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The Role of Pancreatic Stone Protein as a Predictor of Sepsis in the Intensive Care Unit and Its Correlation With D-Dimer, C-Reactive Protein, and Procalcitonin

Darko Sazdov, Ivica Dimitrov, Androniki Bibovska

Abstract

Pancreatic stone protein (PSP) is secreted by pancreatic acinar cells. PSP is a damage-associated molecular pattern (DAMP) produced in response to sepsis. The study aims to determine the association between PSP values and sepsis criteria, SOFA score, mortality, and values of D-dimer, WBC, CRP, and Procalcitonin on admission to the ICU. This is a monocentric retrospective study that includes 63 patients, with a mean age of 62.5±15.5 years, with PSP values measured on admission to the ICU. Patients were stratified as having normal PSP values, low, moderate, or high risk for sepsis and compared with the classification based on the Third International Consensus Definitions for Sepsis and Septic Shock and to their SOFA score and laboratory parameters, including WBC, CRP, D-dimer, and Procalcitonin. A statistically significant difference was confirmed in the distribution of patients with normal PSP values, with low, moderate and high risk for sepsis according to PSP values in terms of not meeting sepsis criteria (p=0.0000), meeting SIRS criteria (p=0.029), meeting sepsis criteria (p=0.022) and meeting septic shock criteria (p=0.000), while, the difference in terms of severe sepsis criteria was not statistically significant (p=0.88). PSP significantly positively correlated with D-dimers, CRP, and PCT (R =0.4462, p=0.0008; R =0.4971, p=0.00003; and R=0.5825, p=0.000001, respectively). PSP may play a role as a predictor of sepsis severity and mortality in patients in the ICU.

Keywords: PSP, sepsis, SOFA, Intensive Care Unit

Introduction

Pancreatic stone protein (PSP) is a 16 kDa C-type lectin protein produced predominantly by the pancreas and intestine. It is a protein secreted by acinar cells to inhibit the growth and nucleation of calcium carbonate crystals in pancreatic juice. Preclinical studies indicate that PSP is actually a molecular pattern produced in response to sepsis (Hu, 2023). Based on this, the question arises about the role of PSP as a predictor of sepsis, especially in intensive care units. Early recognition of sepsis is essential to prevent complications such as multiorgan dysfunction syndrome (MODS) and death in patients hospitalized in intensive care units. No biomarker has yet been successfully implemented in daily practice with good clinical performance. Pancreatic stone protein (PSP) is a promising biomarker in the context of sepsis.

Study Objectives

1. To determine the association of PSP value with sepsis criteria in ICU patients.
2. To determine whether there is a connection between PSP value and D-dimer, WBC, CRP values, and procalcitonin measured in ICU patients.
3. To determine the correlation between PSP and mortality in the ICU.
4. To determine the correlation between PSP values and SOFA score on admission to the ICU.

Methods and Materials

This is a monocentric retrospective study that included 63 patients with an average age of 62.5±15.5 years, who were hospitalized in the general intensive care unit at Acibadem Sistina Hospital in Skopje, North Macedonia.

All patients had the following parameters upon admission to the general intensive care unit:

PSP, sepsis criteria, sofa score, D-dimer, WBC, CRP, procalcitonin.

PSP reference values:

- PSP<100ng/m Normal value
- PSP ≥100-200 ng/ml Low risk of sepsis
- PSP ≥200-300 ng/ml Moderate risk of sepsis
- PSP ≥300ng/ml High risk of sepsis

Sepsis Criteria

Table 1:

Sepsis Criteria - The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3)

SIRS Criteria (≥2 meet SIRS definition)		
Temp >38°C or <36°C	No	Yes
Heart rate >90	No	Yes
Respiratory rate >20 or $PaCO_2$ <32 mm Hg	No	Yes
WBC >12,000/mm^3, <4,000/mm^3, or >10% bands	No	Yes
Sepsis Criteria (SIRS + Source of Infection)		
Suspected or present source of infection	No	Yes
Severe Sepsis Criteria (Organ Dysfunction, Hypotension, or Hypoperfusion)		
Lactic acidosis, SBP <90 or SBP drop ≥40 mm Hg from normal	No	Yes
Septic Shock Criteria		
Severe sepsis with hypotension, despite adequate fluid resuscitation	No	Yes

Reference Values of WBC, D-D-Dimer, CRP, and Procalcitonin

- WBC 4-11 *10^9/L
- CRP <0.5 mg/dl
- PCT <0.5 ng/ml (Normal);
  0.1-0.5 ng/ml (Local inflammation, infection);
  0.5-2ng/ml (Low- systemic anwer on inflammation);
  2-10 ng/ml (Strong - systemic answer on inflammation);
  >10 ng/ml (Bacterial sepsis / septic shock)
- D-Dimer < 0.5 ug/ml F. EU

Inclusion and Exclusion Criteria

Patient data was obtained from the CEREBRAL+ electronic system of Acibadem Sistina Clinical Hospital, which contains all medical documentation for treated patients.

Inclusion criteria

All patients who had PSP taken upon admission to the ICU.

Exclusion criteria

- Death within 24 hours of ICU admission;
- Patients under 18 years of age;

Statistical Analysis

The statistical processing of the data received from the research was made in the statistical program Statistical Package for the Social Sciences (SPSS Inc., Chicago, Illinois) version 25.0. For the data distribution, the Kolmogorov-Smirnov and the Shapiro-Wilk W test were used. Qualitative variables are presented with absolute and relative numbers. Quantitative variables are presented with average, minimum, and maximum values, standard deviation, median, and interquartile range. For comparison of qualitative data, Fisher's exact test was used; quantitative variables were compared with the Kruskal-Wallis test. Correlation between two variables was analyzed with Spearman's rank correlation coefficient.

The statistical significance was defined for $p < 0.05$.

Ethical Aspects of the Study

For this study, the researchers have received approval from the Committee for Ethical Issues at the Acibadem Sistina Hospital No. 02-2673/02.

Results

The study included 63 patients, 42(66.7%) male and 21(33.7%) female. The patients were aged 18 to 89 years, with a mean age of 62.5±15.5 years.

Table 2 shows the distribution of patients according to sepsis criteria.

Table 2:

Distribution of Patients According to Sepsis Criteria

Sepsis criteria	**n (%)**
Doesn't fulfill criteria for sepsis	15 (23.81)
Criteria for SIRS	8 (13)
Criteria for sepsis	16 (25.4)
Criteria for severe sepsis	4 (6.35)
Criteria for septic shock	20 (31.75)

Patients in our study most often had PSP values of 300 ng/ml and higher, and belonged to the group with high risk for sepsis – 34 (53.97%), followed by 12 (19.0%) patients with PSP values of 200 to 300 ng/ml who have a moderate risk for sepsis, 9 (14.29%) patients with PSP values of 100 to 200 ng/ml with a low risk for sepsis, and, 8 (12.7%) patients with PSP values lower than 100 ng/ml who have no risk for sepsis.

Table 3 shows the distribution of PSP values in patients with different sepsis criteria. Patients with normal PSP values most often did not meet the criteria for sepsis (87.5%), patients with low risk for sepsis according to PSP most often met the criteria for SIRS (44.44%), patients with moderate risk for sepsis according to PSP met the criteria for sepsis (58.33 %), patients with high risk for sepsis according to PSP most often met the criteria for septic shock (55.88 %).

A statistically significant difference was confirmed in the distribution of patients with normal PSP values, with low, moderate and high risk for sepsis according to PSP values in terms of not meeting sepsis criteria (p=0.0000), meeting SIRS criteria (p=0.029), meeting sepsis criteria (p=0.022) and meeting septic shock criteria (p=0.000), while the difference in terms of severe sepsis criteria was not statistically significant (p=0.88). The impact of PSP was confirmed as significant for 4 out of 5 sepsis criteria.

Table 3:

Distribution of PSP Values in Patients With Different Criteria for Sepsis

Variable	PSP (Pancreatic stone protein)					p-level
	N	Normal value n (%)	Low risk for sepsis n (%)	Moderate risk for sepsis n (%)	High risk for sepsis n (%)	
Doesn't fulfill criteria for sepsis	15	7 (87.5)	2 (22.22)	2 (16.67)	4 (11.76)	***p=0.0000
Criteria for SIRS	8	1 (12.5)	4 (44.44)	1 (8.33)	2 (5.88)	*p=0.029
Criteria for sepsis	16	0	2 (22.22)	7 (58.33)	7 (20.59)	*p=0.022
Criteria for severe sepsis	4	0	1 (11.11)	1 (8.33)	2 (5.88)	p=0.88
Criteria for septic shock	20	0	0	1 (8.33)	19 (55.88)	***p=0.000
total	63	8	9	12	34	

p (Fisher's exact test), * Sig p<0.05, ***sig p<0.0001

The comparison of patients without risk, with low, moderate, and high risk for sepsis according to PSP in terms of D- D-dimer, leukocyte, C-reactive protein, and procalcitonin values showed a statistically significant difference in terms of D- dimer values (p=0.0045), CRP (p=0.0003) and PCT (p=0.0001), and non-significant for WBC values (p=0.32) (Table 4).

