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Research Article

Retrospective evaluation of histopathological lesion patterns and obstetric indications in placentas referred for pathological examination

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ABSTRACT

Aim: To characterize the distribution of histopathological lesion patterns in placentas referred for pathological examination on obstetric indication, to define the obstetric indications for referral, and to evaluate the associations between lesion patterns and clinical indications at a single tertiary center.

Methods: In this single-center, retrospective, descriptive and analytical archival study, records of placentas obtained from deliveries and submitted for pathological examination on clinical indication between January 2018 and February 2026 were reviewed. Obstetric indications and histopathological findings were extracted from the clinical and pathology reports and classified according to the Amsterdam Placental Workshop Group consensus into maternal vascular, fetal vascular, acute inflammatory, and chronic inflammatory patterns. Associations were assessed with the chi-square or Fisher exact test, and continuous variables were compared using the Mann-Whitney U test.

Results: A total of 399 placentas were analyzed. The mean maternal age was 30.3 years, and 73.9% of placentas were obtained by cesarean section. The most frequent indications were hypertensive disorders of pregnancy (14.3%), preterm birth (13.5%), congenital anomaly (12.8%), and fetal growth restriction (12.5%). Most placentas were reported as normal or non-specific (86.7%), and a defined lesion pattern was identified in 13.3%, among which acute inflammatory (5.3%) and maternal vascular (4.8%) patterns predominated. Acute inflammatory lesions were significantly more common among placentas referred for suspected infection (42.9%; $p = 0.005$), whereas the associations of maternal vascular malperfusion with hypertensive disorders, growth restriction, and diabetes did not reach significance.

Conclusion: In this large single-center series, most referred placentas were histologically unremarkable in routine reporting, with acute inflammatory and maternal vascular lesions predominating among abnormal cases, and acute inflammatory lesions were associated with suspected infection. Standardized sampling and synoptic reporting are needed to realize the diagnostic value of placental examination.

Keywords: placenta, placenta diseases, chorioamnionitis, pregnancy complications, pathology, retrospective studies

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Introduction

The placenta is a highly specialized, dynamic organ that lies at the center of the maternofetal interface and sustains fetal growth and development throughout gestation, while also serving important immunological and barrier functions (1,2). Because the structural and functional integrity of the placenta is tightly coupled to the intrauterine environment, a wide spectrum of obstetric complications, including fetal growth restriction (FGR), hypertensive disorders of pregnancy, preterm birth, gestational diabetes mellitus (GDM), placental abruption, and intrauterine fetal death, leaves identifiable morphological signatures in the placenta. Consequently, histopathological examination of the placenta has become a valuable tool for elucidating the pathophysiological basis of adverse pregnancy outcomes and for informing the management of subsequent pregnancies (2,3).

To standardize the reporting and interpretation of these changes, the Amsterdam Placental Workshop Group Consensus Statement classified placental injury into a limited number of reproducible patterns: maternal vascular malperfusion (MVM), fetal vascular malperfusion (FVM), acute inflammatory lesions (the amniotic fluid infection sequence, i.e., acute chorioamnionitis and the maternal and fetal inflammatory responses), and chronic inflammatory lesions (chiefly villitis of unknown etiology and related chronic patterns). This framework and its subsequent refinements continue to shape contemporary placental diagnostics, although certain entities, such as the definition and clinical significance of basal chronic villitis, remain the subject of ongoing consensus discussion (4).

A growing body of evidence links specific lesion patterns to distinct clinical presentations. Maternal vascular malperfusion lesions have been consistently associated with FGR and with hypertensive disorders of pregnancy, and have more recently been reported as a prominent feature of placentas from GDM pregnancies, particularly when complicated by growth restriction (3,5). Acute inflammatory lesions have been related to spontaneous preterm birth, increased neonatal intensive care requirements, and neonatal morbidity, and may act as independent predictors of adverse neonatal outcome and even of recurrent early preterm birth (6,7). Placental pathology has also been shown to differ between early- and late-onset presentations of placenta-associated complications; for example, early placental abruption is characterized by higher rates of maternal vascular malperfusion and maternal inflammatory response lesions and by more severe neonatal morbidity than late abruption (8). Comparable pattern-specific associations have been described in preeclampsia, including postpartum-onset disease, and in twin gestations complicated by growth restriction and preeclampsia (9,10).

Despite this accumulating knowledge, most published studies focus on a single clinical entity (e.g., GDM, FGR, or abruption in isolation) and are therefore limited in their ability to characterize the overall spectrum of placental pathology encountered in routine obstetric practice. Comprehensive, single-center analyses that evaluate all placentas referred for pathological examination across the full range of clinical indications, and that systematically relate histopathological lesion patterns to those indications, remain comparatively scarce, particularly within our national context.

Accordingly, the present study was designed to characterize the distribution of histopathological lesion patterns in placentas submitted for pathological examination at our institution, to define the obstetric indications for referral and their relative frequencies, and to evaluate the associations between lesion patterns and clinical indications. We specifically sought to determine which clinical presentations are most frequently associated with maternal vascular, fetal vascular, acute inflammatory, and chronic inflammatory lesion groups. In doing so, we

aimed to establish the institutional profile of placental pathology and to compare our findings with those reported in the international literature.

Materials and Methods

Study design and setting

This was a single-center, retrospective, descriptive and analytical archival study conducted at the Department of Medical Pathology, Kocaeli University Faculty of Medicine. Archived records of placentas obtained from deliveries performed at our institution between January 2018 and February 2026 and submitted for pathological examination on clinical indication were reviewed retrospectively. No intervention was performed; the study was based exclusively on the analysis of existing routine clinical and pathological records.

Ethical considerations

The study was approved by the Non-Interventional Clinical Research Ethics Committee of Kocaeli University Faculty of Medicine (approval no. GOKAEK-2026/05/35; project no. 2026/148; date of approval 06 March 2026) and was conducted in accordance with the principles of the Declaration of Helsinki. Because the study was a retrospective review of anonymized archival data with no patient contact, the requirement for written informed consent was waived by the ethics committee. All cases were anonymized and assigned a unique code number, and no identifying information was transferred to the study dataset.

Study population and case selection

All placentas from deliveries performed at our institution during the study period that were referred to the Department of Medical Pathology for histopathological examination on clinical indication were considered for inclusion. Cases were eligible if the delivery was performed at our institution, the placenta was submitted on a clinical indication, a completed histopathology report was available in the archive with adequate macroscopic and microscopic evaluation, and the obstetric indication for referral was retrievable from the clinical records. Specimens representing tubal ectopic pregnancies without a delivered placenta, cases with inadequate sampling or loss of tissue integrity precluding reliable assessment, and records with missing core variables were excluded.

Data sources and variables

Data were retrieved from the Hospital Information Management System, the archival records of the Department of Medical Pathology, electronic pathology reports, and delivery and clinical case files, and were entered into a structured database using a standardized case report form. The recorded variables comprised maternal age, gestational age at delivery, mode of delivery, the obstetric indication for referral, and the macroscopic and microscopic placental findings. Obstetric indications and placental histopathological findings were derived by systematic review of the free-text clinical summaries and pathology reports and were classified into predefined categories. Because a single placenta could be referred for more than one clinical reason, the obstetric indications were treated as non-mutually-exclusive variables. In a subset of cases a fetus was submitted together with the placenta for concurrent fetal autopsy; in these cases the reported fetal findings, including any structural anomalies, were recorded and reviewed alongside the placental diagnosis.

Histopathological classification

Placental lesions were categorized according to the Amsterdam Placental Workshop Group consensus framework into four groups (4): maternal vascular lesions (e.g., infarction, distal villous hypoplasia, increased syncytial knots, and decidual arteriopathy); fetal vascular lesions (e.g., avascular villi, fetal vascular thrombosis,

and stem villous vessel obliteration); acute inflammatory lesions (chorioamnionitis and funisitis); and chronic inflammatory lesions (villitis, chronic intervillositis, and chronic deciduitis). On the basis of the predominant findings, each placenta was assigned to a single mutually exclusive pattern: maternal vascular, fetal vascular, acute inflammatory, chronic inflammatory, combined (two or more groups present), or normal/non-specific. Classification was based on the diagnoses recorded in the original reports, and no re-sectioning, additional staining, or re-review of slides was undertaken.

Statistical analysis

Continuous variables were summarized as mean ± standard deviation and as median (range), and categorical variables as frequency and percentage. The normality of continuous variables was assessed using the Shapiro-Wilk test. Associations between the placental lesion groups (maternal vascular, fetal vascular, acute inflammatory, and chronic inflammatory) and the obstetric indications were evaluated with the Pearson chi-square test or, when expected cell counts were small, the Fisher exact test. Continuous variables were compared between placentas with and without an abnormal lesion pattern using the Mann-Whitney U test, as these data were not normally distributed. All tests were two-sided, and a p value less than 0.05 was considered statistically significant. Analyses were performed using GraphPad Prism (version 8.0.1; GraphPad Software, San Diego, CA, USA).

Results

During the study period, 399 placentas obtained from deliveries and meeting the inclusion criteria were analyzed, after 15 specimens representing tubal ectopic pregnancies without a delivered placenta had been excluded. The mean maternal age was 30.3 ± 6.1 years (median 30 (17-45)). Gestational age at delivery was documented in 347 cases (87.0%), with a mean of 30.7 ± 7.8 weeks (median 34 (5-41)); 271 (78.1) of these deliveries occurred before 37 weeks of gestation. Most placentas were obtained by cesarean section (295 (73.9)), whereas 104 (26.1) followed vaginal delivery. The demographic and clinical characteristics of the cohort are summarized in Table 1.

A single placenta was frequently referred for more than one clinical reason; therefore the indication categories are not mutually exclusive and their sum exceeds 100%. The most common

Table 1. Demographic and clinical characteristics of the study cohort (N = 399).

Characteristic	Value
Maternal age, years, mean ± SD	30.3 ± 6.1
Maternal age, median (range)	30 (17-45)
Gestational age, weeks, mean ± SD[a]	30.7 ± 7.8
Gestational age, median (range)[a]	34 (5-41)
<28 weeks, n	87
28-33 weeks, n	undefined
34-36 weeks, n	undefined
≥37 weeks, n	76
Preterm (<37 weeks), n (%)[a]	271 (78.1)
Mode of delivery, cesarean, n (%)	295 (73.9)
Mode of delivery, vaginal, n (%)	104 (26.1)

SD, standard deviation. [a] Gestational age was available for 347 of 399 cases (87.0%); percentages for gestational-age categories are based on this subset.

Table 2. Obstetric indications for placental referral (N = 399; categories are not mutually exclusive).

Obstetric indication	n	%
Preeclampsia/HDP	57	14.3
Preterm/PPROM	54	13.5
Congenital anomaly	51	12.8
FGR	50	12.5
Multiple gestation	49	12.3
GDM	39	9.8
Oligo/polyhydramnios	35	8.8
Abruption	26	6.5
Placenta previa/accreta	24	6.0
IUFD/fetal loss	13	3.3
Infection susp.	7	1.8
Other/unspecified	120	30.1

documented obstetric indications were hypertensive disorders of pregnancy, including preeclampsia (57; 14.3%), preterm birth or preterm premature rupture of membranes (54; 13.5%), congenital anomalies (51; 12.8%), fetal growth restriction (50; 12.5%), and multiple gestation (49; 12.3%). Gestational diabetes mellitus, amniotic fluid abnormalities, placental abruption, placenta previa or accreta spectrum, intrauterine fetal loss, and suspected infection accounted for the remaining indications. In 120 placentas (30.1%) no specific high-risk indication could be retrieved from the clinical records. The full distribution of indications is presented in Table 2 and Figure 1.

On histopathological review, the great majority of placentas showed no defined pathological pattern and were reported as normal or as showing only non-specific changes (346; 86.7%). At least one of the four Amsterdam lesion groups was identified in 53 (13.3) placentas. Among the abnormal placentas, an acute inflammatory pattern was the most frequent (21; 5.3%), followed by a maternal vascular pattern (19; 4.8%) and a chronic inflammatory pattern (9; 2.3%); a fetal vascular pattern and combined patterns were rare (two cases each). The individual lesions recorded most often were infarction (n = 17), increased syncytial knots (n = 4), chorioamnionitis (n = 22), chronic deciduitis (n = 5), fetal vascular thrombosis (n = 2), calcification (non-specific) (n = 24), perivillous fibrin (n = 5), hemorrhage/hematoma (n = 6). The distribution of lesion patterns is shown in Table 3 and Figure 2.

Table 3. Distribution of placental histopathological lesion patterns (mutually exclusive, N = 399).

Lesion pattern	n	%
Normal/non-specific	346	86.7
Acute inflammatory	21	5.3
Maternal vascular	19	4.8
Chronic inflammatory	9	2.3
Combined	2	0.5
Fetal vascular	2	0.5
Any abnormal pattern[a]	53	13.3

[a] Placentas showing at least one maternal vascular, fetal vascular, acute inflammatory, or chronic inflammatory lesion.

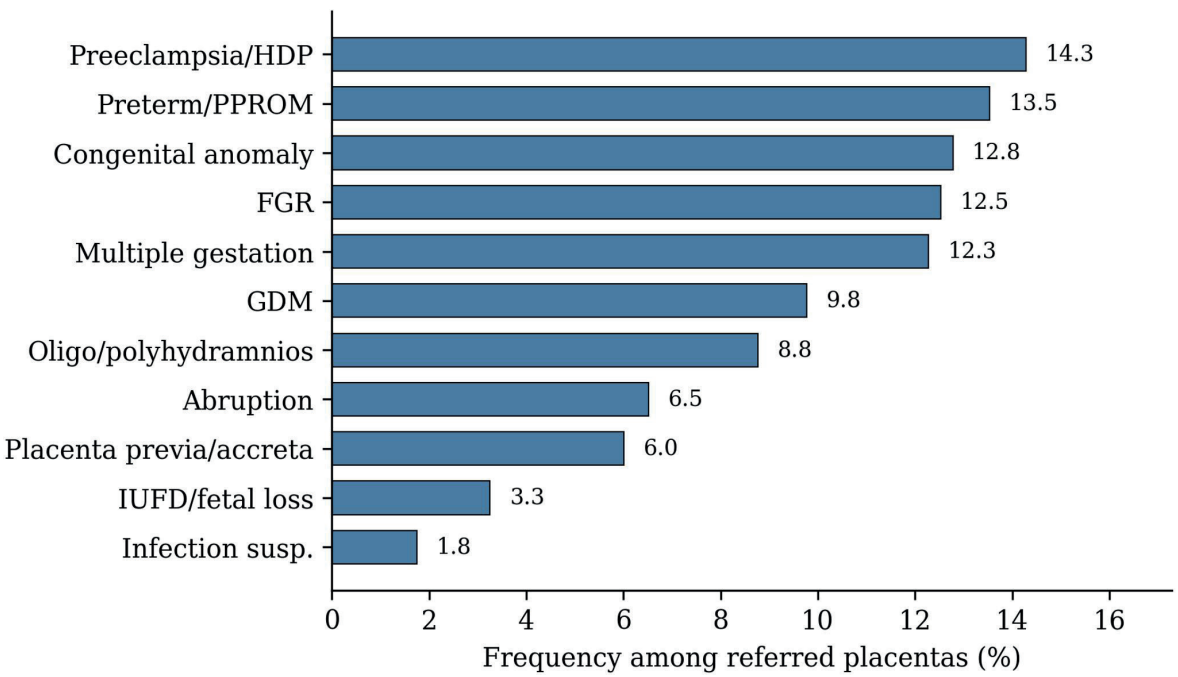

Figure 1. Frequency of documented obstetric indications for placental referral.

