CHIT1 activity	28/32	1.19 (0.83–1.70)	0.348	0.696
Exclude study-visit SSS < 5	MPO activity	28/32	1.27 (0.97–1.66)	0.085	0.254
Earlier BMI-subset exclusion	YKL-40 concentration	29/25	0.97 (0.51–1.84)	0.921	0.921
Earlier BMI-subset exclusion	CHIT1 activity	29/25	1.33 (0.91–1.95)	0.132	0.263
Earlier BMI-subset exclusion	MPO activity	29/25	1.41 (1.08–1.84)	0.012	0.037
Exclude Cook D > 4/*n*	YKL-40 concentration	27/31	1.06 (0.69–1.62)	0.789	0.789
Exclude Cook D > 4/*n*	CHIT1 activity	28/32	1.26 (0.89–1.77)	0.187	0.561
Exclude Cook D > 4/*n*	MPO activity	27/32	1.13 (0.88–1.46)	0.327	0.654

Values are FM/control GMRs with HC3 robust t-based pointwise 95% CIs; *n* is FM/control. Holm correction covers three biomarkers within each model, without adjustment across sensitivity models. Common-range models retain age adjustment; quadratic models add centered age squared; overlap models use group alone with estimated age-overlap weights. BMI models include age and smoking. The model excluding eight records with discrepant BMI values included 29 patients and 25 controls. All 62 participants contributed to the primary biomarker comparisons. Influence exclusions use Cook's distance > 4/*n* separately for each analyte. BMI, body mass index; CHIT1, chitotriosidase-1; CI, confidence interval; GMR, geometric mean ratio; MPO, myeloperoxidase.

4. Discussion

This study brings together serum YKL-40 concentration, CHIT1 activity, and MPO activity in the same participants, alongside clinical symptom scores and routine inflammatory indices. This complementary assessment adds serum CHIT1 activity data to a literature focused largely on circulating proteins and oxidative-stress panels. The three primary comparisons did not meet the multiplicity-adjusted threshold. The estimates provide a quantitative basis for subsequent studies of these biological dimensions of FM.

Our findings should be interpreted within a heterogeneous biomarker literature. Kumbhare et al. identified differences in several blood markers without establishing disease-specific diagnostic biomarkers [3], while Andrés-Rodríguez et al. reported immune alterations with substantial between-study heterogeneity [5]. A 2026 synthesis found a modest pooled high-sensitivity CRP (hsCRP) difference but no significant pooled differences in interleukin-6 or interferon-γ, emphasizing marker-specific findings and methodological

heterogeneity [18]. These reviews support careful attention to recruitment, confounding, and measurement context.

4.1. Interpretation of the Primary Biomarkers

Gerdle et al. studied 32 women with FM and 30 age-matched controls using a plasma multianalyte platform; plasma YKL-40 contributed to multivariate models of pressure-pain sensitivity across the combined sample and body-composition measures [10]. Our study examines serum YKL-40 measured by ELISA together with two enzyme activities. Matrix, platform, and recruitment differences are relevant when comparing these findings.

CHIT1 activity reflects catalysis rather than protein amount [8,19]. Including this measurement alongside YKL-40 distinguishes active chitinase function from chitinase-like protein concentration. The 24-base-pair duplication associated with inherited CHIT1 deficiency can markedly affect activity [20]. This biological variation is relevant to interpreting low activity values.

MPO estimates generally favored higher activity in FM, although the primary CI included one. Koca et al. measured an oxidative-stress panel in 44 patients and 44 demographically comparable controls, with significant findings involving other oxidative markers [11]. Urinary F_2-isoprostane studies address a different compartment and oxidative process [21]. Collection-matrix and handling effects on MPO concentration [22], and evidence of serum MPO-inhibitory activity [23], underscore the biological distinction between enzyme concentration and catalytic activity. Serum provided a common matrix for the three measurements in the present study.

4.2. Hematologic Indices and Clinical Symptom Scores

Evidence for leukocyte-derived indices is also mixed. İlgün et al. reported similar NLR values in patients and controls, whereas Almirall et al. found higher NLR in an age- and sex-matched FM comparison [24,25]. Koca et al. reported higher inflammatory indices in a larger retrospective cohort [26]. A 2026 systematic review synthesized 24 studies and found a pooled NLR difference [27]. Together, these reports place routine leukocyte indices within the broader investigation of peripheral immune features in FM.

Our secondary laboratory findings complement this literature by presenting routine inflammatory indices alongside the three serum measurements. Covariate sensitivity has also been demonstrated for hsCRP [28], supporting consideration of the clinical context when interpreting peripheral biomarker findings.

The clinical scores describe pain distribution, symptom severity, disease impact, and psychological distress in the patient group. These scores provide clinical context for the laboratory comparisons. Previous work on cerebrospinal-fluid and plasma profiles illustrates the value of distinguishing peripheral from central inflammatory compartments [29].

4.3. Limitations

The small, single-center, female-only sample and substantial age imbalance limit precision and generalizability; adjustment cannot fully restore group comparability. Limited age overlap makes adjusted estimates model-dependent, while weighting targets a narrower population with small effective samples. Older age may increase YKL-40 independently of FM [12], while the magnitude of residual bias cannot be quantified. Incomplete disease-duration, medication, sleep, physical-activity, and menopausal information leaves residual confounding. Item-level questionnaire responses and the specific Turkish SCL-90-R adaptation were unavailable. Manufacturer specifications do not replace study-specific validation; incomplete reaction and calibration documentation particularly limits interpretation of

MPO activity. CHIT1 genotyping was not performed. These exploratory cross-sectional findings do not establish equivalence, causality, or diagnostic utility.

4.4. Implications for Further Research

These complementary serum measurements warrant further investigation in age-comparable populations with detailed clinical characterization and standardized laboratory procedures. Longitudinal sampling could clarify their relationship to symptom variation over time. Proteomic evidence likewise supports methodological standardization in FM biomarker research [30]. Literature on microbiota-related lifestyle interventions in chronic widespread pain [31] and physical activity–microbiota relationships [32] further supports including lifestyle measures in future studies. Their relevance to YKL-40, CHIT1, and MPO in FM remains to be established.

5. Conclusions

Age-adjusted comparisons of serum YKL-40 concentration and CHIT1 and MPO activities did not show statistically significant differences between women with fibromyalgia and healthy controls after multiplicity correction. Their joint assessment adds quantitative evidence on complementary protein and enzyme measurements, particularly serum CHIT1 activity, to fibromyalgia biomarker research.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/medicina62122335/s1, Supplementary Statistical Methods; Table S1: Unadjusted primary effect sizes; Table S2: Unadjusted secondary comparisons; Table S3: All 68 exploratory clinical correlations; Table S4: Bootstrap checks for primary models; Table S5: Age-adjusted model diagnostics; Table S6: Outcome-specific sample sizes; Table S7: Exploratory testing families; Table S8: Assay methods and manufacturer performance information; Table S9: Secondary comparisons with all records and the outcome-specific analysis sample; Table S10: Summary of data revisions and analysis populations; Figure S1: Primary biomarker distributions; Figure S2: Age-adjusted primary biomarker comparisons; Figure S3: Age-adjusted secondary comparisons; Figure S4: Exploratory clinical correlations. Checklist S1: STROBE case–control reporting checklist.

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Informed Consent Statement: Written informed consent to participate was obtained from all participants before study-specific procedures.

Data Availability Statement: Aggregate results are provided with the article. De-identified participant data may be requested from Kudret Cem Karayol (kudretcemkarayol@hotmail.com), subject to consent, ethics and institutional permissions, and an appropriate data-use agreement.

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