Post-hoc analyses show significantly higher values of D-dimers, CRP, and PCT in patients with high risk of sepsis compared to patients with normal PSP (p=0.0025, p=0.00021, and p=0.00018, respectively).

D-dimer values in patients at high risk for sepsis versus patients with normal PSP were 9.08 ± 8.8 and 0.97 ± 0.8. ug /ml FEU, respectively; 24.29 ± 12.9 and 4.91 ± 3.9 mg /dl, respectively for CRP; 19.80 ± 28.8 and 1.63 ± 4.4 ng/ml, respectively for PCT (Table 4).

Table 4:

Comparison of PSP With Laboratory Parameter Values

Variable	Statistical parameters	PSP (Pancreatic stone protein)			
		Normal value	**Low risk for sepsis**	**Moderate risk for sepsis**	**High risk for sepsis**
D-Dimer ug /ml FEU	n	6	7	9	31
	mean ± SD	0.97 ± 0.8	4.12 ± 2.6	4.83 ± 4.6	9.08 ± 8.8
	median (IQR)	0.72(0.54-1.01)	3.73(2.3-5.6)	3.48(1.62-7.6)	6.1(2.2-12.6)
	p-level	H=13. 05 * *p=0.0045 post- hoc normal value vs high risk for sepsis **p=0.0025			
WBC * 10^9/L	n	8	9	12	34
	mean ± SD	12.19 ± 6.2	17.06 ± 9.4	18.39 ± 6.7	15.56 ± 9.7
	median (IQR)	10.37(8.6-14.3)	13.3(12.4-20.3)	18.25(14.8-23.7)	13.8(8.6-22.4)
	p-level	H=3. 54 p =0.32			
CRP mg /dl	n	8	9	12	34
	mean ± SD	4.91 ± 3.9	18.53 ± 13.8	15.19 ± 15.21	24.29 ± 12.9
	median (IQR)	3.53(2.9-6.6)	16.6(9.8-20.0)	11.1(4.8-21.2)	24.1(14.9-31.5)
	p-level	H=19. 1 * **p=0.0003 post- hoc normal value vs high risk for sepsis ***p=0.00021			
PCT ng /ml	n	8	9	12	34
	mean ± SD	1.63 ± 4.4	1.32 ± 1.7	9.80 ± 28.5	19.80 ± 28.8
	median (IQR)	0.06(0.04-0.13)	0.79(0.29-1.45)	1.17(0.12-3.33)	4.84(1.31-24.6)
	p-level	H=21. 39 * **p=0.0001 post- hoc normal value vs high risk for sepsis ***p=0.00018			

H (Kruskal-Wallis test, post-hoc Mann-Whitney U test), **sig p<0.01, ***sig p<0.0001

PSP was significantly positively correlated with D-dimer, CRP, and PCT (R =0.4462, p = 0008; R =0.4971, p = 0.00003; and R = 0.5825, p = 0.000001, respectively), and non-significantly correlated with WBC (p = 0.99) (Table 5, Figure 1).

PSP values increased with increasing D – D-dimer, CRP, and PCT values, and vice versa.

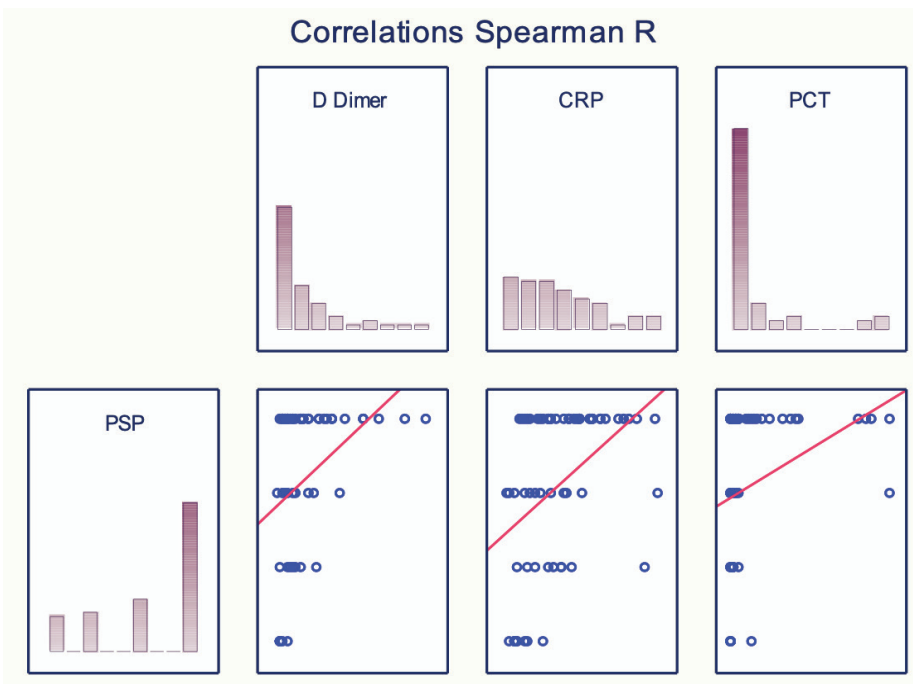
Table 5:

Correlations of PSP with D- dimer, CRP, and PCT

	Spearman R	t(N-2)	p-level
D-Dimer	0.4462	3.5604	0.0008
WBC	0.0014	0.0107	0.99
CRP	0.4971	4.4736	0.00003
PCT	0.5825	5.5504	0.000001

Figure 1

PSP Correlations With D- D-dimer, CRP, and PCT



Patients with high risk of sepsis according to PSP values (44.12%) were the most common, followed by patients with moderate risk of sepsis (33.33%), patients without risk (12.5%), and patients with low risk of sepsis (11.11%). The differences in the distribution of deceased and living patients in relation to PSP were insufficient to be confirmed as statistically significant (p = 0.18) (Table 6).

Table 6:

Distribution of Deceased and Living Patients With Respect to PSP

Deceased	**PSP (Pancreatic stone protein)**					**p-level**
	N	**Normal value n (%)**	**Low risk for sepsis n (%)**	**Moderate risk for sepsis n (%)**	**High risk for sepsis n (%)**	
Yes	21	1 (12.5)	1 (11.11)	4 (33.33)	15 (44.12)	p=0.18
No	42	7 (8 7. 5 8)	8 (88.89)	8 (66.67)	19 (55.88)	

p (Fisher's exact test)

On admission to the ICU, patients' SOFA scores varied significantly depending on the PSP values (p = 0.038). The SOFA score 0-1 had only 1 (11.11%) patient from the group with low risk of sepsis. Patients with normal PSP and low risk of sepsis had a Sofa score of 2-3 more often compared to patients with high and moderate risk (25% vs 22.22% vs 8.82% vs 8.33%). Sofa score of 4 - 5 was most often calculated in patients with moderate risk, followed by patients with normal PSP, with low and high risk of sepsis (58.33% vs 25 % vs 11.11 % vs 8.82 %). Sofa score of 6-7 was most commonly calculated in patients with normal PSP compared to patients with moderate, high and low risk of sepsis according to PSP (25% vs 16.67 % vs 11.76 % vs 11.11 %), Sofa score 8-9 was most often found in patients with normal PSP compared to patients with low, high and moderate risk of sepsis according to PSP (25% vs 22.22 % vs 20.59 % vs 8.33 %), Sofa score 10-11 was more common in patients with high risk compared to patients with low and moderate risk for sepsis (26.47% vs 11.11 % vs 8.33 % vs 0 %), and, Sofa score 12-14 was more common in patients with high risk for sepsis compared to the other groups (23.53 % vs 11.11 % vs 0 % vs 0%) (Table 7).

Patients at high risk for sepsis according to PSP more frequently had SOFA scores of 10-11 and 12-14 compared to other patient groups.

Table 7:

Comparison of the SOFA score with PSP on admission to the intensive care unit

Sofa score on reception in the ICU	PSP (Pancreatic stone protein)					p-level
	N	Normal value n (%)	Low risk for sepsis n (%)	Moderate risk for sepsis n (%)	High risk for sepsis n (%)	
0-1	1	0	1 (11.11)	0	0	
2-3	8	2 (25)	2 (22.22)	1 (8.33)	3 (8.82)	
4-5	13	2 (25)	1 (11.11)	7 (58.33)	3 (8.82)	
6-7	9	2 (25)	1 (11.11)	2 (16.67)	4 (11.76)	*p=0. 038
8-9	12	2 (25)	2 (22.22)	1 (8.33)	7 (20.59)	
10-11	11	0	1 (11.11)	1 (8.33)	9 (26.47)	
12-14	9	0	1 (11.11)	0	8 (23.53)	

P (Fisher's exact test) *sig $p<0.05$

A statistically significant positive correlation was found between PSP and SOFA score on admission (R=0.4343, p=0.0004). PSP values increased with increasing SOFA score, and vice versa (Table 8).

Table 8:

Correlation between PSP and SOFA score reception in the ICU

PSP with SOFA score on reception in the ICU	Spearman R	t(N-2)	p-level
	0.4343	3. 7659	0.0004

Our study included 66.7% male patients and 33.7% female patients with a mean age of 62.5±15.5 years.