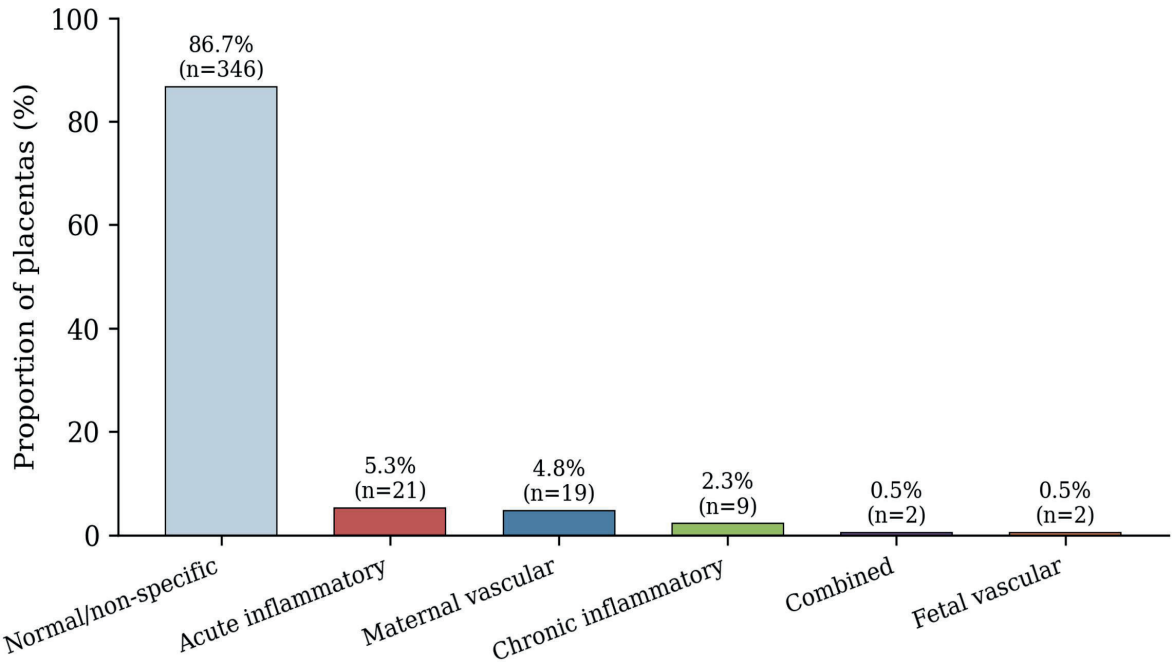

Figure 2. Distribution of placental histopathological lesion patterns.

Table 4. Prevalence of placental lesion groups according to obstetric indication.

Indication	n	MVM, n (%)	AI, n (%)	Any abnormal, n (%)	p[a]
Preeclampsia/HDP	57	4 (7.0)	5 (8.8)	9 (15.8)	0.530
FGR	50	5 (10.0)	3 (6.0)	7 (14.0)	0.826
GDM	39	3 (7.7)	4 (10.3)	6 (15.4)	0.624
Preterm/PPROM	54	2 (3.7)	4 (7.4)	9 (16.7)	0.396
Abruption	26	2 (7.7)	2 (7.7)	6 (23.1)	0.136
IUFD/fetal loss	13	1 (7.7)	2 (15.4)	3 (23.1)	0.394
Infection susp.	7	0 (0.0)	3 (42.9)	3 (42.9)	0.053
Placenta previa/accreta	24	0 (0.0)	0 (0.0)	0 (0.0)	0.057
Multiple gestation	49	1 (2.0)	1 (2.0)	2 (4.1)	0.043
Congenital anomaly	51	3 (5.9)	2 (3.9)	6 (11.8)	0.829
Oligo/polyhydramnios	35	1 (2.9)	1 (2.9)	2 (5.7)	0.202

MVM, maternal vascular malperfusion; AI, acute inflammatory. [a] Fisher exact test comparing the presence of any abnormal pattern in the indicated group versus the remainder of the cohort. Acute inflammatory lesions were significantly associated with suspected infection (3/7; 42.9%; p = 0.005).

The associations between the major lesion groups and the obstetric indications are presented in Table 4. Overall, the presence of any abnormal lesion pattern did not differ significantly across most indications. Maternal vascular lesions were somewhat more frequent among placentas referred for fetal growth restriction (10.0%) and hypertensive disorders (7.0%) than in the remainder of the cohort (Figure 3), but these differences did not reach statistical significance (all $p > 0.05$). Acute inflammatory lesions were significantly more common among placentas referred for suspected infection, being present in 3 of 7 such cases (42.9%) compared with the rest of the cohort (Fisher exact test, $p = 0.005$). Multiple gestation

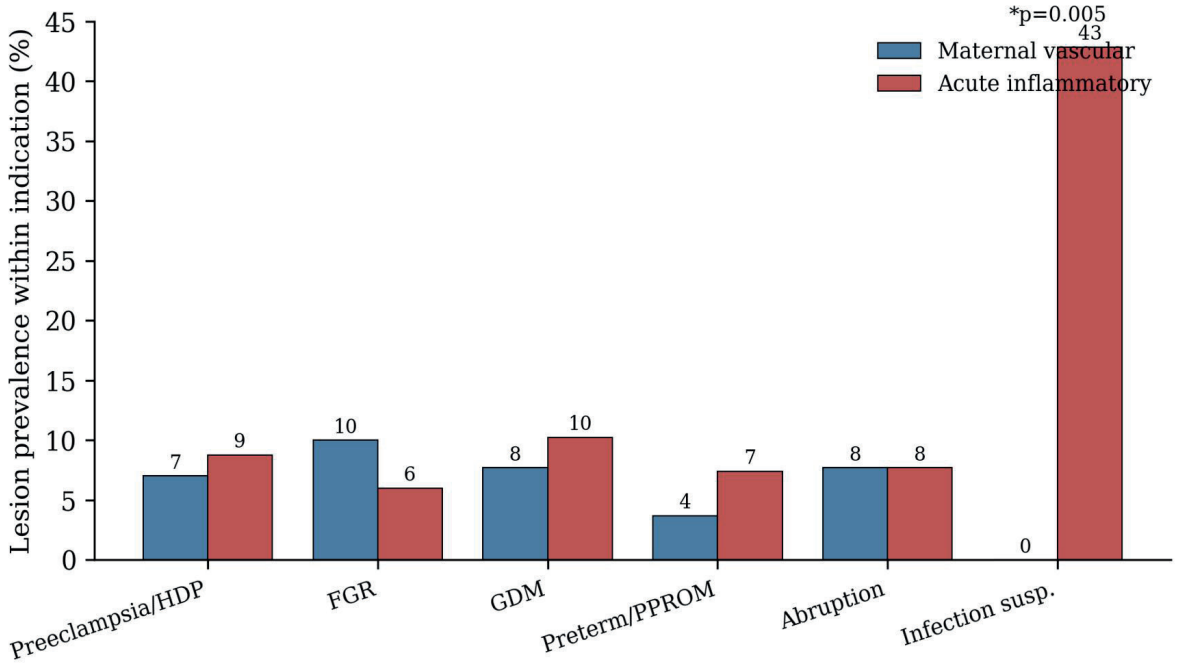

Figure 3. Prevalence of maternal vascular and acute inflammatory lesions within selected obstetric indications
*Fisher exact test, p = 0.005 for acute inflammatory lesions in suspected infection.

was inversely associated with the presence of any abnormal pattern (4.1%; p = 0.043), and no defined lesion pattern was identified in any placenta referred for placenta previa or accreta spectrum. No significant difference in maternal age or gestational age was observed between placentas with and without abnormal patterns, and the abnormal-pattern rate did not differ by mode of delivery (all p > 0.05).

Fourteen of the 399 placentas (3.5%) were submitted together with a fetus for concurrent fetal autopsy, all following a mid-trimester intrauterine loss or termination of pregnancy (gestational age approximately 11 to 22 weeks). A fetal structural anomaly was identified in 8 of these 14 fetuses and comprised central nervous system defects (anencephaly, spina bifida, and holoprosencephaly), a bilateral renal and bladder agenesis sequence, hydrops fetalis, a diaphragmatic hernia accompanied by a ventricular septal defect, dysmorphic features suggestive of aneuploidy, and a disorder of sex development; the remaining 6 fetuses showed growth restriction or nonspecific autolytic changes without a defined structural anomaly. The corresponding placentas were histologically unremarkable in 10 cases and showed only nonspecific coagulative necrosis or immature villi consistent with fetal retention in 3 cases and focal infarction in 1 case. No defined Amsterdam lesion pattern was consistently associated with the fetal anomalies.

Discussion

In this single-center retrospective analysis of 399 placentas submitted for pathological examination on obstetric indication, the majority were reported as histologically normal or as showing only non-specific changes, and a defined Amsterdam lesion pattern was identified in fewer than one in seven placentas. Among the placentas with a defined pattern, acute inflammatory and maternal vascular lesions predominated, whereas fetal vascular and combined patterns were uncommon. The only statistically significant clinicopathological relationship was the concentration of acute inflammatory lesions among placentas referred

for suspected infection. These findings establish the institutional profile of placental pathology at our center and, when read against the international literature, highlight both the value and the practical limitations of routine placental reporting.

The proportion of placentas with a defined lesion pattern in our cohort (13.3%) was substantially lower than that reported in comparable series. In a South African high-risk cohort of 176 placentas, Stevens et al.(11) identified maternal vascular malperfusion in 51.1% and ascending infection in 35.8% of cases, and Al Fahdi et al.(2) reported abnormal findings in 74% of placentas from adverse-outcome pregnancies. Using a standardized request form and a protocol-based workup, Strahler et al.(12) detected at least one pathological finding in 86.3% of clinically indicated singleton placentas. Most strikingly, Romero et al.(13) demonstrated that even placentas from term pregnancies with an entirely normal outcome harbored inflammatory or vascular lesions in 78% of cases, although severe or high-burden lesions were present in only about 1%. Taken together, these data indicate that the apparent prevalence of placental lesions depends heavily on the intensity of sampling and the rigor of reporting rather than on clinical risk alone. Our comparatively low detection rate is therefore best interpreted as a reflection of routine, largely biopsy-level reporting that did not consistently apply the systematic sampling and terminology recommended by the Amsterdam consensus (4,14), rather than as evidence of a genuinely low burden of placental disease.

The significant association between acute inflammatory lesions and suspected infection is biologically coherent and consistent with prior work. Acute chorioamnionitis and funisitis represent the histological correlates of the amniotic fluid infection sequence and of the maternal and fetal inflammatory responses (14). Orsaria et al.(6) linked acute placental inflammation to adverse neonatal outcomes, and Mizrachi et al.(7) reported that isolated acute inflammatory lesions were associated with recurrent early preterm birth. Romero et al.(13) found acute inflammatory lesions to be the most prevalent category even in uncomplicated term pregnancies, with a strong relationship to labor, and Stevens et al.(11) similarly observed ascending infection as one of the two dominant cluster diagnoses in a high-risk population. The clustering of acute inflammatory lesions within our infection-referred group therefore supports the concordance between the clinical indication and the underlying histopathology, even in a dataset with otherwise limited lesion documentation.

In contrast, the anticipated associations between maternal vascular malperfusion and hypertensive disorders, fetal growth restriction, and gestational diabetes were not statistically confirmed, although maternal vascular lesions were numerically more frequent among placentas referred for fetal growth restriction and hypertensive disorders. This diverges from the robust maternal vascular malperfusion and preeclampsia association reported by Stevens et al.(11), the maternal vascular malperfusion findings in gestational diabetes described by Byrne et al.(3), the growth-restricted preterm correlations reported by Dankó et al.(5), and the predominance of maternal vascular malperfusion among indicated singleton placentas found by Strahler et al.(12). A likely explanation is the systematic under-recording of the subtle villous features of maternal vascular malperfusion, such as increased syncytial knots, distal villous hypoplasia, and accelerated villous maturation, in routine reports. Stevens et al.(11), for example, recorded increased syncytial knots in 52.8% of their cases, whereas this feature was documented in only a small minority of ours. The cluster analysis reported by Boujenah et al.(15) likewise linked a vascular placental profile to preeclampsia, HELLP syndrome, and fetal growth restriction, reinforcing the view that these associations exist but require consistent recognition and reporting of maternal vascular malperfusion features to be detected.

No defined parenchymal lesion pattern was identified in any placenta referred for placenta previa or accreta spectrum. This is expected, because the accreta spectrum is defined by abnormal adherence or invasion at the villous and myometrial interface rather than by the parenchymal injury patterns of the Amsterdam classification. In these cases, placental examination is directed toward confirming the depth of invasion, as reflected in the large Turkish accreta-spectrum series reported by Cetinkaya Demir et al.(16), and antenatal assessment increasingly relies on imaging-based prediction of invasion and hemorrhage, as described by Kükrer et al.(17). The absence of Amsterdam patterns in this subgroup therefore reflects the distinct diagnostic purpose of examination in the accreta spectrum rather than a true lack of pathology.

Multiple gestation was inversely associated with the presence of any abnormal pattern in our cohort. This accords with the observations of Strahler et al.(12), who found lower concordance between the clinical indication and the histopathological findings in twin placentas and reported that a substantial proportion of twin placentas were histologically unremarkable, in part because twins are frequently delivered electively at 34 to 38 weeks and are commonly submitted for reasons such as documentation of chorionicity rather than for a specific lesion-associated complication. Matthews et al.(10) similarly noted that placental lesions in twin gestations were most informative when the pregnancies were complicated by growth restriction or preeclampsia. The lower abnormality rate among our multiple gestations is thus consistent with a referral pattern driven by protocol rather than by suspected placental disease.

A subset of the placentas in our series was examined together with a fetus following a mid-trimester loss or termination, most often performed for a congenital anomaly. In these cases the fetal structural anomalies, which were predominantly central nervous system and other malformations, were generally not accompanied by a specific placental lesion pattern, and the placentas were most often histologically unremarkable or showed only changes attributable to fetal retention. This observation is consistent with the concept that many congenital anomalies arise from primary genetic or early embryonic mechanisms rather than from placental malperfusion or inflammation, and it supports the practice of correlating placental findings with a dedicated fetal autopsy rather than relying on the placenta alone to account for an anomalous fetus (18).

Collectively, our findings underscore the importance of standardized sampling and structured reporting if the clinical and prognostic potential of placental examination is to be realized. As emphasized by Turowski et al.(18), placental pathologists employ terminology, such as maternal vascular malperfusion, that is distinct from clinical diagnoses, such as preeclampsia, and the utility of a report depends on clear, standardized diagnoses that clinicians can interpret and act upon. Stevens et al.(11) demonstrated that placental histopathology is frequently underutilized in the formulation of subsequent-pregnancy management plans. The stepwise application of the Amsterdam framework (14) and the adoption of a synoptic reporting template (4,18) would be expected to improve both the detection and the communication of clinically relevant lesions in a high-volume setting such as ours, and to strengthen the clinician and pathologist collaboration on which the effective use of placental pathology ultimately depends.

Limitations

This study has several limitations. First, it was a retrospective, single-center analysis based on routine archival reports, and the lesion classification was derived from the free-text diagnoses recorded at the time of original sign-out rather than from a re-review of histological slides. Lesions that were not explicitly stated in the report could not be captured, which almost certainly led to underestimation of maternal and fetal vascular malperfusion, whose

villous features are easily overlooked without dedicated sampling. The resulting abnormality rate should therefore be regarded as a lower bound that reflects reporting practice rather than the true prevalence of placental disease, as illustrated by the far higher rates in protocol-based cohorts (11-13). Second, sampling was not standardized across the study period, and many specimens were reported at a brief, biopsy-level of detail. Third, the obstetric indications were extracted from free-text clinical notes and treated as non-mutually-exclusive categories; several indications, most notably suspected infection, comprised small numbers, so the significant association between acute inflammatory lesions and infection rests on few cases and warrants cautious interpretation. Fourth, gestational age was unavailable for a minority of cases, and fetal status and placental weight were not reliably recorded, precluding weight-based assessment of maternal vascular malperfusion and correlation with neonatal outcomes. Finally, no neonatal or subsequent-pregnancy outcomes were available, and the sparse event counts precluded multivariable adjustment. In addition, although a fetus was examined together with the placenta in a subset of cases, systematic fetal phenotyping, imaging, and karyotype results were not available for all of these cases, which limited a more detailed correlation between specific fetal anomalies and placental findings.