A statistically significant difference was confirmed in the distribution of patients with normal PSP values, with low, moderate and high risk for sepsis according to PSP values in terms of not meeting sepsis criteria (p=0.0000), meeting SIRS criteria (p=0.029), meeting sepsis criteria (p=0.022) and meeting septic shock criteria (p=0.000), while the difference in terms of severe sepsis criteria was not statistically significant (p=0.88).

PSP is a new biomarker that can be used for screening and diagnosis of infection and sepsis, but also as a prognostic marker for determining the severity and mortality of patients with infection and sepsis.

PSP significantly positively correlated with D-dimers, CRP, and PCT (R=0.4462, p=0.0008; R=0.4971, p=0.00003; and R=0.5825, p=0.000001, respectively), and insignificantly with WBC (p=0.99). (Table 5, Figure 1). PSP values increased with increasing D-dimers, CRP, and PCT values, and vice versa.

Discussion

Pancreatic stone protein/regenerating protein (PSP/Reg) was first discovered in the 1970s and named lithostathine because it was believed to help prevent the formation of calcium pancreatic stones. It was found to have a role in regeneration and cell proliferation and is a part of the so-called regenerating (Reg) gene family. A breakthrough study by Graf et al. (2002) revealed that even physiologic stress and mild stress, such as anesthesia, can produce a measurable elevation of the level of PSP. After these initial findings, Keel et al. (2009) did a study to determine if PSP/Reg levels were dependent on pancreatic involvement. Therefore, they compared the levels of PSP/Reg and several other biomarkers and complement proteins in patients with polytrauma without pancreatic damage and in healthy subjects. PSP/Reg levels are increased after trauma, and this level is correlated to the degree of damage. They also showed that PSP/Reg engages neutrophils and can be used as a biomarker for complications in the period after trauma, indicated by a significant increase of PSP in patients who developed infection (111.4 g/ml) and sepsis (146.4 ng/ml). CRP, IL-6, and PCT levels were also increased, but didn't discriminate between septic and non-septic patients (Keel, 2009).

Several studies have also investigated the potential role of PSP/Reg as a prognostic biomarker, enabling clinicians to better identify high-risk patients who would benefit from more aggressive treatment (Lopes et al., 2022). A study from 2012 evaluated 107 patients admitted to a mixed surgical and non-surgical ICU. 33 were diagnosed with severe sepsis and 74 with septic shock. The authors measured levels of PSP, PCT, CRP, and IL-6 24 hours from admission. They also computed APACHE II (Acute Physiology and Chronic Health Evaluation II), SAPS (Simplified Acute Physiology Score) II and III, and SOFA scores for admitted patients in the first 24 hours after admission. Acute phase protein levels and APCHE II and SAPS II, and SAPS III scores were significantly higher in patients with septic shock compared to patients with severe sepsis. For PSP/Reg, this was a five-fold difference between these two groups of patients. But regardless of disease severity, only levels of PSP/Reg and SPAS II score showed a statistically significant difference between patients

who survived and those who didn't. Our monocentric retrospective study included 63 adult patients admitted to the Surgical Intensive Care Unit and showed similar results. 34 of these patients or 53.97%, had a PSP value of 300 ng/ml and higher, and belonged to the group with high risk for sepsis, followed by 12 patients (19.0%) with a PSP value of 200 to 300 ng/ml and a moderate risk for sepsis, and 9 patients (14.29%) with a PSP value between 100 and 200 ng/ml and a low risk for sepsis. When compared to the clinical criteria for sepsis, our study revealed a statistically significant difference in the distribution of patients with different levels of PSP and clinical signs of SIRS, sepsis, and septic shock.

The use of PSP as a biomarker for sepsis was assessed in different settings and different patient populations alone or in combination, and was compared to other commonly used and new biomarkers.

Klein et al. (2015) did a study to test the ability of PSP/Reg to predict the occurrence of infection in 120 cardiac surgery patients. For this purpose, they measured the levels of PSP, CRP, and Leucocytes on the first 3 days after surgery and found increased levels of PSP/Reg with infection and sepsis, even 48 hours before other biomarkers (Klein et al, 2015).

Klien et al. (2021) also tested the PSP/Reg as a screening tool for infection and sepsis in 90 patients with burns covering more than 15% of their total body surface area (TBSA). In this study, PSP/Reg, CRP, PCT, and Leucocyte levels were measured daily, 3 days after admission, to test the ability to discriminate between patients with inflammation, infection, and sepsis. Levels of PSP/Reg demonstrated a steep increase in those with sepsis, especially septic shock, even 72 hours before other biomarkers (Klein et al., 2021).

A prospective multicenter study in 14 centers across Europe, including 243 ICU patients, confirmed these results (Pugin et al., 2021). Authors compared the clinically established diagnosis of sepsis with the trend of PSP, PCT, and CRP. The results revealed that levels of PSP in patients admitted to the ICU can sometimes increase up to 5 days before the clinical diagnosis of sepsis is made. This was 3 days before PCT and 2 days before CRP. Clinical signs of sepsis correlated with the highest level of PSP, which was above 450 ng/ml on the day of sepsis, compared to the non-sepsis group, where levels of PSP were under 200 ng/ml (Pugin et al, 2021). In our study, we only measured the values of PSP, white blood cells, CRP, and D-dimers at admission. We did not follow up with separate measurements on subsequent days.

In a 2022 study from China by Xiang et al Z and colleagues, 105 emergency department patients were assessed without prior selection. Levels of PSP of 126.4 ng/ml were more effective in detecting infections compared to PCT and CRP. When PSP was combined with CRP and PCT, diagnostic accuracy improved significantly, achieving a perfect AUROC of 1.0 in distinguishing infected from non-infected patients (Xiang et al., 2022).

In a separate investigation by De Hond et al. (2022), conducted in the emergency department of a tertiary hospital, researchers explored the potential of PSP as a diagnostic marker for infection and sepsis. Of the 156 patients included, 47.4% were diagnosed with infection and 16.7% with sepsis. The results supported the clinical utility of PSP/Reg in helping differentiate sepsis from both uncomplicated infections ($p = 0.032$) and non-infectious presentations ($p = 0.022$). In this setting, PSP showed greater diagnostic performance than CRP and white blood cell counts. Additionally, patients with sepsis exhibited significantly elevated PSP levels compared to those with milder infections or no infection in both cohorts (De Hond et al., 2022).

A recent systematic review and meta-analysis conducted by Mai et al. (2024) evaluated the diagnostic performance of Pancreatic Stone Protein (PSP) across 14 studies. The pooled analysis demonstrated a sensitivity of 0.88, a specificity of 0.78, and an AUC of 0.90 (Mai et al., 2024). When compared to other established biomarkers, the pooled sensitivity of PSP in this meta-analysis was notably higher than that reported by Chen et al. (2023) for the neutrophil-to-lymphocyte ratio (NLR), which showed a sensitivity of 0.79, though the specificity was lower than NLR's 0.91. (Chen, 2023) A similar trend was observed in the comparison between PSP and calprotectin, where PSP demonstrated higher sensitivity (0.88 vs. 0.77), while calprotectin exhibited greater specificity (0.85 vs. 0.78) (Gao et al., 2022). In contrast, Poggi et al.'s (2022) meta-analysis of presepsin reported superior diagnostic performance, with pooled sensitivity and specificity values of 0.93 and 0.91, respectively (Poggi et al., 2022).

Different cut-off values have been used to distinguish infection, sepsis, and septic shock in various studies. Prazak et al. (2021), in a meta-analysis, including 5 studies and 631 patients, confirmed infection in 371 631 patients using a PSP cut-off of 44.18 ng/ml. The overall sensitivity and specificity were 0.66 and 0.83, respectively. The cut-off value for risk of sepsis in our study was 100ng/ml, and the risk level increased with higher levels of PSP values. Combining PSP with CRP increased both the sensitivity and specificity for differentiating patients with and without infection (Prazak et al., 2021).

Higher levels of PSP/reg (above 33.9 ng/mL), together with purulent sputum, yielded a specificity of 97% for detecting pathogenic organisms in sputum samples. On the other hand, lower levels and non-purulent sputum had a sensitivity of 92% in excluding bacterial infections (Scherr et al., 2013).

Apart from its use as a diagnostic tool in early detection of infection and sepsis in different patient populations and different settings, studies show a correlation between levels of Pancreatic Stone Protein (PSP) and higher SOFA scores, which suggests a direct link between levels of PSP and the extent of organ dysfunction (Que et al., 2012; Zuercher et al., 2023). We also computed the SOFA score for each patient on admission and compared it to the PSP level. A statistically significant positive correlation was found between PSP and SOFA score on admission (R=0.4343, p=0.0004). PSP values increased with increasing SOFA score, and vice versa, similar to the aforementioned study by Paguin et al (2022) (Table 8).

Zuercher et al. (2023) in their systematic review and individual patient level meta-analysis set to evaluate the performance of PSP in predicting intensive care unit (ICU) mortality and infection severity among critically ill adults admitted to the hospital for infection. Among the 678 patients included, the pooled ICU mortality was 17.8%. PSP was strongly associated with ICU mortality (OR = 2.7, 95% credible interval (CrI) [1.3–6.0] per one standard deviation increase; age, gender, and sepsis severity adjusted OR = 1.5, 95% CrI [0.98–2.8]). The AUC was 0.69 for PSP, 95% confidence interval (CI) [0.64–0.74]. PSP showed a very good discriminative ability for both investigated study endpoints, ICU mortality and infection severity; better in comparison to CRP, similar to PCT. Combinations of biomarkers did not improve their predictive ability (Zuercher et al., 2023). In a different study of only levels of PSP/Reg and SPAS II score and not disease severity, a statistically significant difference was found between patients who survived and those who didn't. In patients with septic shock, PSP/reg was the only biomarker associated with in-hospital mortality (P = 0.049). Risk of mortality increased continuously for each ascending quartile of PSP/reg (Pugin et al., 2021).

The differences in the distribution of deceased and living patients with respect to PSP in our study were insufficient to be confirmed as statistically significant (p=0.18).

Conclusion

Pancreatic Stone Protein (PSP) has emerged as a promising biomarker in the intensive care unit (ICU). It shows promising results for early detection of sepsis, monitoring of systemic inflammation, and prognosis of critically ill patients. Its quick response to infectious and inflammatory stimuli makes it a valuable tool for timely clinical decision-making, especially when traditional markers may lag. Incorporating PSP into routine ICU protocols could enhance diagnostic accuracy, promote early intervention, and ultimately improve patient outcomes. Larger multicenter studies are needed to standardize PSP thresholds and fully establish its role alongside other biomarkers in critical care practice.

References

Chen J, Y. S. (2023). Meta-analysis of the role of neutrophil to lymphocyte ratio in neonatal sepsis. *BMC Infect Dis.*, 23:837.

de Hond, T. A. P., Oosterheert, J. J., van Hemert-Glaubitz, S. J. M., Musson, R. E. A., & Kaasjager, K. A. H. (2022). Pancreatic Stone Protein as a Biomarker for Sepsis at the Emergency Department of a Large Tertiary Hospital. *Pathogens (Basel, Switzerland)*, *11*(5), 559. https://doi.org/10.3390/pathogens11050559

Gao, R. Y., Jia, H. M., Han, Y. Z., Qian, B. S., You, P., Zhang, X. K., Li, W. X., & Huang, L. F. (2022). Calprotectin as a diagnostic marker for sepsis: A meta-analysis. *Frontiers in cellular and infection microbiology*, *12*, 1045636. https://doi.org/10.3389/fcimb.2022.1045636

Graf, R., Schiesser, M., Lüssi, A., Went, P., Scheele, G. A., & Bimmler, D. (2002). Coordinate regulation of secretory stress proteins (PSP/reg, PAP I, PAP II, and PAP III) in the rat exocrine pancreas during experimental acute pancreatitis. *The Journal of surgical research*, *105*(2), 136–144. https://doi.org/10.1006/jsre.2002.6387

Hu, P., Lu, Y. H., Deng, W., Li, Q., Zhao, N., Shao, Q., Wu, L., Wang, X. Z., Qian, K. J., & Liu, F. (2023). The critical role of pancreatic stone protein/regenerating protein in sepsis-related multiorgan failure. *Frontiers in medicine*, *10*, 1172529. https://doi.org/10.3389/fmed.2023.1172529

Keel, M., Härter, L., Reding, T., Sun, L. K., Hersberger, M., Seifert, B., Bimmler, D., & Graf, R. (2009). Pancreatic stone protein is highly increased during posttraumatic sepsis and activates neutrophil granulocytes. *Critical care medicine*, *37*(5), 1642–1648. https://doi.org/10.1097/CCM.0b013e31819da7d6

Klein, H. J., Niggemann, P., Buehler, P. K., Lehner, F., Schweizer, R., Rittirsch, D., Fuchs, N., Waldner, M., Steiger, P., Giovanoli, P., Reding, T., Graf, R., & Plock, J. A. (2021). Pancreatic Stone Protein Predicts Sepsis in Severely Burned Patients Irrespective of Trauma Severity: A Monocentric Observational Study. *Annals of surgery*, *274*(6), e1179–e1186. https://doi.org/10.1097/SLA.0000000000003784

Lopes, D., Chumbinho, B., Bandovas, J. P., Faria, P., Espírito Santo, C., Ferreira, B., Val-Flores, L., Pereira, R., Germano, N., & Bento, L. (2022). Pancreatic stone protein as a biomarker of sepsis. *Critical care (London, England)*, *26*(1), 100. https://doi.org/10.1186/s13054-022-03953-x

Mai, B., Zhou, L., Wang, Q., Ding, B., Zhan, Y., Qin, S., Zhu, N., Li, Z., & Lei, Z. (2024). Diagnostic accuracy of pancreatic stone protein in patients with sepsis: a systematic review and meta-analysis. *BMC infectious diseases*, *24*(1), 472. https://doi.org/10.1186/s12879-024-09347-4

Poggi, C., Lucenteforte, E., Petri, D., De Masi, S., & Dani, C. (2022). Presepsin for the Diagnosis of Neonatal Early-Onset Sepsis: A Systematic Review and Meta-analysis. *JAMA pediatrics*, *176*(8), 750–758. https://doi.org/10.1001/jamapediatrics.2022.1647

Prazak, J., Irincheeva, I., Llewelyn, M. J., Stolz, D., García de Guadiana Romualdo, L., Graf, R., Reding, T., Klein, H. J., Eggimann, P., & Que, Y. A. (2021). Accuracy of pancreatic stone protein for the diagnosis of infection in hospitalized adults: a systematic review and individual patient level meta-analysis. *Critical care (London, England)*, *25*(1), 182. https://doi.org/10.1186/s13054-021-03609-2

Pugin, J., Daix, T., Pagani, J. L., Morri, D., Giacomucci, A., Dequin, P. F., Guitton, C., Que, Y. A., Zani, G., Brealey, D., Lepape, A., Creagh-Brown, B., Wyncoll, D., Silengo, D., Irincheeva, I., Girard, L., Rebeaud, F., Maerki, I., Eggimann, P., & François, B. (2021). Serial measurement of pancreatic stone protein for the early detection of sepsis in intensive care unit patients: a prospective multicentric study. *Critical care (London, England)*, *25*(1), 151. https://doi.org/10.1186/s13054-021-03576-8

Que, Y. A., Delodder, F., Guessous, I., Graf, R., Bain, M., Calandra, T., Liaudet, L., & Eggimann, P. (2012). Pancreatic stone protein as an early biomarker predicting mortality in a prospective cohort of patients with sepsis requiring ICU management. *Critical care (London, England)*, *16*(4), R114. https://doi.org/10.1186/cc11406

Scherr, A., Graf, R., Bain, M., Christ-Crain, M., Müller, B., Tamm, M., & Stolz, D. (2013). Pancreatic stone protein predicts positive sputum bacteriology in exacerbations of COPD. *Chest*, *143*(2), 379–387. https://doi.org/10.1378/chest.12-0730

Xiang, Z., Chen, X., Zhou, X., Qin, Y., Zhao, X., Wang, Y., Li, Q., & Huang, B. (2022). Development and application of a novel aldehyde nanoparticle-based amplified luminescent proximity homogeneous assay for rapid quantitation of pancreatic stone protein. *Clinica chimica acta; international journal of clinical chemistry*, *535*, 120–130. https://doi.org/10.1016/j.cca.2022.08.020

Zuercher, P., Moser, A., Garcia de Guadiana-Romualdo, L., Llewelyn, M. J., Graf, R., Reding, T., Eggimann, P., Que, Y. A., & Prazak, J. (2023). Discriminative performance of pancreatic stone protein in predicting ICU mortality and infection severity in adult patients with infection: a systematic review and individual patient level meta-analysis. *Infection*, *51*(6), 1797–1807. https://doi.org/10.1007/s15010-023-02093-w

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Analysis of an Educational dataset using Classification Algorithm in Conjunction with Wrapper Feature Selection Methods

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Abstract

Feature selection plays a critical role in improving the efficiency, accuracy, and interpretability of machine learning models, particularly when dealing with high-dimensional datasets. Among various approaches, wrapper-based feature selection methods are known for their ability to capture feature interactions by directly optimizing model performance. This study presents a comprehensive comparative analysis of six wrapper feature selection techniques—Recursive Feature Elimination (RFE), Sequential Forward Selection (SFS), Sequential Backward Selection (SBS), Genetic Algorithm (GA), Particle Swarm Optimization (PSO), and Differential Evolution (DE)—in conjunction with five widely used classification algorithms: Decision Tree, K-Nearest Neighbour, Random Forest, Logistic Regression, and Support Vector Machine. Experiments are conducted on an educational dataset comprising 395 student records with 30 attributes obtained from the UCI repository, using different feature subset sizes (all features, top 20, top 15, and top 10). Model performance is evaluated using accuracy, precision, recall, F1-score, and AUC. The results demonstrate that wrapper methods significantly enhance classification performance while reducing dimensionality, with GA and RFE consistently emerging as the most effective techniques across multiple classifiers. DE also shows strong performance, particularly with Logistic Regression and Random Forest, whereas PSO generally underperforms in terms of AUC. Furthermore, reducing the feature set does not adversely affect predictive accuracy and, in several cases, leads to improved generalization. The findings confirm the effectiveness of wrapper methods for educational data mining and provide practical insights for selecting optimal feature–classifier combinations.