Conclusion

In this large single-center series, most placentas referred for pathological examination on obstetric indication were reported as normal or non-specific, and among the placentas with a defined pattern, acute inflammatory and maternal vascular lesions predominated. Acute inflammatory lesions were significantly associated with suspected infection, whereas the expected associations of maternal vascular malperfusion with hypertensive disorders, fetal growth restriction, and gestational diabetes did not reach significance. The marked discrepancy between our detection rate and those of protocol-based cohorts indicates that routine reporting under-recognizes Amsterdam lesion patterns and argues for standardized sampling and synoptic reporting to realize the diagnostic and prognostic value of placental examination. Establishing this institutional profile provides a foundation for improving clinician and pathologist communication and for future prospective studies incorporating systematic histological workup and neonatal outcomes.

Ethical approval

The study was approved by the Non-Interventional Clinical Research Ethics Committee of Kocaeli University Faculty of Medicine (approval no. GOKAEK-2026/05/35; project no. 2026/148; date 6 March 2026).

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: BYB; data collection: BYB, GK; analysis and interpretation of results: BYB, GV, YD; draft manuscript preparation: BYB. All authors reviewed the results and approved the final version of the article.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Research Article

Cytotoxic effects of cisplatin, curcumin, and a lipid-based vitamin E formulation on MCF-7 breast cancer cells

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ABSTRACT

Aim: To characterize, for each agent individually, the dose-dependent cytotoxic activity of cisplatin, curcumin, and a lipid-based vitamin E formulation in MCF-7 breast cancer cells, as groundwork for subsequent combination research.

Methods: MCF-7 cells were exposed to a range of escalating concentrations of cisplatin, or of curcumin and vitamin E, over an extended exposure period, after which viability was determined using the MTT colorimetric assay. For curcumin, background absorbance arising from its intrinsic pigmentation was corrected using acellular compound-only wells. Statistical comparisons were performed using the Mann-Whitney U test.

Results: Both cisplatin and curcumin significantly lowered MCF-7 viability relative to untreated controls in a dose-dependent fashion across all concentrations tested. A pronounced drop in viability was already evident with cisplatin at its lowest tested concentration. After correcting for background absorbance, curcumin still showed a marked cytotoxic effect. The lipid-based vitamin E formulation, however, showed no such concentration-dependent decline; at several doses, apparent viability was actually higher than in controls, an effect more consistent with the formulation's poor aqueous solubility than with a true biological response.

Conclusion: Both cisplatin and curcumin exhibited consistent, reproducible cytotoxicity toward MCF-7 cells, whereas the tested lipid-based vitamin E formulation proved unsuitable for assessment under conventional aqueous culture conditions. Collectively, these single-agent concentration-response data lay the methodological groundwork for future studies combining cisplatin, curcumin, and more water-compatible vitamin E formulations in breast cancer models.

Keywords: breast neoplasms, cisplatin, curcumin, vitamin e, mcf-7 cells, cell survival

Introduction

According to GLOBOCAN 2022 data, breast cancer is the most commonly diagnosed cancer in women and the leading cause of female cancer mortality worldwide (1). Despite progress in systemic treatment, breast cancer continues to impose a substantial global burden, underscoring the need for more effective, better-tolerated therapies (2).

Among platinum-based coordination compounds, cisplatin occupies a central place in chemotherapy regimens for its strong antineoplastic action against solid tumors, including breast cancer, exerted primarily

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through intrastrand DNA cross-link formation that blocks replication and triggers apoptosis (3,4). Nonetheless, its clinical use is limited by dose-dependent adverse effects- kidney damage, nerve toxicity, hearing loss, and vestibular impairment- together with the development of resistance over time (3,5). These limitations have fueled interest in combination approaches capable of sustaining or boosting cisplatin's anticancer effect while lowering the required dose (5).

Interest in naturally occurring bioactive molecules as chemotherapy adjuncts has grown considerably. Curcumin, a polyphenolic compound from *Curcuma longa*, was first reported to possess antitumor activity in 1985 (6) and has since been investigated extensively across cancer cell lines and animal models (7). In breast cancer, curcumin acts through multiple mechanisms, modulating proliferative and apoptotic signaling and elevating reactive oxygen species (ROS) levels, while also sensitizing cells to agents such as doxorubicin and allowing apoptosis to occur at reduced concentrations (8), making it a rational candidate for cisplatin-sparing combinations.

A related line of investigation concerns vitamin E, a family of lipid-soluble tocopherols and tocotrienols known for context-dependent antioxidant and pro-oxidant activity and for their capacity to modulate cancer-related gene pathways (9,10). In breast cancer cell models, combining vitamin E with doxorubicin enhanced the drug's antiproliferative effect while sparing normal cells, although no formal synergism was established (10). This dual profile supports a rationale for including vitamin E in cisplatin-based protocols while underscoring the need for careful validation.

Although cisplatin, curcumin, and vitamin E have each been studied individually, and curcumin has previously been paired with various chemotherapeutic agents as well as with vitamin C (11,12), no prior study has evaluated combinations of all three agents together in breast cancer cells. Notably, vitamin C has featured far more prominently than vitamin E in this line of research, leaving the latter's antitumor potential comparatively underexplored (9-13). Establishing dependable single-agent dose-response data for each compound is a necessary precondition for meaningfully evaluating their combined effects.

This study was therefore designed to characterize the dose-dependent cytotoxic effects of cisplatin, curcumin, and vitamin E in the MCF-7 breast cancer cell line using the MTT viability assay, as a preliminary step toward future combination work. We report concentration-response profiles for cisplatin and curcumin, together with a methodological observation regarding the technical limitations of a lipid-based vitamin E formulation under standard cell culture conditions, a finding with direct practical relevance for future *in vitro* combination studies involving this vitamin.

Materials and Methods

Cell line and culture conditions

MCF-7 human breast cancer cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS), 1% penicillin-streptomycin (10,000 U/mL penicillin, 10 mg/mL streptomycin), and 1% L-glutamine, at 37°C under a humidified atmosphere with 5% CO_2.

Drugs and treatment groups

The dose-dependent cytotoxic effects of cisplatin, curcumin, and vitamin E on MCF-7 cells were each evaluated separately. Vitamin E was administered as a commercially available lipid-based preparation (Vi-Plex E, OSEL İlaç Sanayi ve Ticaret A.Ş., Istanbul, Turkey), with the tested concentrations expressed relative to the total formulation rather than to its α-tocopherol content alone. Each agent was

tested at four concentrations: 0.95, 1.9, 3.8, and 7.6 μg/mL for cisplatin, and 12.5, 25, 50, and 100 μg/mL for curcumin and vitamin E. Cisplatin was applied directly in its ready-to-use liquid form. Curcumin was dissolved in dimethyl sulfoxide (DMSO) to prepare stock solutions, while vitamin E (Vi-Plex E) was dissolved in ethanol before being added to the cultures. All intermediate dilutions were prepared in FBS-free DMEM; for vitamin E, this corresponded to final in-well ethanol concentrations of 0.0083%, 0.0167%, 0.0333%, and 0.0667% (v/v), respectively, remaining well within levels generally considered non-cytotoxic for cultured cells. For curcumin, this corresponded to final in-well DMSO concentrations of 3.125%, 6.25%, 12.5%, and 25% (v/v), respectively. A DMSO-only vehicle control was tested at each of these matched concentrations, including the highest (25%), and showed no cytotoxicity at any concentration; it was therefore not presented as a separate figure. No corresponding ethanol-only vehicle control was prepared for the vitamin E experiments. Each treatment condition was tested in quadruplicate wells (n=4) within each plate, across 4 independent experiments. The control condition consisted of an untreated MCF-7 group.

Cell viability (MTT) assay

Cell viability was determined with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay, which relies on the conversion of MTT into insoluble formazan crystals by mitochondrial dehydrogenase enzymes present in viable cells (14). Cells were plated in 96-well dishes at 1×10^4 cells per well and allowed to attach for at least 1 h at 37°C in 5% CO_2, with attachment verified before proceeding (typically reaching approximately 80% cell adherence within 1–4 h), before being exposed to the test agents for 72 h. At the end of this exposure period, each well received 10 μL of MTT solution (5 mg/mL), and the plates were kept at 37°C in 5% CO_2 for 4 h in the dark. After removing the supernatant, 100 μL of DMSO was added to dissolve the formazan crystals that had formed. Absorbance readings were then taken at 570 nm with a microplate spectrophotometer, and percentage viability and cytotoxicity values were calculated as shown in Equations 1 and 2:

$$\%\ \text{Viability} = (\text{A}_{\text{sample}}/\text{A}_{\text{control}}) \times 100 \quad (1)$$

$$\%\ \text{Cytotoxicity} = 100 - [(\text{A}_{\text{sample}}/\text{A}_{\text{control}}) \times 100] \quad (2)$$

in which A_{sample} and $A_{control}$ denote the absorbance values recorded for treated and untreated control wells, respectively (14).

Since curcumin's inherent yellow pigmentation can interfere with absorbance measurements at 570 nm, cell-free wells containing curcumin alone at each test concentration were also included as negative controls. The absorbance recorded in these compound-only wells was then subtracted from that of the matching cell-containing wells, isolating the true cell-viability signal from compound-related background absorbance.

Statistical analysis

All data were processed in GraphPad Prism (version 5.04) and are reported as mean ± standard error of the mean (SEM). Each treatment group was compared against the untreated control using the non-parametric Mann-Whitney U test, with statistical significance defined as $p<0.05$ (marked as * in the figures; ** denotes $p<0.005$). Each condition was tested in quadruplicate wells within a plate in each of 4 independent experiments; the mean of the quadruplicate wells from each independent experiment was treated as a single data point, and comparisons were performed on these experiment-level means (n=4 vs. n=4). Given this sample size, GraphPad Prism computed p-values using the asymptotic (Gaussian) approximation rather than the exact method.

Results

Cisplatin reduces MCF-7 cell viability in a dose-dependent manner

At every concentration tested, cisplatin significantly reduced MCF-7 cell viability relative to the untreated control ($p < 0.005$ for all doses), with viability declining progressively as the concentration increased (Figure 1). A roughly 50% loss of viability occurred at 0.95 μg/mL, making this an appropriate working concentration for subsequent experiments.

Curcumin reduces MCF-7 cell viability

Curcumin likewise produced a significant reduction in MCF-7 viability at every concentration examined, relative to the untreated control ($p < 0.005$) (Figure 2A), with a pronounced decline evident across the whole concentration range. Although the resulting dose-response curve was not perfectly linear, all tested concentrations still showed a statistically significant cytotoxic effect compared with the control group. Given that curcumin's yellow pigmentation can interfere with 570 nm absorbance readings, acellular curcumin-only wells were used to correct for this compound-related background (see Section 2.3); after applying this correction, curcumin continued to show a clear cytotoxic effect across the concentrations tested (Figure 2B). Notably, at the lowest concentration examined (12.5 μg/mL), curcumin lowered cell viability to a greater extent than cisplatin did at its lowest tested concentration (0.95 μg/mL).

Vitamin E (lipid-based formulation) does not exhibit a cytotoxic effect in MCF-7 cells

Unlike cisplatin and curcumin, the lipid-based vitamin E formulation (Vi-Plex E) produced no cytotoxic effect in MCF-7 cells. At several of the tested concentrations, apparent viability was actually higher than in the untreated control, and some of these differences reached statistical significance (Figure 3); nonetheless, no dose-dependent decline in viability was observed at any point. Given this outcome, the vitamin E formulation was not pursued further in the study.

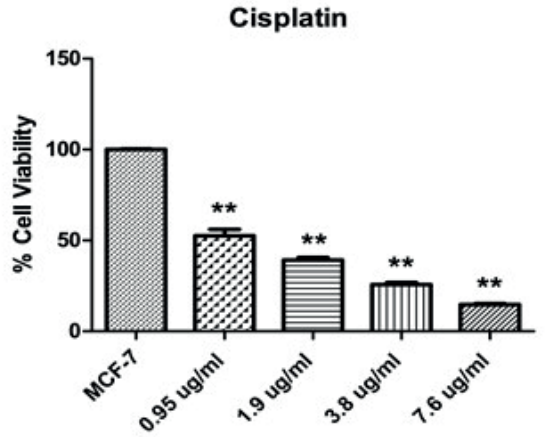

Figure 1. Cisplatin's effect on MCF-7 cell viability, as measured by the MTT assay. Cells were exposed to increasing concentrations of cisplatin (0.95, 1.9, 3.8, and 7.6 μg/mL), and viability values are shown relative to the untreated control.

**$p < 0.005$ versus untreated control. Values represent mean ± SEM from 4 independent experiments.

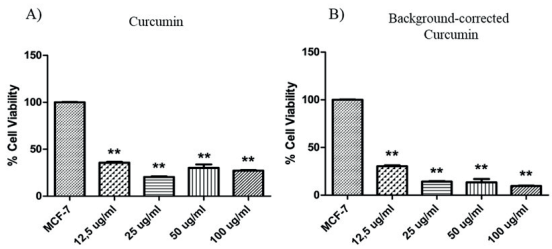

Figure 2. Curcumin's effect on MCF-7 cell viability, assessed via the MTT assay. (A) Cells were exposed to rising concentrations of curcumin (12.5, 25, 50, and 100 μg/mL); viability was measured by the MTT assay and is expressed relative to the untreated control. (B) Viability values after subtracting the absorbance recorded in acellular curcumin-only wells from that of the matching cell-containing wells, correcting for compound-related background.

Values represent mean ± SEM from 4 independent experiments; **$p < 0.005$ relative to the untreated control.

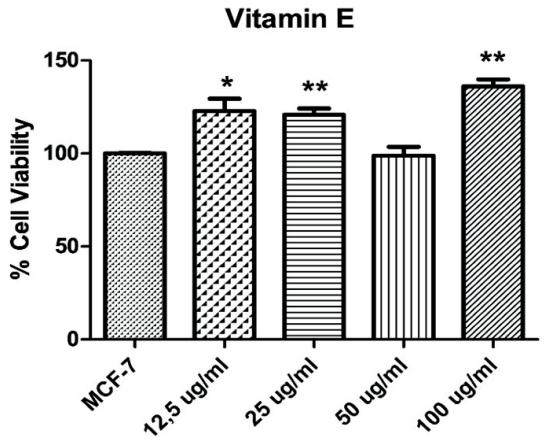

Figure 3. Effect of the lipid-based vitamin E preparation (Vi-Plex E) on MCF-7 cell viability, measured by the MTT assay. Cells were exposed to increasing concentrations of vitamin E (12.5, 25, 50, and 100 μg/mL), and viability, assessed by the MTT assay, is expressed relative to the untreated control. Values are given as mean ± SEM of 4 independent experiments.

*$p < 0.05$; **$p < 0.005$ versus untreated control.

Discussion

In this work, we assessed how cisplatin, curcumin, and a lipid-based vitamin E preparation individually affect the viability of MCF-7 breast cancer cells, using the MTT assay as the readout. Both cisplatin and curcumin produced substantial declines in cell viability, while the vitamin E preparation failed to do so. Together, these single-agent concentration-response findings offer a working framework for subsequent combination experiments.

As a chemotherapeutic agent for solid tumors including breast cancer, cisplatin continues to be used extensively, largely because of its ability to generate DNA cross-links that trigger apoptotic cell death. In line with this mode of action, all tested cisplatin concentrations produced a significant drop in MCF-7 viability, and the 0.95 μg/mL dose was sufficient to reduce viability by roughly half, marking it as an appropriate concentration for subsequent work. While reported IC_{50} figures differ between studies depending on exposure duration and assay setup, the response observed here is in line with the well-documented sensitivity of MCF-7 cells to cisplatin (4,5).