Keywords: Classification Algorithms, Feature Selection, Performance Metrics, Wrapper Methods

Introduction

Excellent academic records are constantly needed by prestigious universities, and their students are their most valuable asset. The primary focus of universities is student performance, which serves as the foundation for producing top-notch graduates and post-graduate students who will lead their countries and assume responsibility for the social and economic advancement of society. Furthermore, because it directly affects the hiring process and subsequently staff productivity, market companies are primarily concerned with university and student performance. Binmat et. al. (2014) discussed that graduates who work hard during their academic careers are able to meet the demands of their employers. The curriculum and learning assessments are used to gauge student success. Feature selection helps address this challenge by identifying the most relevant attributes for prediction. Among existing approaches, wrapper-based feature selection methods are particularly effective because they evaluate feature subsets directly using classification models. Although computationally intensive, these methods often yield more accurate and reliable predictions compared to traditional statistical techniques.

In this work, six wrapper feature selection methods are systematically evaluated in combination with five commonly used classification algorithms to predict student academic performance. The analysis is performed using different feature subset sizes to assess the trade-off between dimensionality reduction and predictive accuracy. The study aims to identify robust feature–classifier combinations that can support effective decision-making in educational data mining applications.

This paper provides a comprehensive analysis of wrapper methods. The key objectives of the research paper are the comparative evaluation of wrapper feature selection methods and the analysis of computational efficiency and model performance. The following is the paper's outline: The literature on the feature selection method related to the wrapper method is presented in Section II. The paper's research approach is presented in Section III. The discussions and results are shown in Sections IV and V. Section VI contains a description of the study's conclusion.

Literature Review

This section presents a review of the literature on Wrapper methods of Feature Selection and Classification Algorithms used to classify the instances in conjunction with wrapper methods.

Review of Literature Using Wrapper Methods

Techniques for feature selection can be broadly divided into three categories: filter, wrapper, and embedding. While integrated approaches integrate selection into the learning algorithm, filters rank features according to statistical measures. Wrappers use model performance metrics to assess subsets of features.

Several surveys, including Chandrashekar and Sahin (2014), have reviewed the taxonomy and applications of feature selection methods, highlighting their critical role in reducing overfitting and improving model interpretability. Dash and Liu (1997) emphasized the importance of integrating domain knowledge to enhance feature selection processes. As Li et al. (2017) discussed, recent advances have focused on scalable methods to handle large datasets.

Moslehi & Haeri (2020) proposed a hybrid filter-wrapper technique for feature subset selection that combines particle swarm optimization (PSO) with evolutionary-based genetic algorithms (GA). The suggested method's primary goal is to minimize the amount of time spent searching and the complexity of calculations in order to find the best answer to the feature selection problem for high-dimensional datasets.

Similarly, to balance efficiency and performance, Tang et al. (2014) highlighted the importance of hybrid approaches that integrate filter and wrapper methods. Maldonado and Weber (2009) proposed that wrapper algorithms can computationally enhance the performance of feature selection when combined with Filter Selection methods.

Singh and Karthikeyan (2024) determined that the performance of Ant colony optimization with random forest (ACO-RF) is superior for feature selection. In order to predict university student dropout, the ACO-RF wrapper technique for feature selection is suggested in research. Neural networks and machine learning methods are eventually used to validate the feature that ACO-RF chose. With a 94% accuracy rate, the neural network outperformed competing machine learning techniques.

Two-stage feature selection procedures using machine learning techniques were presented by Patel et al. (2024). The sequential backward feature selection approach is used in the second stage to show the dataset's correctness, while the wrapper method is used in the first stage to choose a combination of feature subsets from the dataset.

The classifier selection has a significant impact on the quality of the feature subset that is produced when creating a wrapper feature selection model. In a recent study, Xue et. al. (2015) conducted significant experiments to focus on the computational elements of wrappers using several classifiers. Xue et. al. (2016) carried out an interesting survey of feature selection strategies emphasizing evolutionary techniques and their salient features, such as the fitness function design, benefit mechanisms, and model representation.

Review of Literature on Classification Algorithms Used in Conjunction With Wrapper Methods

By repeatedly training a model on several subsets and assessing performance using a user-defined criterion (such as accuracy or F1-score), wrapper approaches find the optimum feature subset. Typical wrapper methods consist of: (i) Recursive Features Elimination (RFE), (ii) Step Forward Selection (SFS), (iii) Step Backward Selection (SBS), (iv) Genetic Algorithm (GA), (v) Particle Swarms Optimization (PSO), (vi) Differential Evolution (DE). These are frequently used in conjunction with classifiers such as Random Forests, Decision Trees, K-Nearest Neighbors, Support Vector Machines, and Logistic Regression.

Applying RFE with Random Forest to student datasets, Feng & Xu (2019) discovered that it enhanced model interpretability, which in turn improved dropout prediction. When comparing SFS with SBS in pattern recognition, Jain and Zongker (1997) discovered that forward selection typically outperforms SBS for sparse datasets.

Asif et al. (2017) achieved notable accuracy gains in their selection of factors influencing student academic performance in educational datasets by combining SFS with Decision Trees. In their evaluation of GA-based feature selection, Xue et al. (2016) demonstrated how well it traverses the search space, particularly when used with classifiers such as SVM and RF. Khan and Jawaid (2020) enhanced AUC scores by using GA with Random Forest to choose the best attributes for identifying students who were likely to fail.

The ability of PSO to handle huge feature spaces with less computation than GA was highlighted by Chandrashekar and Sahin (2014). Tomasevic et al. (2019) enhanced early student dropout prediction by using PSO on academic data. Using DE with classification models on biological and educational datasets, Islam et al. (2018) demonstrated faster convergence and competitive accuracy. DE has been less popular in educational contexts, but its use is expanding because of its ease of use and capacity to search globally.

Research Methodology

In this Research paper, various wrapper methods are applied on the dataset namely, Forward Selection, Backward Selection, Recursive Feature Elimination (RFE), Particle Swarm Optimization (PSO), Differential Evolution (DE) and Genetic Algorithm (GA) by taking all features and then by selecting Top 20, Top 15 and Top 10 features to evaluate the dataset by using different wrapper methods and then calculating the performance metrics for the same with different Classification Algorithms namely Logistic Regression, Decision Tree, Random Forest, SVM and KNN. The dataset is taken from the UCI data repository, which consists of 30 attributes/ features and a target variable christened as "Passed". The data is partitioned into two parts, i.e., 80 % training data set and 20% testing data set.

Objective/Questions

The main research objective is to examine the impact of wrapper feature selection algorithms on the effectiveness of classification algorithms applied to the best set of predictors. The following research questions will be addressed by this study:

Research Question 1: How do different wrapper-based feature selection methods (RFE, SFS, SBS, GA, PSO, and DE) affect the predictive performance of classification models when applied to educational datasets?

Research Question 2: Which wrapper feature selection techniques consistently identify the most informative features for predicting student academic performance?

Research Question 3: How does the choice of classification algorithm (Decision Tree, KNN, Random Forest, Logistic Regression, and SVM) influence the effectiveness of wrapper-based feature selection methods?

Research Question 4: What is the impact of reducing feature dimensionality (top 20, top 15, and top 10 features) on model accuracy, robustness, and generalization performance?

Dataset Description

The dataset comprises 395 student records with 30 features for each record. This dataset has been used in many studies and is available publicly on many data repositories such as Kaggle. It was previously used to check the students' academic success and passing rates. There are three categories of attributes in this dataset (i) demographic features (sex, age, address, family size, Parent status, health), (ii) aca-

demic background features (school, study time, failures, school support, paid, activities, nursery, higher studies, absences) and (iii) social-economic features (Mother's education, Father's education, Father's job, Mother's job, family support, reason, guardian, travelling time, internet used, romantic, family relation, free time, gout for outing, Weekday alcohol consumption, Weekend alcohol consumption). In the previous research paper, the dataset was used to study the impact of feature selection by using seven classification algorithms, and it was concluded that by selecting different features, the behavior of classification algorithms remains almost the same with all features, as well as with the Top 10 and Top 8 features.

Wrapper Methods

Wrapper methods utilize predictive models to assess feature subsets. These methods are computationally intensive but often yield superior results due to their consideration of feature interactions. Notable techniques include:

Recursive Feature Elimination (RFE): Recursively eliminating the least significant features and using the remaining features to construct models is how RFE works. It removes features gradually and assigns a feature priority based on how much it contributes to the model's performance. Through this procedure, overfitting decreases, model accuracy improves, and interpretability is enhanced. Guyon and Elisseeff (2003) introduced RFE, demonstrating its effectiveness in identifying informative genes in bioinformatics. The algorithm and formula of RFE are given in the following.

Algorithm (Guyon et al., 2009):

Step1: Train the model on the full feature set.

Step2: Compute the importance of features.

Step3: Remove the least important feature.

Step4: Repeat until the desired features remain.

Formula (Kohavi & John,1997)

Let $F=\{f_1,f_2,\ldots,f_n\}$ be the feature set.
For every step:
Train model M on F
Rank features using Importance, let say ()
Eliminate feature that are least important by using f_k=argminImportance(fi)

Genetic Algorithms (GA): By simulating natural selection, the Genetic Algorithm (GA) effectively finds the ideal subset of traits. In order to evolve feature subsets that optimize model performance while reducing redundancy, GA uses selection, crossover, and mutation. Holland (1992) described the foundational principles of genetic algorithms, which have since been applied to feature selection for optimizing search spaces, as detailed by Siedlecki and Sklansky (1989). The algorithm of GA is as follows:

Algorithm (Holland, 1992):
Step1: Generate initial population of chromosomes $X \in \{0,1\}^n$
Step2: Evaluate fitness: $f(X_i)$
Step3: Select parents via tournament or roulette
Step4: Crossover:
X_{new} =crossover (X_i, X_j)
Mutation:
$X_{new}[k] = 1 - X_{new}[k]$ with probability p_m
Step5: Form a new population and repeat (Khan & Jawaid, 2020; Siedlecki & Sklansky,1989)

Step Forward Selection (SFS): SFS follows a greedy approach by gradually identifying the most pertinent features and adding them one at a time according to their contribution to model performance. Sequential Forward Selection (SFS) is utilized in wrapper methods for feature selection. It lessens computational complexity while enhancing accuracy. Kohavi and John illustrated that using an iterative process, we begin with a blank set of features and continue to add features that enhance our model the most with each iteration. The stopping criterion is when the performance of the model does not increase with the addition of a new variable, as discussed by Siedlecki and Sklansky (1989). The algorithm and Formula of SFS are as follows:

Algorithm [Guyon and Elisseeff, 2003]:

Step1: Initialization: Let the original data set is $F = \{f_1, f_2, \ldots, f_n\}$. Initialize with an empty feature set, let's say $S = \emptyset$

Step2: **Candidate Feature Selection**: For each feature $f \notin F \setminus S$, train a model using a feature subset $S \cup \{f\}$ and evaluate model performance with J(.) (e.g., accuracy, Precision, AUC, etc.)

Step3: Feature Selection: Select the feature f^* that maximizes the elevation matrix as

$$f^* = \underset{f \in F \setminus S}{arg\max} \; (J(S \cup \{f\}))$$

Step 4: Update the feature subset:
S = S {f*}
Step 5: if |S| =k (desired number of features or performance improved is less than a limit stop
Otherwise
Go to Step 2

Formula:

$$f* = \underset{f \in F \setminus S}{\text{argmax}} \; J(S \cup \{f\})$$

Where J is performance functions, i.e., accuracy, F1-Score, etc. (Pudil et. al., 1994). F\S .is F minus S.

Step Backward Selection (SBS): Sequential Backward Selection (SBS) follows the Dimensionality Reduction technique for eliminating the least significant features to enhance model performance. SBS is utilized in wrapper approaches for feature selection. To make sure that only the most essential features are left, it begins with the entire list of features and removes each one individually. The criterion for terminating is until the removal of the feature results in no discernible improvement in the model's performance, as discussed by Kohavi and John (1997). The algorithm and Formula of SBS are as follows:

Algorithm (Jain & Zongenkar, 1997):
Step1: **Initiation:** Start with the full feature set S=F
Step2: **Evaluation:** For each feature f ∈ S, evaluate J (S\ {f})
Step3: **Elimination:** Remove features that least affect performance.
Step4: **Iteration:** Elimination: Repeat steps 2-3 until k features remain.

Formula:

$$f* = \underset{f \in S}{\text{argmax}} J \quad \text{(Guyon and Elisseeff,2003)}$$

Particle Swarm Optimization (PSO): The optimization method known as Particle Swarm Optimization (PSO) is modeled after the social behavior of fish schools or flocks of birds. Since its introduction by Kennedy and Eberhart (1995), it has been extensively used to solve a variety of optimization issues, such as combinato-

rial and continuous optimization. PSO is utilized for feature selection in wrapper methods as it effectively identifies the most appropriate subset of features by modeling a swarm's behavior. In order to improve model performance and decrease redundancy, it strikes a balance between exploration and exploitation. The algorithm and Formula of PSO are as follows:

Algorithm (Waheed et al., 2020):
Step1: **Initialize** particles with random positions $\in \{0,1\}^n$
Step2: **Evaluate** fitness f () (e.g., model accuracy)
Step3: **Update velocity**: $v_i = w\, v_i + (pbest_i - x_i) + (gbest - x_i)$
Step4: **Update position**: $x_i = sigmoid(v_i) > rand$
Step5: **Update** pbest and gbest

Repeat until max iterations (Kennedy & Eberhart,1995)

Differential Evolution (DE): Another population-based optimization technique that was first presented by Storn and Price (1997) is called Differential Evolution (DE). In contrast to PSO, DE is predicated on the idea that improved solutions can be evolved through the recombination and mutation of individuals within the population. It works very well for optimization issues with real values. To determine the best choice, DE looks at a large number of feature subsets. It works effectively with rich in features datasets. Finding the ideal feature combination to enhance model performance and managing high-dimensional datasets are two areas where DE excels. DE improves predictive performance through the selection of the most pertinent features. The algorithm and Formula of DE are as follows:

Algorithm (Storn & Price, 1995):
Step1: Initialize population $X_i \in [0,1]^n$
Step2: Mutation: $V_i = X_{r1} + F \cdot (X_{r2} - X_{r3})$
Ui[j]={Vi[j] if r and j<CR
Xi[j]otherwise
Step3: Selection:
Xi= {U_i if $f(U_i) > f(X_i)$
Xi otherwise
Step 4: Repeat until convergence [Das and Suganthan,2011] [Sharma and Kaur, 2020]

Kohavi and John (1997) highlighted the trade-off between computational cost and selection accuracy in their novel work on wrapper techniques.

Wrapper Methods in Conjunction with Classification Algorithm

RFE Pseudocode for Classification Algorithm (Guyon et. al. 2002; Pedregosa et. al. 2011)

Input: Take D as the dataset, C as the classifier, and k as the number of features to select.

Output: Select Specific features $F_{selected}$
F –For all features
Continue until $|F| == k$.
Use features to train classifier C on the dataset. F
Calculate feature importance scores (such as Gini importance or coefficients) from C.
Take away F's least significant feature.
F_{min}=arg min I(f)
$f \in F$
Return $F_{selected}$ = F at the end

SFS Pseudocode for Classification Algorithm (Jain & Zongekar, 1997; Guyon & Elisseeff, 2003; Pudil et al., 1993)

Input: Take D as the dataset, C as the classifier, and k as the number of features to select.

Output: Let selected features be $F_{selected}$
$F_{selected} \leftarrow \{\}$
While $|F_{selected}| < k$ do
best_score ← -∞
For each feature, f not in F_selected:
$F_{temp} \leftarrow F_{selected} \cup \{f\}$
Train classifier C on F_{temp}
score ← Evaluate(C on validation set)
If score > best_score:
best_score ← score
best_feature ← f
Add best_feature to $F_{selected}$
End
Return $F_{selected}$

SBS Pseudocode for Classification Algorithm (Jain & Zongekar, 1997; Guyon & Elisseeff, 2003)

Input: Take D as the dataset, C as the classifier, and k as the number of features to select.

Output: Let selected features be $F_{selected}$
$F_{selected}$ ← All features
While |F_selected| > k:
best_score ← -∞
For each feature f in $F_{selected}$:
F_{temp} ← $F_{selected}$ \ {f}
Train classifier C on F_{temp}
score ← Evaluate(C on validation set)
If score > best_score:
best_score ← score
worst_feature ← f
Remove worst_feature from $F_{selected}$
End
Return $F_{selected}$

GA Pseudocode for Classification Algorithm (Waheed et. al., 2020)

Input: Take D as the dataset, C as the classifier, and k as the number of features to select.

Output: Selected feature subset
Initialize population of N binary chromosomes (length = number of features)
For each generation from 1 to G:
For each chromosome in the population:
Select features where gene == 1
Train classifier C using selected features
Evaluate fitness using F1 score or Accuracy
Select top individuals via tournament/roulette
Perform crossover and mutation to create a new population
Return the chromosome with the highest fitness → selected feature subset

PSO Pseudocode for Classification Algorithm (Waheed et. al., 2020; Sivanandam & Deepa, 2007; Goldberg,1989)

Input: Take D as the dataset, C as the classifier, S as the swarm size, and T
Output: Best feature subset
Initialize S particles (binary vector positions & velocities)
For t = 1 to T:

For each particle:
Decode binary vector → selected features
Train classifier C with selected features
Compute fitness (e.g., F1 score)
Update personal best (pBest) and global best (gBest). For each particle:
Update velocity:
$v = \omega * v + c_1 * r_1 * (pBest - current) + c_2 * r_2 * (gBest - current)$
Update position using sigmoid(v) and threshold
Return gBest position (best feature subset)

DE Pseudocode for Classification Algorithm (Storn & Price, 1995; Das & Suganthan, 2011; Sharma & Kaur, 2020)

Input: Take D as the dataset, C as the classifier, P as the population size, and G as the generations.