A similarly pronounced reduction in MCF-7 viability was seen with curcumin at every concentration examined, in agreement with existing evidence that curcumin restrains breast cancer cell growth through several routes—altering oxidative stress balance, blocking proliferative signaling, inducing cell-cycle arrest, and promoting apoptosis (6-8). Unlike cisplatin, the pre-correction concentration-response pattern was not strictly linear, an outcome most plausibly attributable to curcumin's strong yellow pigmentation, a recognized source of interference in colorimetric absorbance-based assays (15). To address this, acellular wells containing curcumin alone were included, allowing background absorbance attributable to the compound itself to be quantified and subtracted. Once this correction was applied, curcumin's cytotoxic effect remained clearly evident across the full concentration range, supporting the reliability of the compound-only control approach for pigmented substances. We note that this approach is an approximation; a more rigorous correction would require quantifying background absorbance directly from the formazan signal itself.

Notably, at their respective lowest tested doses (12.5 μg/mL for curcumin versus 0.95 μg/mL for cisplatin), curcumin caused a larger drop in viability than cisplatin did; these concentrations are not molar-equivalent, so cross-compound comparisons should be interpreted cautiously. Even so, this observation aligns with existing literature describing curcumin's strong antiproliferative activity in breast cancer models (6-8), as well as evidence that curcumin can potentiate chemotherapy-induced cytotoxicity, including that of cisplatin, by shifting oxidative stress balance and interfering with DNA repair processes such as FEN1 function (8,16).

The lipid-based vitamin E preparation (Vi-Plex E) behaved quite differently: rather than showing a dose-dependent decline in viability, several tested concentrations produced apparent viability values above those of the control wells. We interpret this as more likely a technical artifact than a genuine biological effect. Given that α-tocopherol is a highly lipophilic molecule with poor water solubility (17), lipid-based preparations of this kind may not disperse evenly within aqueous culture medium, which could distort assay readings. It should also be noted that, because an ethanol-only vehicle control was not included, some contribution from the ethanol solvent itself to these results cannot be ruled out. In addition, redox-active compounds such as α-tocopherol can nonenzymatically reduce the MTT tetrazolium salt independent of cell viability, which, alongside solubility, may further contribute to the above-control readings observed in Figure 3. Furthermore, vitamin E's antitumor efficacy is known to vary considerably depending on the specific isoform and formulation used; tocotrienols and derivatives such as α-tocopheryl succinate, for example, have demonstrated stronger antiproliferative and pro-apoptotic activity than native α-tocopherol in breast cancer models (9,10,13,18,19). These results do not argue against vitamin E's anticancer potential broadly, but indicate that this specific lipid-based α-tocopherol formulation did not generate an interpretable cytotoxic signal under the tested conditions.

All experiments described here were performed in the MCF-7 line, a commonly used and well-characterized luminal A model of hormone receptor-positive breast cancer. Like any single-cell-line system, it cannot capture the full molecular and phenotypic heterogeneity seen across breast cancer subtypes; so applying these results to other subtypes - triple-negative disease, for instance- will require confirmation in additional cell models. In addition, each dose group was compared against the untreated control using a separate Mann-Whitney U test without correction for multiple comparisons, which may inflate the type I error rate; the reported p-values should therefore be interpreted with appropriate caution. Absorbance was recorded at a single wavelength (570 nm) without a paired reference/background wavelength (typically 630-690 nm); background from curcumin's intrinsic pigmentation was instead addressed through the acellular compound-only wells described in Section 2.3, but the absence of a reference-wavelength subtraction step is acknowledged as an additional methodological limitation. Because agents differing markedly in physicochemical behavior (e.g., pigmentation versus lipophilicity) require optimized solubilization and co-culture conditions for reliable combination in an aqueous system, we first established single-agent concentration-response profiles as necessary groundwork. While this approach does not allow us to draw conclusions about synergistic or additive drug interactions, the individual-agent data we generated supply the reference profiles needed to design future combination experiments on a rational basis.

Building on this work, later studies could broaden the scope to additional breast cancer cell lines covering other molecular subtypes-triple-negative models such as MDA-MB-231 among them - so that luminal and triple-negative responses can be compared directly. Substituting the lipid-based vitamin E formulation used here with alternatives better suited to aqueous environments, such as D-α-tocopheryl polyethylene glycol succinate (TPGS) or tocotrienol-based preparations, may allow for a more dependable assessment of vitamin E-based approaches (19,20). Once an appropriate formulation has been identified, two-drug (cisplatin-curcumin) and eventually three-drug combinations could be examined using the Chou–Talalay combination index approach (21), supplemented by assays targeting apoptosis and other modes of cell death.

Conclusion

Taken together, this work characterizes the dose-dependent cytotoxicity of cisplatin and curcumin in MCF-7 breast cancer cells, establishing both as suitable candidates for future combination studies. Cisplatin's viability-reducing effect followed a clear dose-dependent pattern consistent with its known DNA cross-linking mechanism, and curcumin's antiproliferative activity proved reproducible once an appropriate background-correction step was applied to account for its natural pigmentation. The lipid-based vitamin E formulation examined here, by contrast, did not produce an interpretable cytotoxic signal, an outcome attributable primarily to its limited aqueous solubility rather than to any absence of biological activity; formulations such as TPGS or tocotrienol derivatives, better suited to aqueous systems, merit consideration in subsequent work. Although combination experiments fell outside the scope of the present study, the concentration-response data presented here provide a basis for subsequent investigations of cisplatin-curcumin-vitamin E combinations in MCF-7 and other breast cancer cell models.

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Ethical approval

This study did not require ethics committee approval, as it involved only in vitro experiments on an established human breast cancer cell line (MCF-7) and did not involve human participants, patient data, or animal subjects.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: GEC; data collection: GEC and ÇB; analysis and interpretation of results: GEC and ÇB; draft manuscript preparation: GEC. All authors reviewed the results and approved the final version of the article.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Research Article

General surgery residency placements in Türkiye: institutional, geographic, and temporal patterns from 2019 to 2025

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ABSTRACT

Background: Declining interest in surgical specialties has raised concerns regarding the sustainability of the surgical workforce worldwide. In Türkiye, general surgery residency training is centrally allocated through the Medical Specialty Examination (TUS), yet longitudinal national data on placement dynamics remain limited.

Aim: To evaluate temporal, institutional, and geographic patterns of general surgery residency placements in Türkiye between 2019 and 2025.

Methods: This retrospective descriptive study analyzed 2,617 general surgery placement records obtained from fourteen consecutive TUS cycles conducted between 2019 and 2025. Quota fill rates were evaluated according to hospital type, geographic region, metropolitan status, supplementary placement process, and quota category.

Results: The overall mean quota fill rate was 48.3%, while 47.9% of quota records remained completely unfilled. Fill rates showed an overall downward trend across the study period, despite year-to-year variation, with the lowest annual levels observed in 2024–2025. City hospitals demonstrated the highest mean fill rate (82.8%), followed by training and research hospitals (59.2%) and university hospitals (40.5%). Metropolitan provinces showed higher observed fill rates than non-metropolitan provinces (54.7% vs. 23.8%). Nearly all quota records entering the supplementary placement process remained unfilled (99.9%).

Conclusion: General surgery residency placements in Türkiye demonstrate an overall decline over the study period, accompanied by marked institutional and geographic variation. Lower observed fill rates in university hospitals and non-metropolitan regions suggest an uneven distribution of residency placement outcomes across the training system. These findings highlight the need for continued monitoring and targeted workforce planning to support the sustainability of the future general surgery workforce.

Keywords: general surgery, residency training, medical workforce, TUS, surgical education, workforce planning

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Introduction

Modern health systems depend on a balanced mix of clinical specialties, yet the global surgical workforce is widely projected to fall short of demand driven by population ageing, rising procedural volumes, and shifting case complexity. The World Health Organization estimates a global shortfall of approximately 10 million health workers by 2030, with surgical disciplines disproportionately affected across high- and low-income settings (1). In parallel, ageing of the surgical workforce has emerged as an additional challenge. Ellison et al. demonstrated that demographic changes and increasing cancer burden are expected to substantially increase future demand for general surgeons (2). Cross-specialty match analyses further document a narrowing of the applicant-to-position ratio in general surgery relative to surgical subspecialties and integrated programmes, with applicant interest tilting toward non-surgical fields perceived as offering greater lifestyle control (3,4). Declining fill rates and rising reliance on supplementary placement rounds in radiation oncology, orthopaedics, and other comparable specialties have been described as concrete signals of this realignment (5).

The determinants underlying these shifts are well characterised. Systematic reviews identify lifestyle, work–life balance, discipline interest, financial expectations, and gender as dominant drivers of specialty choice, with weighting variable across countries (6). Persistent gender disparities continue to constrain surgical recruitment (7). Systemic factors — geographic maldistribution, training-site characteristics, and programme prestige — further shape where newly trained specialists ultimately practise (2,8).

Türkiye occupies a distinctive position within this picture. Specialty entry is governed by the centralised, biannual Tıpta Uzmanlık Sınavı (TUS), whose results, combined with candidates' ranked preferences, determine placement across university hospitals, training and research hospitals, and the more recently introduced city hospitals (9). Despite the post-2003 Health Transformation Programme expanding training capacity, Türkiye still trails OECD averages in specialist density, and surgical specialties have shown waning appeal. A national survey of TUS candidates demonstrated that fewer than 30% of medical graduates intended to pursue surgery, with malpractice risk and workplace violence as significant negative determinants (10). A workforce analysis ranked general surgery 29th of 35 clinical specialties in candidate preference at the 2017 fall TUS, with 26% of residents leaving training before completion (11). These patterns sit within a broader physician-migration crisis: applications for Good Standing Certificates from the Turkish Medical Association rose from 59 in 2012 to 2,685 in 2022, with a structural break around 2017–2018 coinciding with overlapping economic, political, and health-system pressures (12). Reported push factors include demanding working conditions, workplace violence, systemic dysfunction, and burnout, the latter highly prevalent among attending Turkish surgeons (13,14).

What remains absent from the literature is a systematic, multi-year empirical analysis of TUS placement outcomes. Existing Turkish studies have examined single cycles, surveyed candidates, or described the broader workforce, but none has quantified how fill rates, score distributions, and supplementary-placement dynamics have evolved across consecutive cycles, or how they vary by institution type, geography, and quota structure. This gap is consequential because placement outcomes — fill rates, fully unfilled centres, and score floors and ceilings — operationalise specialty preference into tangible workforce signals that survey instruments cannot easily capture.

Methods

This retrospective descriptive study analyzed general surgery residency placement data

obtained from fourteen consecutive Tıpta Uzmanlık Sınavı (TUS) cycles conducted between 2019 and 2025 in Türkiye. All placement data were retrieved from publicly accessible datasets published on the official website of the Measurement, Selection and Placement Center (ÖSYM), the national authority responsible for residency examination and placement procedures (15).

The study dataset included institutional quota characteristics, placement outcomes, quota fill rates, placement score distributions, hospital types, geographic regions, metropolitan status, supplementary placement status, quota size, and quota category. Hospital types were classified as university hospitals, training and research hospitals, and city hospitals. Geographic analyses were performed according to the official regional classification of Türkiye. Metropolitan status was determined based on official metropolitan municipality designation.

The unit of analysis was an individual quota record, defined as a general surgery quota line for a specific institution, TUS examination period, and quota category. Thus, the same institution could contribute more than one record across different examination periods or quota categories. For each record, the fill rate was calculated as the number of filled positions divided by the number of available positions and expressed as a percentage. The overall mean fill rate represents the unweighted arithmetic mean of these record-level fill percentages rather than the position-level proportion obtained by dividing the total number of filled positions by the total number of available positions.

Quota fill rates were evaluated across examination periods, institutional categories, geographic regions, and quota structures. Fully filled, partially filled, and fully unfilled quota records were analyzed separately. Domestic and international quota lines were also examined comparatively.

Because the study used exclusively publicly accessible, fully anonymized secondary data and did not involve direct human participation, patient information, or identifiable personal data, ethics committee approval and informed consent were not required in accordance with national research regulations and institutional ethical standards. The study was conducted in accordance with the principles of the Declaration of Helsinki (16).

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics (IBM Corp., Armonk, NY, USA). The analysis was primarily descriptive because the dataset comprised nationwide administrative placement records covering all available general surgery quota lines during the study period rather than a sample drawn for hypothesis testing. Continuous variables were summarized using mean, median, minimum, maximum, and measures of dispersion where appropriate, while categorical variables were expressed as frequencies and percentages. Fill rates were examined across examination periods, hospital types, geographic regions, metropolitan status, supplementary placement status, quota size, and quota category. Temporal changes were evaluated descriptively across consecutive examination periods and calendar years. Accordingly, comparisons between groups were interpreted as observed differences within the study dataset and were not intended to imply statistical significance or causality.

Results

A total of 2,617 placement records obtained from fourteen consecutive TUS examination periods conducted between 2019 and 2025 were included in the analysis. Annual record counts ranged from 314 (12.0%) in 2020 to 485 (18.5%) in 2025, with a balanced distribution between spring (n = 1,319; 50.4%) and autumn (n = 1,298; 49.6%) examination periods. University hospitals contributed 1,839 records (70.3%),

training and research hospitals 534 (20.4%), and city hospitals 244 (9.3%). The largest regional contribution was from Marmara (29.6%), followed by Central Anatolia (21.7%), the Black Sea (14.7%), Aegean (13.0%), Eastern Anatolia (7.3%), Mediterranean (6.7%), Southeastern Anatolia (6.5%), and Cyprus (0.6%). Metropolitan provinces accounted for 79.4% of records. The median quota size was 2 positions (range: 1–18); single-position quotas comprised 43.6% of records and quotas of ≥6 positions 5.7%. Domestic quotas represented 72.9% of records and international quotas 27.1% (Table 1).

The pooled mean fill rate across the study period was 48.30%, with 45.3% of quota records fully filled and 47.9% fully unfilled. Period-specific mean fill rates ranged from 38.99% (2021 spring) to 68.47% (2020 autumn). The highest values were observed in 2020 autumn (68.47%), 2019 autumn (66.07%), and 2020 spring (58.83%); the lowest were observed in 2021 spring (38.99%), 2023 autumn (39.59%), and 2024 autumn (39.92%). The proportion of fully unfilled quota records ranged from 30.4% (2020 autumn) to 57.7% (2024 autumn) (Table 2).

Mean fill rates differed across hospital types. City hospitals had a mean fill rate of 82.83%, with 82.0% of quota records fully filled and 15.6% fully unfilled. Training and research hospitals had a mean fill rate of 59.24% (54.9% fully filled; 36.7% fully unfilled). University hospitals had a mean fill rate of 40.54% (37.7% fully filled; 55.4% fully unfilled) (Table 3).

Mean fill rates ranged from 3.33% (Cyprus) to 60.87% (Marmara). Values in the remaining regions were 58.09% in the Mediterranean, 53.89% in Central Anatolia, 47.81% in the Aegean, 35.64% in Southeastern Anatolia, 28.77% in the Black Sea, and 26.69% in Eastern Anatolia. The proportion of fully unfilled quota records was lowest in Marmara (36.1%), the Mediterranean (40.0%), and Central Anatolia (43.7%), and highest in Cyprus (93.3%), the Black Sea (67.5%), and Eastern Anatolia (62.8%) (Table 4).

Table 1. Descriptive characteristics of the study dataset (n = 2617 placement records).

Variable	n	%
Examination year		
2019	341	13.0
2020	314	12.0
2021	376	14.4
2022	335	12.8
2023	344	13.1
2024	422	16.1
2025	485	18.5
Examination period		
Spring	1319	50.4
Autumn	1298	49.6
Supplementary placement		
No	1733	66.2
Yes	884	33.8
Hospital type		
University hospital	1839	70.3
Training and research hospital	534	20.4
City hospital	244	9.3
Geographic region		
Marmara	775	29.6
Central Anatolia	568	21.7
Black Sea	385	14.7
Aegean	339	13.0
Eastern Anatolia	191	7.3
Mediterranean	175	6.7
Southeastern Anatolia	169	6.5
Cyprus	15	0.6
Provincial status		
Metropolitan	2077	79.4
Non-metropolitan	540	20.6
Quota size group		
1 position	1141	43.6
2 positions	526	20.1
3–5 positions	801	30.6
≥6 positions	149	5.7
Quota type		
Domestic quota	1908	72.9
International quota	709	27.1

Data were compiled from ÖSYM TUS placement guides (2019–2025). No missing values. Median quota size was 2 (range: 1–18). ÖSYM: Assessment, Selection and Placement Centre (Ölçme, Seçme ve Yerleştirme Merkezi); TUS: Medical Specialty Examination (Tıpta Uzmanlık Sınavı).

Table 2. Quota fill profile by examination year and period.

Year	Period	n	Mean fill rate (%)	Fully filled quota records (%)	Fully unfilled quota records (%)
2019	Spring	172	53.58	52.9	45.3
2019	Autumn	169	66.07	65.1	32.5
2020	Spring	166	58.83	57.8	39.8
2020	Autumn	148	68.47	67.6	30.4
2021	Spring	182	38.99	32.4	52.7
2021	Autumn	194	42.00	40.2	54.6
2022	Spring	181	57.67	53.0	38.1
2022	Autumn	154	46.08	38.3	44.2
2023	Spring	169	50.02	47.9	46.7
2023	Autumn	175	39.59	32.6	51.4
2024	Spring	209	41.09	39.2	56.5
2024	Autumn	213	39.92	38.5	57.7
2025	Spring	240	46.09	42.9	50.0
2025	Autumn	245	40.04	37.6	57.1
Pooled	—	2617	48.30	45.3	47.9

Fill rate was calculated for each quota record as the number of placed candidates divided by the number of available positions. Percentages for fully filled and fully unfilled categories were calculated using the number of quota records within each examination period as the denominator. The pooled mean fill rate represents the unweighted mean of record-level fill percentages.

Table 3. Quota fill profile by hospital type.

Hospital type	n (%)	Mean fill (%)	Fully filled (%)	Partially filled (%)	Unfilled (%)
City hospital	244 (9.3)	82.83	82.0	2.5	15.6
Training and research hospital	534 (20.4)	59.24	54.9	8.4	36.7
University hospital	1839 (70.3)	40.54	37.7	6.9	55.4

Percentages for fully filled, partially filled, and unfilled categories were calculated using quota records within the corresponding group as the denominator.

Table 4. Quota fill profile by geographic region.

Geographic region	n (%)	Mean fill (%)	Fully filled (%)	Partially filled (%)	Unfilled (%)
Marmara	775 (29.6)	60.87	58.3	5.5	36.1
Mediterranean	175 (6.7)	58.09	57.1	2.9	40.0
Central Anatolia	568 (21.7)	53.89	52.1	4.2	43.7
Aegean	339 (13.0)	47.81	43.4	8.3	48.4
Southeastern Anatolia	169 (6.5)	35.64	31.4	11.2	57.4
Black Sea	385 (14.7)	28.77	26.5	6.0	67.5
Eastern Anatolia	191 (7.3)	26.69	18.8	18.3	62.8
Cyprus	15 (0.6)	3.33	0.0	6.7	93.3

Percentages for fully filled, partially filled, and unfilled categories were calculated using quota records within the corresponding geographic region as the denominator.

Table 5. Fill profile by metropolitan status and quota size.

Variable	n (%)	Mean fill (%)	Fully filled (%)	Unfilled (%)
Panel A. Provincial status				
Metropolitan	2077 (79.4)	54.68	51.9	41.8
Non-metropolitan	540 (20.6)	23.76	20.2	71.1
Panel B. Quota size				
1 position	1141 (43.6)	41.72	41.7	58.3
2 positions	526 (20.1)	44.01	41.3	53.2
3–5 positions	801 (30.6)	57.70	51.3	33.8
≥6 positions	149 (5.7)	63.35	55.0	24.8

Quota size groups were defined according to the total number of announced positions within each quota record. Percentages for fully filled and unfilled categories were calculated using quota records within the corresponding provincial-status or quota-size group as the denominator.

Table 6. Mean quota fill rates by examination year and hospital type (%).

Year	University hospital	Training & research hospital	City hospital	Overall mean
2019	53.17	85.37	95.45	59.77
2020	55.69	88.89	95.65	63.37
2021	32.40	59.94	88.62	40.54
2022	44.56	67.09	86.56	52.34
2023	34.76	56.09	82.50	44.72
2024	30.20	46.83	79.17	40.50
2025	34.09	51.10	70.37	43.04

All values are unweighted mean record-level fill percentages.

Mean fill rates were 54.68% in metropolitan provinces (51.9% fully filled; 41.8% fully unfilled) and 23.76% in non-metropolitan provinces (20.2% fully filled; 71.1% fully unfilled). When stratified by quota size, mean fill rates were 41.72% for single-position quotas, 44.01% for two-position quotas, 57.70% for 3–5 position quotas, and 63.35% for quotas of ≥6 positions (Table 5).

Mean fill rates declined across all three hospital types between 2019 and 2025. In university hospitals, mean fill rates decreased from 53.17% in 2019 to 34.09% in 2025, with a low of 30.20% in 2024. In training and research hospitals, mean fill rates decreased from 85.37% in 2019 to 51.10% in 2025, with a low of 46.83% in 2024. In city hospitals, mean fill rates decreased from 95.45% in 2019 to 70.37% in 2025. The overall annual mean fill rate ranged from 40.50% (2024) to 63.37% (2020) (Table 6).

Among quotas with at least one placed candidate (n = 1,364), the median minimum placement score ranged from 47.88 (2024) to 53.00 (2025), and the median maximum placement score ranged from 49.88 (2020) to 55.25 (2021 and 2025). Full ranges of minimum scores spanned 45.05 to 68.90 across years, and maximum scores spanned 45.10 to 73.68 (Table 7).

Of all quota records, 1,733 (66.2%) were filled within the principal placement round, with a mean fill rate of 72.93% (68.3% fully filled; 31.7% fully unfilled). The remaining 884 quota records (33.8%) entered the supplementary placement stage; in this round their mean fill rate was 0.10%, and 99.9% remained fully unfilled (Table 8). Stratified by hospital type, the rate of entering the supplementary stage was 11.9% for city hospitals, 24.3% for training and research hospitals, and 39.4% for university hospitals (Table 9).

Table 7. Distribution of minimum and maximum scores in filled quotas by examination year.

Year	n	Minimum score (median, IQR)	Maximum score (median, IQR)	Min (range)	Max (range)
2019	207	50.45 (47.12–54.12)	51.88 (47.56–56.67)	45.12–66.34	45.12–69.12
2020	203	48.12 (46.12–51.34)	49.88 (46.45–53.45)	45.12–63.45	45.12–66.34
2021	174	51.54 (47.52–55.84)	55.25 (51.14–59.54)	45.09–68.90	45.12–73.68
2022	198	50.59 (47.52–55.01)	54.68 (49.45–58.69)	45.09–68.22	45.12–73.68
2023	175	48.45 (46.16–52.46)	52.13 (49.13–56.73)	45.09–65.62	45.13–73.68
2024	182	47.88 (45.88–50.12)	50.67 (47.51–53.03)	45.12–61.12	45.12–61.12
2025	225	53.00 (50.00–56.12)	55.25 (52.12–58.97)	45.05–68.90	45.10–70.12

Only quotas with at least one placed candidate are included; fully unfilled quotas have no score record. IQR: interquartile range.

Table 8. Impact of the supplementary placement process on quota fill.

Placement stage	n (%)	Mean fill (%)	Fully filled (%)	Unfilled (%)
Did not enter supplementary stage	1733 (66.2)	72.93	68.3	31.7
Entered supplementary stage	884 (33.8)	0.10	0.1	99.9

Percentages were calculated using quota records within each placement-stage category as the denominator. Entry into the supplementary stage followed non-filling during the principal placement round; therefore, the observed association should not be interpreted as a causal effect of supplementary placement.

Table 9. Supplementary information: rate of entering the supplementary stage by hospital type.

Hospital type	No supplementary (%)	With supplementary (%)
City hospital	88.1	11.9
Training and research hospital	75.7	24.3
University hospital	60.6	39.4

Percentages represent the proportion of quota records within each hospital type that entered the supplementary placement stage.

Table 10. Comparison of domestic and international quota profiles.

Panel A. Overall fill profile

Quota type	n (%)	Mean fill (%)	Fully filled (%)	Partially filled (%)	Unfilled (%)
Domestic	1908 (72.9)	58.24	54.2	9.1	36.6
International	709 (27.1)	21.56	21.3	0.6	78.1

Panel B. Distribution by year

Year	Domestic n (%)	International n (%)	Domestic mean fill (%)	International mean fill (%)	International share (%)
2019	257 (75.4)	84 (24.6)	71.53	23.81	24.6
2020	252 (80.3)	62 (19.7)	67.46	46.77	19.7
2021	285 (75.8)	91 (24.2)	47.35	19.23	24.2
2022	265 (79.1)	70 (20.9)	56.92	35.00	20.9
2023	257 (74.7)	87 (25.3)	51.88	23.56	25.3
2024	260 (61.6)	162 (38.4)	56.50	14.81	38.4
2025	332 (68.5)	153 (31.5)	57.65	11.33	31.5

Of international quota positions, 94.1% were located in university hospitals and 5.8% in training and research hospitals; city hospitals offered no international quota in practice (n = 1). The share of international quotas increased in 2024 (from 19.7% to 38.4%), and this structural change should be considered when interpreting overall fill trends.

Domestic quotas (n = 1,908; 72.9%) had a mean fill rate of 58.24% (54.2% fully filled; 9.1% partially filled; 36.6% fully unfilled). International quotas (n = 709; 27.1%) had a mean fill rate of 21.56% (21.3% fully filled; 0.6% partially filled; 78.1% fully unfilled). The share of international quotas was 24.6% in 2019, 19.7% in 2020, 24.2% in 2021, 20.9% in 2022, 25.3% in 2023, 38.4% in 2024, and 31.5% in 2025. Mean fill rates for international quotas ranged from 11.33% (2025) to 46.77% (2020), and mean fill rates for domestic quotas ranged from 47.35% (2021) to 71.53% (2019). Of international quota positions, 94.1% were located in university hospitals and 5.8% in training and research hospitals (Table 10).

Discussion

Across the seven-year study period, general surgery placement patterns changed in several notable ways. Mean fill rates showed an overall downward pattern despite year-to-year fluctuations, and clear differences were observed across hospital types, geographic regions, and metropolitan status. Quota records that entered supplementary placement also showed persistent non-filling. These findings are broadly consistent with previous reports describing declining interest in surgical careers and changing residency recruitment patterns (3-5).

Differences across hospital types were among the most notable findings of the study. City hospitals had the highest observed mean fill rate (82.8%), followed by training and research hospitals (59.2%) and university hospitals (40.5%). University hospitals, despite their traditional role as major training quota records, showed lower observed fill rates than the other hospital categories. Career preferences are shaped by a range of personal, professional, and working-environment factors, and these may interact with institutional characteristics when candidates rank training positions (6,10). Several factors may therefore contribute to the differences observed in the present study. University hospitals accounted for 70.3% of all quota records, while international quotas, which had lower observed fill rates, were concentrated predominantly in university hospitals. In addition, hospital type, metropolitan location, quota category, and other institutional characteristics may overlap. Therefore, the observed differences between hospital types should not be interpreted as independent effects of institution type alone.

The geographic gradient is even more dramatic, with mean fill ranging from 60.9% in Marmara to 3.3% in Cyprus, and a 31-percentage-point gap between metropolitan (54.7%) and non-metropolitan (23.8%) provinces. This pattern is consistent with the broader geographic maldistribution of the surgical workforce described in other settings (8). In the Turkish context, persistently low fill rates in Eastern Anatolia and the Black Sea region may contribute to ongoing geographic imbalances in the surgical workforce. The Cyprus finding should be interpreted more cautiously because of the small number of observations.

The temporal trajectory aligns with the push factors documented in recent Turkish literature. Although annual fill rates fluctuated over the study period, the overall pattern was downward across all three hospital types between 2019 and 2025, with the largest absolute decrease observed in university hospitals (53.2% to 34.1%) and the lowest overall annual level recorded in 2024. Yıldız and Khan reported that fewer than 30% of TUS candidates intended to pursue surgery, with malpractice risk and workplace violence as significant deterrents (10), while Aydın and colleagues documented a more than 45-fold rise in Good Standing Certificate applications across the same window (12). Burnout among attending Turkish surgeons (14) and the broader brain-drain literature (13) complete the picture. The present results extend that evidence base by demonstrating that these macro-level pressures translate directly into measurable placement-level outcomes. A similar pattern has also been observed in general surgery subspecialty placements in Türkiye, where occupancy rates declined over time despite

an increase in available positions, suggesting that reduced filling may extend beyond core residency recruitment to later stages of surgical training (17).

Two aspects of the results deserve closer attention. Quota records entering the supplementary placement stage showed a very high rate of persistent non-filling, with 99.9% remaining fully unfilled. However, entry into the supplementary stage occurs after failure to fill during the principal placement round, and this association should therefore not be interpreted as evidence that the supplementary process itself causes poor filling. Rather, supplementary placement appears to identify quota records with a particularly high likelihood of remaining unfilled. Separately, international quota records showed a notable mismatch between increasing quota availability and declining observed fill rates. The share of international quotas increased from 19.7% in 2020 to 38.4% in 2024, while their mean fill rate decreased over the same period. This pattern may indicate that expansion in available positions was not matched by a corresponding increase in placement demand. Such findings should be considered within the broader context of specialty-choice determinants and changing physician career preferences reported in Türkiye and elsewhere (6,10-13,17).

Notably, placement score distributions among filled quota records remained relatively stable across the study period, with median minimum scores ranging from 47.9 to 53.0. This finding suggests that the observed decline in fill rates was not accompanied by a marked shift in the score distribution among successfully placed candidates. However, because completely unfilled quota records have no placement score data, these findings should be interpreted with caution and may be subject to selection effects.

This study has several limitations. First, the ecological analysis was based on administrative placement records and did not include candidate-level characteristics such as age, sex, socioeconomic background, individual preferences, or reasons for specialty choice. Second, hospital type, geographic location, metropolitan status, and quota category may be interrelated, and the descriptive design does not allow these factors to be separated as independent determinants of fill rates. Third, candidate preference-ranking data were not available, limiting interpretation of why particular institutions or regions were selected or avoided. In addition, placement outcomes do not necessarily reflect subsequent residency enrollment, retention, transfer, resignation, or completion. Finally, the observational design does not permit causal attribution of the observed patterns to specific policy, economic, institutional, or workforce-related changes.

Conclusion

Between 2019 and 2025, general surgery residency placements in Türkiye showed an overall decline in fill rates, although the pattern was not uniform from year to year. The findings also revealed substantial variation across hospital types, geographic regions, metropolitan status, and quota categories. Lower fill rates were particularly evident in university hospitals and non-metropolitan areas, while quota records entering supplementary placement frequently remained unfilled. These findings should be interpreted as descriptive national patterns rather than independent or causal effects of institutional or geographic characteristics. Continued monitoring of placement trends, together with candidate-level preference and follow-up data, may help clarify the factors shaping general surgery recruitment and support more targeted workforce planning.