Output: Best feature subset
Initialize population of binary vectors (length = number of features)
For generation = 1 to G:
For each target vector x in the population:
Randomly select $r_1, r_2, r_3 \neq x$
Mutation: $v = r_1 + F * (r_2 - r_3)$
Crossover: u = crossover(x, v, CR)
Binarize u with a threshold
If fitness(u) > fitness(x):
Replace x with u
Select the best individual in the population
Return as selected feature subset
The core mathematical functions are shown in the following table.

Algorithm	**Core Mathematical Function**
RFE	$S_{t+1}=S_t \setminus \arg\min I(f)$
SFS	$S_{t+1}=S_t \cup \arg\max J(S \cup f)$
SBS	$S_{t+1}=S_t \setminus \arg\max J(S - f)$
PSO	$v^{\{t+1\}} = \omega v^t + c_1 r_1 (pbest-x) + c_2 r_2 (gbest-x)$
DE	$v = x_{r1} + F(x_{r2}-x_{r3})$
GA	$P(c_i) = J(c_i)/ \sum J$

Experimental Setup

For the purpose of analysis, the Python is used. Its high-level interactive nature and growing ecosystem of scientific libraries make it an attractive choice for algorithmic development and exploratory data analysis, as told by Dubois (2007) and Millman and Aivazis (2011). Nonetheless, its usage is expanding in both academic and industrial settings due to its general-purpose nature. Scikit-learn leverages this rich environment to provide state-of-the-art implementations of many well-known machine learning techniques while maintaining an easy-to-use interface that is closely related to the Python programming language.

Results and Analysis

Empirical studies have shown that wrapper methods can significantly improve the performance of machine learning models by selecting informative features that have a high predictive power. For example, in a study by Liu and Yu (2005), the authors applied wrapper methods for gene selection in cancer classification tasks and achieved better classification accuracy compared to filter-methods. Similarly, in a study by Saeys et al. (2007), the authors compared the performance of wrapper methods with filter methods in feature selection for microarray data analysis. They found that wrapper methods were more effective in selecting informative features for class prediction and outperformed filter methods in terms of model accuracy and generalization performance.

Ranks of the Features Using Six Wrapper Methods

The ranks of the features as identified by six wrapper methods are given in Table 1. It is evident from the ranks that there is a variation in ranks. Wrapper methods, as mentioned above, evaluate a feature subset based on the performance of the classification algorithms, which makes their ranking highly sensitive to search strategy, optimization goals, and interaction among the features. Deterministic methods such as RFE, SFS, and SBS often yield identical ranks since they rely on greedy, stepwise inclusion or exclusion of the features under fixed evaluation criteria. Meta-heuristic methods such as PSO, DE, and GA engage stochastic population-based searches that discover more broadly, capture non-linear relation dependencies, and higher-order interactions among features, leading to different ranks frequently.

Table 1:

Rank Comparison of Six Wrapper Methods

Feature	RFE Rank	SFS Rank	SBS Rank	PSO Rank	DE Rank	GA Rank
school	27	27	27	10	1	5
sex	11	11	11	3	4	6
age	14	14	14	12	14	8
address	16	16	16	6	11	19
famsize	22	22	22	1	5	7
Pstatus	28	28	28	20	20	24
Medu	5	5	5	30	28	10
Fedu	3	3	3	21	21	29
Mjob	1	1	1	28	13	16
Fjob	2	2	2	7	8	11
reason	4	4	4	14	10	1
guardian	6	6	6	4	12	14
traveltime	29	29	29	18	9	28
studytime	20	20	20	11	23	18
failures	7	7	7	5	26	27
schoolsup	21	21	21	8	18	17
famsup	19	19	19	25	24	12
paid	17	17	17	24	2	13
activities	12	12	12	17	19	26
nursery	23	23	23	26	3	4
higher	25	25	25	22	6	15
internet	24	24	24	15	7	25
romantic	15	15	15	23	29	23
famrel	18	18	18	19	17	22
freetime	13	13	13	9	30	20
goout	10	10	10	2	15	21
Dalc	30	30	30	27	16	2
Walc	26	26	26	16	22	30
health	8	8	8	13	27	9
absences	9	9	9	29	25	3

Wrapper methods are applied to various classification algorithms to check for the accuracy of models. Various algorithms like RF, SVM, KNN, LR, and DT are applied on the same dataset using a hybrid feature selection algorithm, resulting in similar results (Saxena & Singh, 2025).

Figure 1

Feature Importance Rank by using all Methods

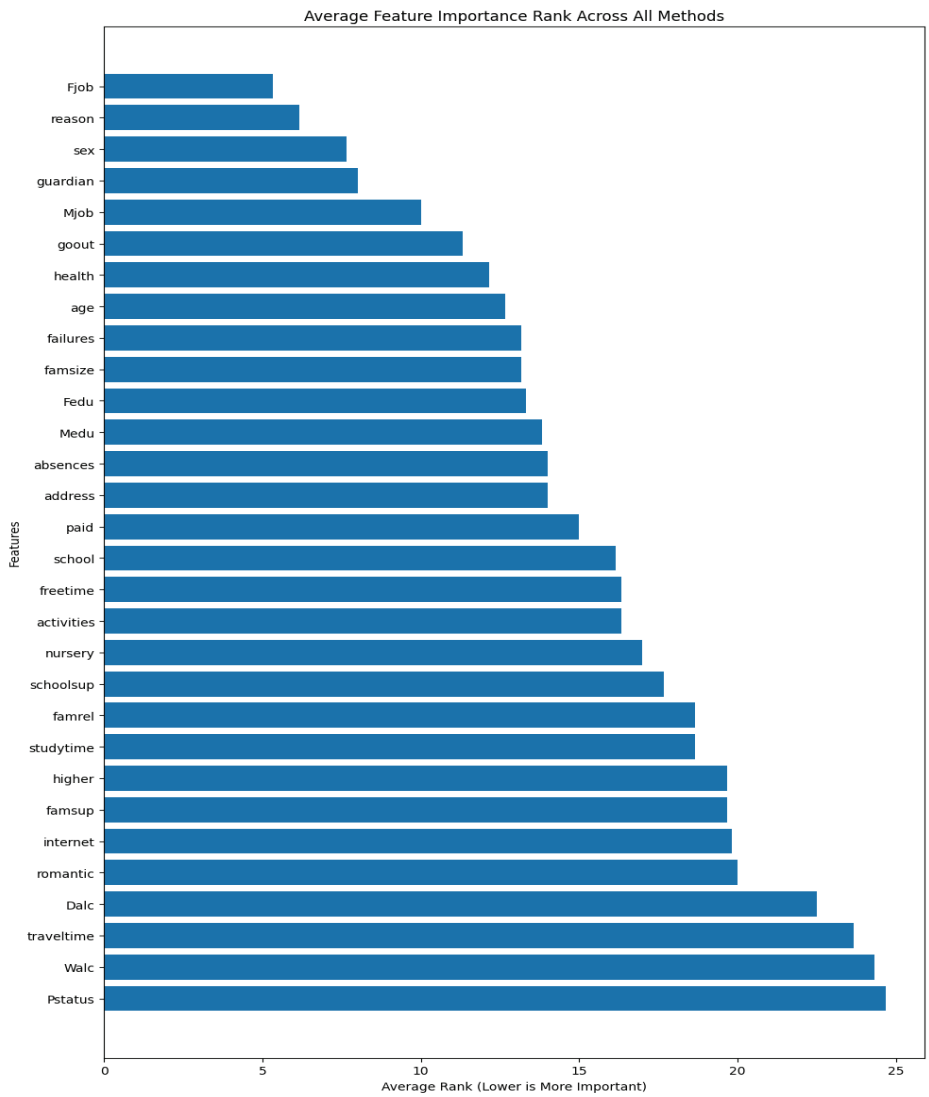


Figure 1 represents the rank of features by using Wrapper methods. It can be seen that the top features to select for the prediction of student academic performance are: Fjob, reason, guardian, Mjob, goout, paid, Medu, Fedu, and absences.