Ethical approval

Ethics committee approval was not required because this study analyzed publicly available, fully anonymized secondary data obtained from the official website of the Measurement,

Selection and Placement Center (ÖSYM). The study did not involve human participants, identifiable or re-identifiable personal data, or any intervention. The study was conducted in accordance with relevant ethical principles and the Declaration of Helsinki.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: SC, SD, SB; data collection: SC, AY, FY, MSS; analysis and interpretation of results: SC, SD, AY, SB; draft manuscript preparation: SC, SD, MSS; critical revision: SD, FY, SB; supervision: SB. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Research Article

Psychometric validation of the Turkish Trypophobia Questionnaire and determinants of trypophobia symptoms in a community-based sample

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ABSTRACT

Background: Trypophobia describes aversive responses to clustered visual patterns, but its epidemiology and cross-cultural measurement remain insufficiently studied. This study evaluated the psychometric properties of the Turkish Trypophobia Questionnaire (TQ) and examined factors associated with symptom severity.

Methods: This cross-sectional study included 261 community participants aged ≥15 years. Reliability and construct validity were assessed using internal consistency measures, exploratory and confirmatory factor analyses, and parallel analysis. Independent associations with TQ scores were examined using multivariable linear regression with HC3 robust standard errors.

Results: The TQ showed high internal consistency (Cronbach's α=0.968; McDonald's ω=0.970), but 61.7% of participants had the minimum score. Parallel analysis favored one factor, whereas one-factor CFA showed poor fit (CFI=0.768, TLI=0.726, RMSEA=0.230). Higher scores were independently associated with female sex and psychiatric disorders, while age and educational level showed inverse associations.

Conclusions: The Turkish TQ showed strong internal consistency, but its structural validity remains uncertain because of the marked floor effect and poor CFA fit. Further validation using independent samples and methods appropriate for ordinal data is needed.

Keywords: trypophobia, psychometric validation, reliability, construct validity, Türkiye

Introduction

Trypophobia refers to a marked aversive response to clustered visual patterns, particularly groups of holes, bumps, pores, or similar repetitive structures. Although its name implies a fear response, affected individuals frequently describe disgust, visual discomfort, and unpleasant bodily sensations rather than fear alone (1-3). This distinction has shaped the current view of trypophobia as a perceptual–emotional phenomenon rather than a conventional specific phobia. Its associations with disgust processing, anxiety-related traits, and other forms of emotional vulnerability have also raised broader questions about how visual characteristics acquire aversive emotional significance (3-5).

Available epidemiological data suggest that trypophobic responses are not uncommon, although their reported frequency varies

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considerably. Studies in different populations have estimated prevalence at approximately 10–18%, with differences in sampling, assessment procedures, and diagnostic thresholds likely contributing to this variability (1,6,7). Demographic associations are similarly inconsistent. Some studies report greater symptom severity among women and younger individuals, but these findings have not been consistent across populations (4,7,8). Cultural context and exposure to trypophobia-related material, particularly through digital media, may further influence how people perceive and report these visual patterns (7,8).

Two main explanations have been proposed for the aversion produced by clustered patterns. One focuses on their visual properties, particularly spatial-frequency characteristics capable of producing visual discomfort (1). The other places greater emphasis on disease avoidance. From this perspective, clustered patterns may resemble visual cues associated with infection, parasites, or skin lesions and consequently evoke disgust and avoidance as part of the behavioral immune response (9-11). The latter account is supported by evidence that aversion becomes particularly strong when such patterns are presented on skin or other biologically meaningful backgrounds (12,13). Experimental work also indicates that disgust is generally more prominent than fear during exposure to trypophobic stimuli (5,11). Physiological and neurophysiological responses reported during stimulus exposure further suggest that the experience involves both perceptual and affective processes (14,15).

Trypophobia has also been associated with psychological characteristics extending beyond the immediate response to visual stimuli. Higher levels of anxiety sensitivity, trait anxiety, disgust sensitivity, neuroticism, depressive symptoms, and psychological distress have been reported among individuals with stronger trypophobic responses (4,7,16). These associations do not establish trypophobia as a psychiatric disorder, but they suggest that individual differences in emotional vulnerability may contribute to symptom severity. Understanding these relationships is therefore relevant when examining trypophobia at the population level.

The Trypophobia Questionnaire (TQ) is widely used to quantify these responses in research settings (17). The original instrument showed good psychometric performance, and subsequent work, including a Japanese adaptation and Rasch analysis, has provided additional evidence regarding its reliability and measurement characteristics (18,19). Nevertheless, questions remain concerning its latent structure and its performance across languages and populations. Such issues are particularly important for a construct whose expression may be influenced by cultural and contextual factors.

Evidence from Türkiye remains scarce, and a Turkish version of the TQ has not been adequately evaluated in a community sample. This limits both the assessment of trypophobia within the Turkish population and comparisons with findings reported elsewhere. Therefore, the present study evaluated the psychometric properties of the Turkish TQ in a community-based sample and examined the distribution of trypophobia symptoms and their demographic and clinical correlates. We specifically assessed reliability and construct validity and explored whether symptom severity varied according to age, sex, educational level, psychiatric/neurological history, and self-reported phobia status.

Materials and Methods

Study design and participants

This cross-sectional study was conducted in Tokat Province, Türkiye, to evaluate trypophobia symptoms and the psychometric properties of the Turkish version of the Trypophobia Questionnaire (TQ). Participants

were recruited voluntarily from the community and completed a self-administered questionnaire. Individuals aged ≥15 years who could complete the questionnaire independently were eligible. Questionnaires with missing data were excluded, leaving 261 participants with complete data for analysis.

Ethical approval

The study was approved by the Clinical Research Ethics Committee of Gaziosmanpaşa University Faculty of Medicine (Approval No. 17-KAEK-158; Meeting No. 2017/16; December 12, 2017) and conducted in accordance with the Declaration of Helsinki. All participants received information about the study and provided written informed consent. Participation was voluntary and responses were anonymous.

Questionnaire

The questionnaire collected age, sex, educational level, physician-diagnosed psychiatric or neurological disorders, and self-reported phobias. Education was recorded in seven ordered categories, from illiterate to doctoral degree. Participants were also asked whether they had a specific phobia, including trypophobia. Thus, the variable "any phobia" was based on self-report and could overlap with the TQ outcome.

The 14-item TQ was completed after participants viewed standardized trypophobia-evoking images (Figure 1). Items assessed emotional and somatic responses to clustered-hole stimuli and were rated from 1 (not at all) to 5 (extremely). Total scores ranged from 14 to 70, with higher values indicating more severe trypophobia symptoms.

Statistical analysis

Analyses were performed using IBM SPSS Statistics version 29 (IBM Corp., Armonk, NY, USA), with psychometric analyses conducted in R (version 4.x). Continuous data are reported as mean ± standard deviation or median (interquartile range), as appropriate, and categorical data as numbers and percentages. Normality was assessed with the Shapiro–Wilk test. Because TQ scores were non-normally distributed, two-group comparisons used the Mann–Whitney U test, comparisons across educational categories used the Kruskal–Wallis test, and associations with age were examined using Spearman's correlation.

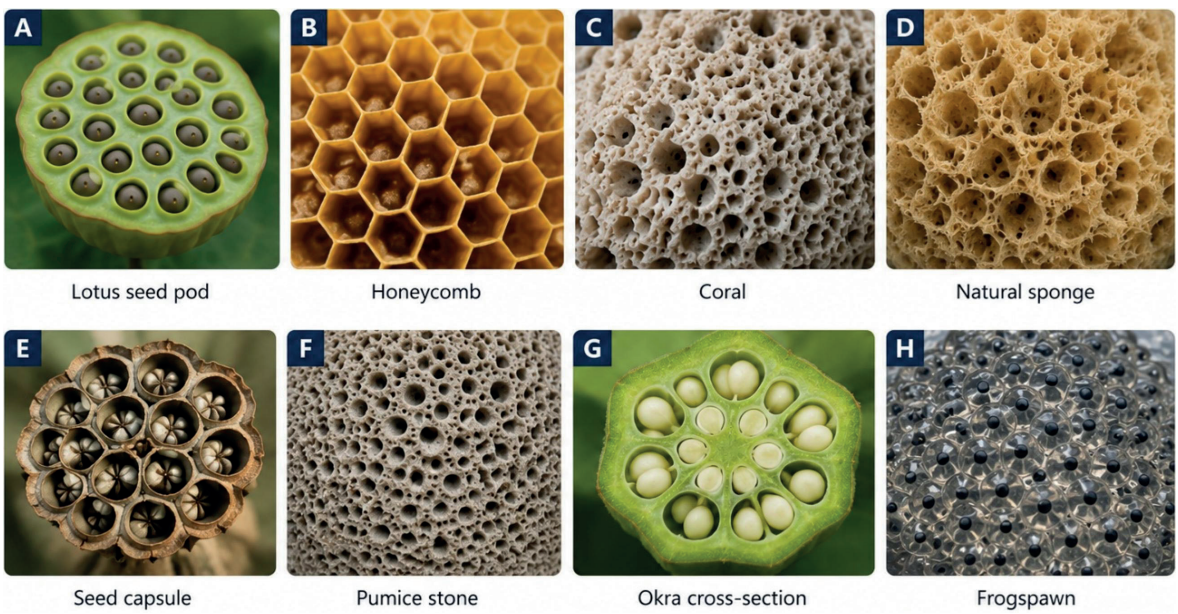

Figure 1. Representative clustered visual stimuli used to illustrate trypophobia-related patterns.

Internal consistency was evaluated with Cronbach's alpha, McDonald's omega, corrected item–total correlations, and alpha if item deleted. Construct validity was examined using the Kaiser–Meyer–Olkin measure, Bartlett's test of sphericity, parallel analysis, exploratory factor analysis (EFA), and one-factor confirmatory factor analysis (CFA). CFA fit was evaluated using the Comparative Fit Index (CFI), Tucker–Lewis Index (TLI), Root Mean Square Error of Approximation (RMSEA), and Standardized Root Mean Square Residual (SRMR).

A multivariable linear regression model was used to examine associations of age, sex, educational level, physician-diagnosed psychiatric/neurological disorders, and self-reported phobia with total TQ scores. Education was entered as an ordered seven-level variable. Because model residuals showed heteroscedasticity and non-normality, HC3 heteroscedasticity-consistent robust standard errors were used. Results are reported as unstandardized coefficients (B), robust standard errors, 95% confidence intervals, variance inflation factors, and p-values.

Results

Participant characteristics

A total of 261 participants with complete data were included in the study. The mean age of the participants was 36.62 ± 12.54 years, with a median age of 36 years (IQR: 26–44; range: 15–74). Of the participants, 147 (56.3%) were male and 114 (43.7%) were female. At least one psychiatric disorder was reported by 25 participants (9.6%), and 56 participants (21.5%) reported at least one phobia (Table 1).

Distribution of scale scores

The mean total TQ score was 20.57 ± 11.98, with a median of 14 (IQR: 14–22) and an observed range of 14–66 (Figure 2). A total of 161 of 261 participants (61.7%) obtained the minimum possible total score of 14, constituting a pronounced observed floor effect and indicating limited discrimination at the low-symptom end in this sample. The Shapiro–Wilk test indicated that normality was not met ($W = 0.623$, $p < 0.001$); therefore, non-parametric methods were used for group comparisons.

Table 1. Demographic and clinical characteristics of the participants.

Variable	Category	n	%	Mean ± SD	Median (IQR)	Range
Age (years)	Overall	261	—	36.62 ± 12.54	36 (26–44)	15–74
Sex	Male	147	56.3	—	—	—
	Female	114	43.7	—	—	—
Educational level	Illiterate	11	4.2	—	—	—
	Primary school	58	22.2	—	—	—
	High school	43	16.5	—	—	—
	Associate degree	28	10.7	—	—	—
	Bachelor's degree	109	41.8	—	—	—
	Master's degree	9	3.4	—	—	—
	Doctoral degree	3	1.1	—	—	—
Psychiatric disorder	No	236	90.4	—	—	—
	Yes	25	9.6	—	—	—
Any phobia	No	205	78.5	—	—	—
	Yes	56	21.5	—	—	—

SD, standard deviation; IQR, interquartile range.

Table 2. Descriptive statistics and reliability of the trypophobia scale.

Measure	n	Mean ± SD	Median (IQR)	Range	Cronbach's α	McDonald's ω	Distribution
Total score	261	20.57 ± 11.98	14 (14–22)	14–66	0.968	0.970	Non-normal; Shapiro–Wilk W = 0.623, p < 0.001
Mean item score	261	1.47 ± 0.86	1.00 (1.00–1.57)	1.00–4.71	—	—	—

SD, standard deviation; IQR, interquartile range.

Table 3. Item analysis and one-factor solution results.

Item	Mean	SD	Corrected item–total correlation	Cronbach's α if item deleted	Factor loading	Communality	Interpretation
Something crawling on my skin	1.460	0.930	0.766	0.967	0.768	0.590	Strong
Desire to scratch	1.650	1.160	0.805	0.966	0.792	0.628	Strong
Feeling uncomfortable	1.660	1.190	0.802	0.967	0.795	0.632	Strong
Disgust	1.640	1.160	0.883	0.965	0.878	0.771	Strong
Fear	1.490	1.040	0.857	0.965	0.869	0.755	Strong
Itching sensation	1.460	0.950	0.831	0.966	0.843	0.711	Strong
Shivering	1.380	0.900	0.848	0.966	0.872	0.760	Strong
Goosebumps	1.570	1.090	0.832	0.966	0.841	0.707	Strong
Irritability	1.380	0.940	0.811	0.966	0.842	0.709	Strong
Anxiety	1.420	0.980	0.842	0.966	0.865	0.748	Strong
Nausea	1.410	0.980	0.824	0.966	0.834	0.696	Strong
Feeling like going crazy	1.300	0.830	0.843	0.966	0.874	0.763	Strong
Panic	1.300	0.830	0.827	0.966	0.854	0.729	Strong
Avoiding objects with holes	1.460	1.130	0.729	0.968	0.744	0.554	Strong

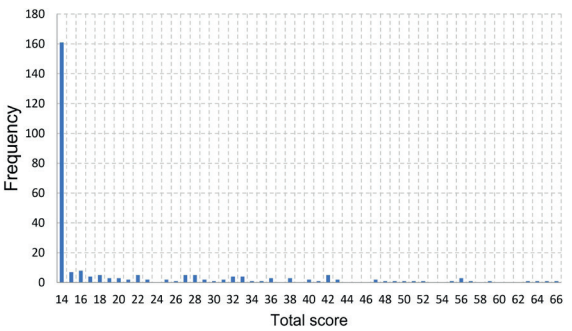

Figure 2. Distribution of total Trypophobia Scale scores.

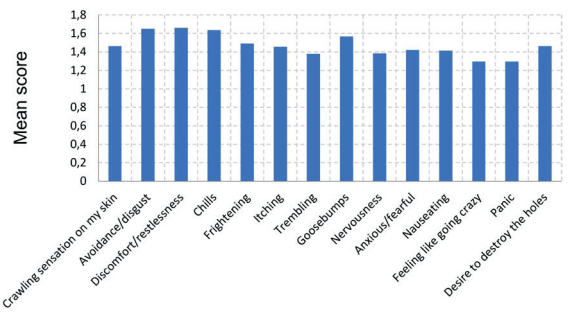

Figure 3. Mean scores of the questionnaire items.

Reliability and item analysis

The scale demonstrated excellent internal consistency (Cronbach's α = 0.968; McDonald's ω = 0.970). Corrected item–total correlations ranged from 0.729 to 0.883, factor loadings in the one-factor solution ranged from 0.744 to 0.878, and communalities ranged from 0.554 to 0.771. Removal of any individual item did not increase Cronbach's α coefficient (Table 2, Table 3, and Figure 3).

Construct validity

The Kaiser–Meyer–Olkin (KMO) measure was 0.926, indicating excellent sampling adequacy, and Bartlett's test of sphericity was statistically significant [$\chi^2(91) = 4567.30$, $p < 0.001$]. With 261 participants for 14 items, the achieved sample size corresponded to approximately 18.6 participants per item. Parallel analysis suggested a single-factor structure, with the first factor explaining 72.1% of the total variance (Figure 4). The reported one-factor CFA used a Pearson/maximum-likelihood approach and showed inadequate fit [$\chi^2(77) = 1137.71$; CFI = 0.768, TLI = 0.726, RMSEA = 0.230], whereas SRMR was acceptable (0.072) (Table 4). Because the items are ordinal and responses showed a pronounced floor effect, these results should be regarded as an internal structural evaluation rather than independent confirmation. Independent validation using polychoric correlations with WLSMV or ESEM is warranted.

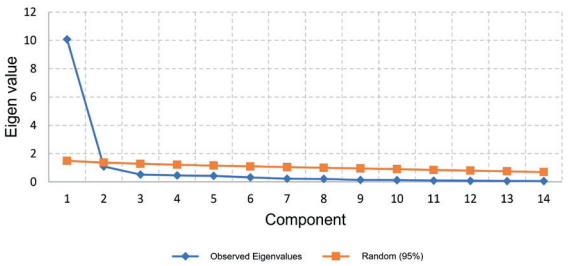

Figure 4. Results of the parallel analysis.

Group comparisons

Women had significantly higher total scores than men [median (IQR): 14 (14–30) vs. 14 (14–17); U = 6863.5, p = 0.004, r = −0.181]. Participants with a psychiatric disorder had significantly higher scores than those without a psychiatric disorder [27 (14–40) vs. 14 (14–19); U = 4259.0, $p < 0.001$, r = 0.444]. Likewise, participants reporting any phobia had higher total scores than those without a phobia [14 (14–33) vs. 14 (14–18); U = 6896.0, p = 0.008, r = 0.201]. No significant differences were observed among educational groups (H = 9.560, p = 0.144), and no significant correlation was found between age and the total score ($\varrho = -0.021$, p = 0.732) (Table 5).

Table 4. Construct validity and factor analysis results.

Indicator	Value	Interpretation	CFA index	Value	Recommended threshold	Result	Note
KMO	0.926	Excellent sampling adequacy	χ^2 (df = 77)	1137.71	p < 0.001	Significant	Pearson/ML confirmatory analysis
Bartlett's test	$\chi^2(91) = 4567.30$; p < 0.001	Suitable for factor analysis	CFI	0.768	≥ 0.90	Poor fit	
Parallel analysis	One factor	A unidimensional structure was suggested	TLI	0.726	≥ 0.90	Poor fit	
First eigenvalue	10.089	Explained 72.1% of the total variance	RMSEA	0.230	≤ 0.08	Poor fit	
			SRMR	0.072	≤ 0.08	Acceptable	

Although the parallel analysis suggested a one-factor structure, the overall fit of the one-factor confirmatory factor analysis (CFA) was poor. For ordinal Likert-scale items, CFA based on polychoric correlations using the weighted least squares mean and variance adjusted (WLSMV) estimator or exploratory structural equation modeling (ESEM) is recommended in an independent sample. Performing both exploratory factor analysis (EFA) and CFA on the same sample does not constitute independent validation evidence.

Table 5. Group comparisons and correlation analysis of total Trypophobia Questionnaire scores.

Comparison	Group 1	Group 2	Test	Statistic	p	Effect size	Interpretation
Sex	Male: 14 (14–17)	Female: 14 (14–30)	Mann–Whitney U	U = 6863.5	0.004	r = −0.181	Higher scores in females
Psychiatric disorder	Yes: 27 (14–40)	No: 14 (14–19)	Mann–Whitney U	U = 4259.0	<0.001	r = 0.444	Moderate effect size
Any phobia	Yes: 14 (14–33)	No: 14 (14–18)	Mann–Whitney U	U = 6896.0	0.008	r = 0.201	Small effect size
Educational level	Seven groups	—	Kruskal–Wallis	H = 9.560	0.144	—	Not significant
Age vs. total score	—	—	Spearman's correlation	ϱ = −0.021	0.732	—	Not significant

Table 6. Multivariable determinants of total Trypophobia Questionnaire scores: Linear regression with HC3 robust standard errors.

Variable	B	Robust SE	z	p	95% CI	VIF	Interpretation
Age (years)	−0.112	0.054	−2.091	0.037	−0.217 to −0.007	3.43	Negative association
Female (reference: male)	3.315	1.491	2.223	0.026	0.392 to 6.237	1.79	Positive association
Educational level (1–7)	−1.649	0.558	−2.956	0.003	−2.743 to −0.556	3.75	Negative association
Psychiatric disorder (yes)	10.157	3.569	2.846	0.004	3.161 to 17.153	1.14	Strongest positive association
Any phobia (yes)	3.840	2.102	1.827	0.068	−0.280 to 7.959	1.39	Borderline; not statistically significant

Model summary: n = 261; R^2 = 0.158; adjusted R^2 = 0.141; F = 6.384; p < 0.001.
The model identifies statistical associations and should not be interpreted as demonstrating causality.

Multivariable analysis

The multivariable linear regression model using HC3 robust standard errors was statistically significant (F = 6.384, p < 0.001) and explained 15.8% of the variance in the total score (R^2 = 0.158; adjusted R^2 = 0.141). Age (B = −0.112, 95% CI: −0.217 to −0.007; p = 0.037) and educational level (B = −1.649, 95% CI: −2.743 to −0.556; p = 0.003) were independently associated with lower total scores. Female sex (B = 3.315, 95% CI: 0.392–6.237; p = 0.026) and the presence of a psychiatric disorder (B = 10.157, 95% CI: 3.161–17.153; p = 0.004) were independently associated with higher total scores. The presence of any phobia did not reach statistical significance (B = 3.840, 95% CI: −0.280 to 7.959; p = 0.068) (Table 6).

Main findings

In this sample, the trypophobia scale demonstrated excellent internal consistency and a strong common factor structure. However, the pronounced floor effect and the poor fit of the one-factor CFA model suggest that the construct validity of the scale should be re-evaluated in an independent sample using ordinal analytical approaches. Female sex and the presence of a psychiatric disorder were independently associated with higher trypophobia scores.

Discussion

The present study evaluated the Turkish version of the TQ in a community sample and examined factors associated with trypophobia symptoms. The distribution of scores was notably uneven, with 61.7% of participants

obtaining the minimum possible score and a smaller group reporting substantially greater symptoms. Despite this pronounced floor effect, the questionnaire showed excellent internal consistency and a strong dominant factor in exploratory analyses. The one-factor CFA, however, showed poor fit. These findings suggest that the TQ items capture closely related experiences, but they also show that high reliability should not be taken as evidence of a satisfactory underlying structure.

Reported rates of trypophobia differ across populations and study methods (1,6-8). We did not use a diagnostic cut-off, so our data cannot be used to estimate prevalence. Notably, 61.7% of participants had the minimum TQ score, whereas higher scores were concentrated in a much smaller group. This strong floor effect also suggests limited discrimination among individuals with few or no symptoms.

Women had higher TQ scores than men, and this association remained significant after adjustment. Similar sex differences have been reported in previous studies, although the pattern has not been consistent across populations (4,7,8). Psychiatric/neurological disorders showed an even stronger relationship with TQ scores. Participants reporting such a diagnosis had substantially higher scores, in line with previous studies linking trypophobia with anxiety sensitivity, depressive symptoms, psychological distress, disgust sensitivity, and neuroticism (4,7,16). This association may indicate that trypophobic responses occur alongside broader emotional vulnerability, although the cross-sectional design does not allow the direction of this relationship to be determined.

One possible explanation involves disgust and disease avoidance. Clustered patterns may resemble visual signs associated with skin disease, parasites, or contamination and may therefore evoke avoidance responses related to the behavioral immune system (9-11). Experimental studies reporting stronger aversion when clustered patterns appear on human skin or other biologically relevant backgrounds support this possibility (12,13). However, disgust sensitivity, anxiety, physiological responses, and behavioral immune activation were not directly measured in the present study. These mechanisms therefore remain possible explanations rather than conclusions that can be drawn from our data.

The psychometric findings deserve particular attention. Cronbach's alpha (0.968) and McDonald's omega (0.970) showed excellent internal consistency, while parallel analysis identified a dominant factor explaining 72.1% of the variance. In contrast, the one-factor CFA showed poor fit, particularly for CFI, TLI, and RMSEA. This apparent discrepancy is not necessarily unexpected because internal consistency and structural validity reflect different measurement properties (20,21). Highly correlated items can produce high reliability even when a strict unidimensional model does not adequately represent the response structure. The pronounced floor effect may also have contributed to the poor CFA performance because strongly skewed ordinal responses can affect conventional covariance-based estimation (22,23). The present findings therefore support the presence of a strong common component but do not confirm strict unidimensionality. Future validation using polychoric correlations and estimators appropriate for ordinal data, such as WLSMV, would provide a more appropriate test of the scale structure.

Age and education require more cautious interpretation. Age was not associated with TQ scores in the unadjusted analysis but showed a small inverse association after adjustment, making a simple age effect difficult to establish. Higher education was also independently associated with lower scores, although the highest educational categories included relatively few participants. Possible explanations involving habituation, cognitive appraisal, or sociocultural exposure cannot be tested with the present data. Interpretation of

the self-reported "any phobia" variable is also complicated by the inclusion of trypophobia itself in this category. Although participants reporting any phobia had higher scores in the unadjusted analysis, the association did not remain statistically significant in the multivariable model. A strength of the study is that these relationships were examined together rather than relying solely on unadjusted comparisons. The community-based sample, item-level psychometric assessment, complementary measures of reliability and factor structure, and use of robust standard errors further strengthen the analysis.

Limitations

This study has several limitations. Participants were volunteers from a single province, which may limit generalizability, and psychiatric and neurological diagnoses were self-reported. The cross-sectional design prevents causal interpretation. In addition, the available records did not allow full reconstruction of the translation/adaptation process or all details of stimulus presentation. The pronounced floor effect may have reduced discrimination at the lower end of the scale and affected the factor-analytic results. Finally, EFA and CFA were conducted in the same sample, and the one-factor CFA showed poor fit. The Turkish TQ therefore requires confirmation in an independent sample using methods appropriate for ordinal data.

Conclusion

The Turkish TQ showed excellent internal consistency, although its structural validity remains uncertain because of the marked floor effect and poor one-factor CFA fit. TQ scores were higher among women and participants with self-reported psychiatric/neurological disorders, while age and education showed inverse associations after adjustment. Further validation in independent samples using ordinal psychometric methods is needed.

Ethical approval

The study was approved by the Clinical Research Ethics Committee of Gaziosmanpaşa University Faculty of Medicine (Approval No. 17-KAEK-158; Meeting No. 2017/16; December 12, 2017).

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: HA; data collection: SSG,HA; analysis and interpretation of results: SSG; draft manuscript preparation: HA. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Review

The role of xanthan gum in necrotizing enterocolitis: clinical implications and safety for preterm neonates

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ABSTRACT

Necrotizing enterocolitis (NEC) is severe inflammatory disease that predominantly affects preterm infants and is closely related to shaping the gut microenvironment and immune system. Early-life feeding plays a critical role in the pathophysiology of NEC. Xanthan gum (XG) has viscosity-stabilizing properties and resistance to breast milk amylase. Therefore, XG has therefore been used in the management of dysphagia in infants. However, its safety in the intestines of premature infants remains controversial. This study aims to review current scientific evidence on the use of XG in NEC and to evaluate potential pathophysiological mechanisms from a clinical perspective. Current data suggest that fermentation of XG by the colonic microbiota into short-chain fatty acids may contribute to mucosal injury in the immature gut, although a causal relationship has not been established. Clinical studies show that NEC cases associated with XG-based thickeners have a later onset than classical NEC, with approximately half of cases developing post-discharge. Therefore, regulatory authorities such as the FDA, EFSA, and JECFA have published restrictive guidelines on the use of XG in neonates. In this context, it is crucial that XG-based products be carefully evaluated, especially in at-risk infants, and that the decision to thicken the product be made through a multidisciplinary, individualized approach that considers the infant's intestinal maturity.

Keywords: food additives, necrotizing enterocolitis, preterm infants, xanthan gum

Introduction

Food additives have been used for many years to enhance the appearance, taste, texture, and shelf life of food products (1,2). The impact of additives on intestinal health is a frequently addressed topic in research. Studies suggest that food additives can modulate the gut microbiota, which are essential for maintaining the intestinal barrier (2). In addition to their effects on the microbiota, various studies have shown that several food additives may induce intestinal damage (3).

Necrotizing enterocolitis (NEC) is characterised by severe intestinal inflammation, particularly in preterm neonates (4). Frequently encountered in neonatal intensive care units, this condition carries a high risk of mortality (5). NEC can rapidly worsen, leading to serious complications such as damage to intestinal tissue, perforation, sepsis, or shock. Major risk factors are prematurity and very low birth weight (VLBW) (4). Consistently, gut bacterial imbalance and intestinal immaturity also contribute to the problem (6). In addition, hypoxia, reduced blood flow, invasive breathing support, and

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prolonged antibiotic use can increase the risk. From a practical standpoint, feeding premature infants formula rather than breast milk is another important risk factor. This highlights the importance of components in formula, especially thickeners among food additives (7).

To prevent NEC, various preventive strategies have been developed or implemented in clinical practice. These include breastfeeding, nutritional strategies, and the use of specific food additives in breast milk, including probiotics, prebiotics, glutamine, arginine, and lactoferrin (8-10). Apart from that, the effects of gum-based thickeners, such as Xanthan Gum (XG), among additives commonly used in premature infants with dysphagia, on intestinal health are a subject of considerable debate (11). Clinical findings indicate that XG-associated NEC cases have a later onset and more pronounced colonic involvement. On the other hand, multiple observational studies report no increased risk of NEC with XG-based thickeners (12,13). These conflicting findings highlight the need to reassess the safety of XG-based thickeners in neonatal nutrition. Already, the potential implications of XG for NEC and the current regulatory perspectives on this substance have not yet been adequately discussed in the literature. This study aims to critically evaluate the potential physiological effects of XG relevant to NEC pathogenesis in preterm neonates. In addition, legal regulations regarding XG over the last 20 years have also been examined.

Preterm Infant Physiology

The suboptimal development of the intestinal barrier in preterm infants arises from both deficiencies in structural proteins and an imbalance in the microbial population. This issue becomes more pronounced as birth weight and gestational age decrease (14). Preterm infants have a less developed and permeable intestinal barrier. This immaturity, along with their weaker immune systems and less developed gut bacteria, makes them more vulnerable to serious health issues like NEC (15,16). In preterm infants, the expression of epithelial barrier-supporting proteins such as mucin-5AC (MUC5AC), trefoil factors (TFF2 and TFF3), and neutrophil defensin 3 (DEFA3) is lower in the fecal proteome compared to term infants. This deficiency in preterm infants indicates a thinner, less stable mucus layer, weakening the intestinal barrier (14). Intestinal barrier development is also closely related to the microbiota (17). An inverse relationship has been observed between the deficiency of barrier proteins in preterm infants and the production of bacterial oxidative stress proteins by opportunistic pathogens (e.g., *Enterococcus spp.* and *Klebsiella spp.).* In term infants, beneficial bacteria such as *Bifidobacterium*, which are more abundant, protect by producing short-chain fatty acids (SCFAs) that stimulate barrier function. However, colonization by these bacteria is generally delayed in preterm infants (18).

In preterm infants, reduced intestinal motility and impaired carbohydrate absorption may promote excessive lactose fermentation by potentially pathogenic bacteria, such as *Clostridium butyricum*, leading to overproduction of butyric acid and subsequent mucosal injury. This excessive production is thought to upregulate inducible nitric oxide synthase (iNOS) gene expression, which is associated with mucosal damage, thereby contributing to the development of NEC (19-21).

At normophysiological concentrations, butyrate exerts protective effects by activating the Notch Receptor 1 (Notch1) signaling pathway and enhancing the expression of key negative regulators of intestinal inflammation, such as Single Ig IL-1 Related Receptor (SIGIRR) and Tumor Necrosis Factor Alpha-Induced Protein 3 (A20) (22). Furthermore, butyrate, acetate, and propionate have been shown to have anti-inflammatory effects in intestinal tissue. These effects involve suppressing proinflammatory cytokines interleukin-6 (IL-6) and interleukin-8 (IL-8) and inhibiting inflammatory signaling

pathways, such as Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells (NF-κB) (23,24). Consistently, clinical evidence indicates that levels of butyrate, propionate, and acetate are significantly reduced in infants diagnosed with NEC (23). This situation suggests that the impact of butyrate on intestinal health is highly dose- and context-dependent.

Physicochemical Properties and Digestibility of Xanthan Gum in Infant Feeding

XG is a high molecular weight, naturally occurring heteropolysaccharide produced by the aerobic fermentation of *Xanthomonas campestris*, a Gram-negative bacterial species. Described in the literature as a non-toxic, biodegradable, and biocompatible polymer, XG has a wide range of applications in medicine and pharmaceutical sciences due to these properties. XG was cleared for food use in the United States by the US Food and Drug Administration (FDA) in 1969 and is currently regulated as a direct food additive under 21 CFR 172.695 (25).

XG is used in patients' nutritional and therapeutic processes primarily due to its thickening and stabilizing properties. As a high molecular weight polysaccharide, XG has the capacity to significantly increase the viscosity of liquids even at low concentrations (26). XG solutions exhibit pseudoplastic flow behavior. This property means that their fluidity increases when a mechanical force, such as mixing or swallowing, is applied, and they thicken again when the force is removed. This rheological property facilitates the control of food in the mouth, especially during oral intake. Furthermore, XG maintains high elasticity and structural stability under shear stress (27).

XG's high stability to pH and ionic strength changes ensures it retains its structural integrity when combined with various food and beverage matrices (26,28). Finally, XG is entirely resistant to the amylase enzyme found in human saliva and breast milk. While rice cereal-based thickeners rapidly degrade when added to breast milk, XG can remain stable for hours.

XG is not digested in the human stomach or small intestine and therefore reaches the colon intact. In the colon, it is fermented by anaerobic microbiota, producing SCFAs and serving as an energy source for colonic bacteria. This property makes XG a suitable carrier for targeted colon drug-delivery systems (29). In a study involving healthy adults, daily consumption of 15 g of XG for 10 days significantly increased SCFA production in stool samples than basal levels (30).

Clinical Evidence Linking Xanthan Gum to NEC Risk

In clinical settings, NEC cases have been reported in infants receiving specific XG-based commercial thickening agents. These cases appear to have a later onset than classical NEC. While typical NEC cases generally occur around day 45 of life, the median age of onset in XG-associated cases has been reported as 66 days (12). In addition, whereas most NEC cases develop in the hospital setting, approximately 50% of XG-associated cases have been reported to occur after hospital discharge, representing a distinctive clinical pattern. These cases have also been characterized by greater colonic involvement (12,31,32).

A retrospective study conducted at Boston Children's Hospital evaluated 20 infants who received XG-based thickening agents and reported no cases of NEC. The authors attributed this finding to the low baseline incidence of NEC within the studied population (33).

Similarly, a 2022 retrospective cohort study by Abdulezer and colleagues compared the safety of XG-based commercial thickeners and rice cereal-based thickeners in 53 infants with dysphagia. The results showed no statistically significant differences between the two groups regarding the incidence of diarrhea, constipation, excessive weight gain, or obesity during the 3-6 month and 6-12 month follow-up periods. Both types of thickeners exhibited comparable safety profiles; however, prolonged use of rice cereal-based products was linked to a

higher prevalence of constipation. Importantly, no cases of NEC were reported among the infants (13).

Proposed Mechanisms Potentially Linking Xanthan Gum to Necrotizing Enterocolitis

The mechanisms proposed to link XG exposure to NEC are summarised in Figure 1. These comprise altered fermentation kinetics, bile acid accumulation, and activation of inflammatory signalling pathways. It should be emphasised that these pathways are hypothesised on the basis of preclinical and indirect clinical data and do not establish a causal relationship (Figure 1).

Fermentation Kinetics and Short-Chain Fatty Acid (SCFA) Accumulation

XG provides superior stability compared with rice cereal for thickening breast milk and infant formula. However, its use involves clinically relevant considerations. Through colonic fermentation, XG may modulate SCFA production, with implications for NEC risk. Therefore, clinical decision-making regarding thickener use should consider not only safety outcomes but also technical and feeding-related factors, including variability in preparation, time to achieve the desired viscosity, and infant oral sensory responses (34). The current lack of robust randomized controlled trials underscores the need for individualized risk–benefit assessment and for further high-quality clinical studies to inform evidence-based recommendations.

XG is broken down by anaerobic bacteria, including *Bacteroides*, *Bifidobacterium*, and *Eubacteria*, when it reaches the colon. It produces sugars, hydrogen gas, and SCFAs such as acetate, propionate, and butyrate at the end of fermentation in the colon (32,35). Consistent with these findings, XG increased acetate, propionate, butyrate, and total SCFAs in the gut of male mice in an *in vivo* study (36).

The role of SCFAs in NEC pathogenesis is controversial, as excessive levels may be toxic to intestinal tissue. High doses of SCFAs can cause intestinal mucosal damage, similar to NEC. For instance, high levels of butyric acid may contribute to the development of NEC by increasing the expression of the iNOS gene, which is responsible for mucosal injury (37). Similarly, intraluminal SCFA administration in neonatal rats consistently triggers mucosal damage. These injuries bear a striking resemblance to the clinical features of human NEC (38).

Although the information presented so far suggests that SCFAs are harmful to NEC pathogenesis, studies have shown that premature infants, who are more susceptible to NEC, have lower SCFA concentrations in their intestines than full-term infants (22). SCFAs mitigate inflammatory damage by reducing the production of IL-6, IL-8, and tumor necrosis

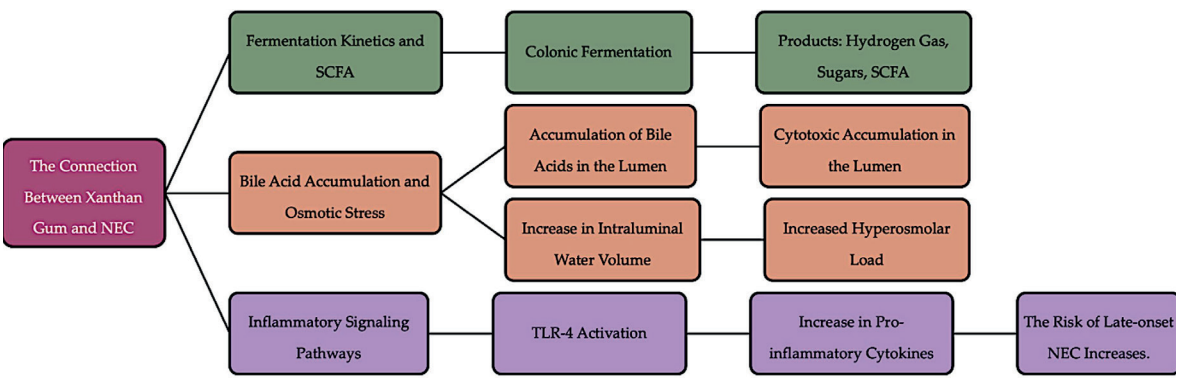

Figure 1. Conceptual framework of the proposed mechanisms potentially linking XG exposure to NEC in preterm infants.

factor-alpha (TNF-α), and so strengthening intestinal barrier integrity (37). For example, butyrate activates the Notch1 signaling pathway in the intestinal epithelium and suppresses pro-inflammatory Toll-like receptor (TLR) signaling. This process helps develop immune tolerance to bacterial colonization in the newborn gut and reduces the risk of NEC (22).

In brief, the relationship between SCFAs and NEC is complex, described in the literature as both a protective shield and a disease-triggering factor under certain conditions. The conclusion drawn from the reviewed scientific literature is that SCFAs produced under controlled conditions via beneficial bacteria have a protective function against NEC. Uncontrolled SCFA accumulation resulting from pathogenic overgrowth or the addition of exogenous fermentable substances, such as XG, increases the risk of tissue necrosis. However, the current findings were contradictory. Given the limited number of randomized controlled clinical trials, further research is needed in this area.

Bile Acid–Induced Toxicity and Osmotic Stress

XG consumption has been found to enhance bile acid excretion in humans via fecal matter (32). For instance, in a study conducted on rats, those fed an XG diet had a higher bile acid pool than the control group ($p<0.001$) (39). This increase leads to the accumulation of bile acids in the intestinal lumen. The accumulation of cytotoxic bile acids in the intestine has been identified as a critical component for NEC development in neonatal mouse models.

XG, a high-molecular-weight polysaccharide, also has a strong water-retaining capacity (40). According to sources, it increases the amount of water in the intestinal lumen of infants (32). This property leads to strong osmotic effects within the intestine. For example, studies on rats have shown that XG causes a 400% increase in intraluminal water and a 150% increase in sugar in the distal part of the small intestine. The underdeveloped intestines of premature infants may not tolerate this hyperosmolar load within the lumen, potentially leading to intestinal injury (41). In addition, the accumulation of intraluminal sugars, leading to the production of SCFA and hydrogen gas, further enhances the effect of the generated osmotic load. However, XG use does not always result in NEC among neonates (33).

XG increases the amount of water, sugars, bile acids, and SCFAs in the intestinal lumen, creating excessive stimulation and osmotic stress on the sensitive intestinal tissue of premature infants, which plays a role in NEC pathogenesis. However, given conflicting findings, well-designed randomized controlled clinical trials and cohort studies are needed in this area.

Activation of TLR-4 and Pro-inflammatory Signaling Pathways

XG can induce the production of pro-inflammatory cytokines, such as TNF-α and interleukin-12 (IL-12), in macrophages by acting as a TLR4 ligand. Overactivation of the TLR-4 signaling pathway is directly linked to mucosal damage and tissue necrosis, which are key components of NEC. It has been proposed that XG may contribute to the risk of late-onset NEC in preterm infants through this pathway (32).

The anti-inflammatory effect of XG appears to differ between paediatric and adult populations. XG, which is used in the treatment of osteoarthritis, a disease characterized by intense inflammation, decreased the Osteoarthritis Research Society International Score (OARSI) and inflammatory cytokine concentration after intra-articular injection (42). In support of these findings, pediatric gastroenterology authorities approach the use of thickeners cautiously. XG is not recommended for use in premature infants due to the risk of NEC, especially in the late-onset form (43).

Aside from underlying inflammation mechanisms, excessive accumulation of SCFAs in the lumen from XG intake can lead to mucosal

damage, irritation, and an inflammatory cascade. At the end of this, the risk of NEC can be increased (12,32).

The pro-inflammatory activity of XG on NEC is antithetical to its effect on osteoarthritis, where inflammation is intense. Studies on osteoarthritis have demonstrated that XG exhibits a high binding affinity for the TLR4 receptor, thereby significantly reducing cytokine release and the synthesis of inflammatory proteins (44). This discrepancy may be due to differences in age between patient groups with NEC and osteoarthritis.

Although some studies have demonstrated that XG has pro-inflammatory effects on NEC, studies in adults have shown the contrary. Therefore, more clinical research is needed in this area.

Regulatory and Legal Considerations

The North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition / European Society for Paediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN/ESPGHAN) (2009) guidelines focus on the use of rice, corn, and potato starches, as well as guar gum and locust bean gum, as thickeners in commercial antiregurgitation formulas. The guidelines state that while thickeners reduce vomiting, they do not reduce acid reflux attacks and that their long-term use in infants requires special attention. Additionally, at the time the study was published, the risk of NEC in premature infants was associated not with XG (45).

The use of a specific XG-based thickening agent in premature infants has been subjected to extensive safety review and resulted in severe restrictions by the FDA. The FDA issued its first formal warning in May 2011 regarding reports of potentially related adverse events such as NEC. The warning concerned 17 reported cases of NEC in premature infants born before 37 weeks, 5 of which were fatal. The agency's investigations determined that some of the cases occurred after the infants were discharged from the hospital and transitioned to a feeding regimen containing the product. Consequently, the product was strongly discouraged both in the hospital and for the first 30 days after discharge. The FDA is actively investigating whether other thickening agents pose similar risks and is requesting prompt reporting of symptoms such as bloating or bloody stools. XG-based thickeners are contraindicated for *ad libitum* (addition at home or in the hospital) use in infants born before 37 weeks (46).

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) first evaluated XG in 1974. In 1986, the committee assigned it a status of "not specified" for Acceptable Daily Intake (ADI). This decision was made due to the absence of any observed adverse effects, which eliminated the need for a specific numerical value for ADI. During its 82nd meeting in 2016, JECFA concluded that the use of XG in infant formulas, follow-on formulas, and special medical purpose infant formulas at a maximum concentration of 1,000 mg/L does not raise any safety concerns. This scientific data supports the use of the substance in food as a thickener, stabilizer, emulsifier, and foaming agent (47).

The 2017 EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) assessment found that XG (E 415) is safe for infants and children older than 12 weeks. Its use in special medical purpose infant formulas at concentrations up to 1,500 mg/L was found to be clinically well tolerated, not affecting infant growth characteristics, and not disrupting mineral balance. The report emphasized that this additive is not absorbed from the intestines and does not pose any genotoxic risk. However, it was explicitly noted that these 2017 results did not include infants younger than 12 weeks at that time, and a separate risk assessment is needed for this age group (48). Additionally, the 2018 NASPGHAN/ESPGHAN guidelines recommend thickening milk as a first-line treatment for regurgitation. While rice grains are recommended for formula milk, it is emphasized that breast milk cannot be

thickened with grains because of enzymes, and that commercial products with age restrictions, varying by brand, should be used (43).

In 2023, EFSA re-evaluated the safety of XG. The published report concluded that its use is safe up to a concentration of 1200 mg/L in special medical-purpose infant formulas. Technical updates included recommendations to ensure that the bacteria used in production do not contain live cells, that the product is free of residual enzyme activity, to add the *Cronobacter sakazakii* test to the microbiological criteria, and to change the substance description to "water-dispersible". Furthermore, it was reaffirmed that the risk of NEC in premature infants is associated not with XG added as a controlled food additive at the factory, but with thickeners added directly and in high doses (*ad libitum*) to breast milk or formula (49).

Conclusion

Nutrition in the early stages of life is important for establishing a healthy intestinal environment at the cellular and molecular levels. Dietary additives influence the delicate balance of this microenvironment and can affect intestinal barrier integrity and gut microbiota composition. In this context, the potential impact of XG-based thickeners, commonly used in dysphagia management, on the pathophysiology of NEC in premature infants is a critical area of investigation.

Because the neonatal intestine is physiologically unique, nutritional modifications must prioritize intestinal maturity and safety over the technical ease of application. The available evidence argues specifically against using XG-based thickening agents added directly, and in uncontrolled amounts, to human milk or infant formula in preterm infants and in infants younger than 12 weeks of corrected age, particularly in the home setting. This caution should not be generalized as a universal contraindication to all XG-containing products: XG incorporated at controlled, regulator-approved concentrations during the industrial manufacture of formulas for special medical purposes has not been associated with the same risk. Decisions regarding feed thickening should therefore be made on an individual basis. In addition, a multidisciplinary approach involving pediatricians and dietitians is required to assess the infant's specific microbial and developmental requirements. Furthermore, parents must be educated to monitor for symptoms like abdominal distension and bloody stools, particularly when products are used in a home setting. Ultimately, the preferred clinical approach must protect the immunological and microbial integrity of human milk while focusing future research on personalized feeding protocols and precise dosage ranges based on intestinal maturity.

Author contribution

The authors confirm contribution to the paper as follows: Review conception and design: GT, EG; literature review: GT, EG; draft manuscript preparation: GT, EG. All authors reviewed the results and approved the final version of the manuscript.

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