Performance of Classification Algorithms with all Features

The performance evaluation matrices of classification algorithms with all features of the data set are presented in Table 2. It can be inferred from the results given in table 2 that accuracy is highest for the combination of SVM (PSO); Precision is highest for the combination of Decision Tree (PSO); Recall is maximum for the combination of SVM (RFE) and SVM (SBS); F1-Score is highest for the combination SVM (RFE), SVM (SBS), and SVM (PSO); and AUC is highest for Random Forest (DE)

Table 2:

Performance Metrics with all Features using Classification Algorithm

Classification Model	**Accuracy**	**Precision**	**Recall**	**F1 Score**	**AUC**
Decision Tree					
Decision Tree (RFE)	**0.64**	0.74	**0.71**	**0.73**	0.60
Decision Tree (SFS)	0.52	0.67	0.56	0.61	0.50
Decision Tree (SBS)	0.59	0.71	0.66	0.68	0.55
Decision Tree (PSO)	**0.64**	**0.75**	0.70	0.72	**0.61**
Decision Tree (GA)	0.59	0.69	**0.71**	0.70	0.52
Decision Tree (DE)	0.59	0.69	0.70	0.70	0.53
KNN					
KNN (RFE)	0.65	0.69	0.88	0.77	0.53
KNN (SFS)	0.61	0.67	0.84	0.74	0.48
KNN (SBS)	0.66	0.69	0.88	0.77	0.57
KNN (PSO)	0.61	0.66	0.84	0.74	0.48
KNN (GA)	**0.67**	**0.70**	**0.89**	**0.78**	0.60
KNN (DE)	0.64	0.69	0.84	0.76	**0.61**
Logistic Regression					
Logistic Regression (RFE)	0.66	**0.71**	0.84	0.77	0.58
Logistic Regression (SFS)	0.66	0.70	0.86	0.77	0.59
Logistic Regression (SBS)	0.64	0.69	0.83	0.75	0.58
Logistic Regression (PSO)	0.64	0.69	0.84	0.76	0.54

Classification Model	**Accuracy**	**Precision**	**Recall**	**F1 Score**	**AUC**
Logistic Regression (GA)	**0.68**	**0.71**	0.90	**0.79**	**0.60**
Logistic Regression (DE)	0.65	0.69	0.85	0.76	0.59
Random Forest					
Random Forest (RFE)	0.66	0.70	0.86	0.77	0.55
Random Forest (SFS)	0.62	0.69	0.80	0.74	0.57
Random Forest (SBS)	**0.70**	**0.72**	**0.90**	**0.80**	0.54
Random Forest (PSO)	0.58	0.66	0.78	0.71	0.56
Random Forest (GA)	0.67	0.71	0.86	0.78	0.59
Random Forest (DE)	0.66	0.71	0.84	0.77	**0.62**
SVM					
SVM (RFE)	0.70	0.70	**0.96**	**0.81**	0.55
SVM (SFS)	0.67	0.69	0.93	0.79	0.57
SVM (SBS)	**0.71**	**0.71**	**0.96**	**0.81**	0.54
SVM (PSO)	0.70	**0.71**	0.94	**0.81**	0.57
SVM (GA)	0.67	0.70	0.91	0.79	0.60
SVM (DE)	0.67	0.69	0.93	0.79	**0.61**

Performance of Classification Algorithms With Top 20 Features

The performance evaluation matrices of classification algorithms with the top 20 features of the data set are presented in Table 3. It can be seen from the results that accuracy is highest for the combination of Logistic Regression (RFE); Precision is highest for the combination of Random Forest (SBS); Recall is maximum for the combination of SVM (RFE); F1-Score is highest for the combination of SVM (RFE); and AUC is highest for Random Forest (SBS). The performance of SVM classifiers in combination with RFE is best. It has an F1-score of 0.81 and a recall of 1.0.

Table 3:

Performance Metrics Calculation for Top 20 Features Using Classification Algorithm

Classification Model	Accuracy	Precision	Recall	F1 Score	AUC
Decision Tree					
Decision Tree (RFE)	0.61	**0.70**	0.74	0.72	0.54
Decision Tree (SFS)	0.53	0.67	0.60	0.63	0.49
Decision Tree (SBS)	**0.62**	**0.70**	**0.75**	**0.73**	0.55
Decision Tree (PSO)	**0.62**	**0.70**	**0.75**	**0.73**	0.55
Decision Tree (GA)	0.55	0.68	0.64	0.66	0.49
Decision Tree (DE)	0.61	0.73	0.66	0.69	**0.58**
KNN					
KNN (RFE)	**0.65**	0.69	0.85	**0.76**	0.52
KNN (SFS)	0.62	0.67	**0.87**	0.75	0.54
KNN (SBS)	0.59	0.66	0.81	0.73	**0.56**
KNN (PSO)	0.61	0.67	0.81	0.74	**0.56**
KNN (GA)	**0.65**	**0.70**	0.84	**0.76**	0.52
KNN (DE)	0.59	0.67	0.79	0.72	0.50
Logistic Regression					
Logistic Regression (RFE)	**0.70**	0.72	**0.91**	**0.80**	**0.62**
Logistic Regression (SFS)	0.62	0.67	0.85	0.75	0.60
Logistic Regression (SBS)	0.66	0.69	**0.91**	0.78	0.64
Logistic Regression (PSO)	0.66	0.69	0.89	0.78	0.59
Logistic Regression (GA)	0.69	0.71	0.90	**0.80**	0.57
Logistic Regression (DE)	0.66	0.69	**0.91**	0.78	0.57
Random Forest					
Random Forest (RFE)	0.65	0.69	0.87	0.77	0.57
Random Forest (SFS)	0.59	0.66	0.81	0.73	0.58
Random Forest (SBS)	0.68	0.72	0.87	**0.79**	**0.66**
Random Forest (PSO)	**0.68**	0.71	**0.89**	**0.79**	0.64
Random Forest (GA)	0.58	0.67	0.74	0.70	0.57
Random Forest (DE)	0.63	0.69	0.83	0.75	0.55
SVM					
SVM (RFE)	**0.68**	0.68	**1.00**	**0.81**	0.56
SVM (SFS)	0.65	0.67	0.92	0.78	0.61

Classification Model	Accuracy	Precision	Recall	F1 Score	AUC
SVM (SBS)	0.65	0.67	0.92	0.78	0.61
SVM (PSO)	0.67	0.68	0.94	0.79	**0.62**
SVM (GA)	0.69	0.70	0.95	0.80	0.58
SVM (DE)	0.67	0.68	0.94	0.79	0.54

Performance of Classification Algorithms With Top 15 Features

The following inference can be drawn from the performance evaluation matrices of classification algorithms with the top 15 features presented in Table 4.

The combination with the highest accuracy is Random Forest (GA). The precision is highest for the combination of Decision Tree (SBS). The evaluation matrix recall is the maximum for the combination of SVM (RFE). F1-Score is highest for the combination SVM (RFE). AUC is highest for KNN (DE). The performance SVM (RFE) combination of classifiers and feature selection algorithm is best based on Recall (0.98) and F1-Score (0.81).

Table 4:

Performance Metrics for Top 15 Features Using Classification Algorithm

Classification Model	Accuracy	Precision	Recall	F1 Score	AUC
Decision Tree					
Decision Tree (RFE)	0.52	0.64	0.66	0.65	0.45
Decision Tree (SFS)	0.54	0.69	0.58	0.63	0.46
Decision Tree (SBS)	0.66	**0.76**	**0.72**	**0.74**	0.62
Decision Tree (PSO)	0.56	0.70	0.60	0.65	0.53
Decision Tree (GA)	0.54	0.67	0.63	0.65	0.49
Decision Tree (DE)	**0.66**	0.76	**0.72**	0.74	0.63
KNN					
KNN (RFE)	0.62	0.66	**0.89**	0.76	0.54
KNN (SFS)	0.59	0.66	0.83	0.73	0.48
KNN (SBS)	0.63	0.68	0.87	0.76	0.59
KNN (PSO)	0.61	0.66	0.87	0.75	0.58
KNN (GA)	**0.64**	0.68	**0.89**	0.77	0.51
KNN (DE)	0.70	**0.72**	**0.89**	**0.80**	**0.69**

Classification Model	Accuracy	Precision	Recall	F1 Score	AUC
Logistic Regression					
Logistic Regression (RFE)	**0.67**	**0.70**	0.89	**0.78**	0.57
Logistic Regression (SFS)	0.65	0.68	**0.91**	0.77	0.55
Logistic Regression (SBS)	0.66	0.69	**0.91**	**0.78**	**0.66**
Logistic Regression (PSO)	0.66	0.69	0.89	**0.78**	0.63
Logistic Regression (GA)	0.66	0.69	**0.91**	**0.78**	0.58
Logistic Regression (DE)	**0.67**	**0.70**	0.89	**0.78**	0.65
Random Forest					
Random Forest (RFE)	0.59	0.66	0.83	0.73	0.55
Random Forest (SFS)	0.53	0.65	0.66	0.65	0.53
Random Forest (SBS)	0.70	**0.75**	0.83	0.79	0.60
Random Forest (PSO)	0.67	0.73	0.81	0.77	**0.62**
Random Forest (GA)	**0.71**	0.73	**0.89**	**0.80**	0.58
Random Forest (DE)	0.68	0.72	0.87	0.79	0.70
SVM					
SVM (RFE)	0.70	0.69	**0.98**	**0.81**	0.54
SVM (SFS)	0.66	0.68	0.92	0.78	0.51
SVM (SBS)	0.65	0.68	0.91	0.77	**0.62**
SVM (PSO)	0.65	0.68	0.91	0.77	0.60
SVM (GA)	0.69	**0.70**	0.95	0.80	0.56
SVM (DE)	0.68	0.69	0.96	0.80	0.63

Performance of Classification Algorithms With Top 10 Features

The following inference can be drawn from the performance evaluation matrices of classification algorithms with the top 10 features presented in Table 5.

Accuracy is highest for the combination of Random Forest (SBS). Precision is highest for the combination of Random Forest (SBS). The value of recall is maximum for the combination of SVM (RFE). F1-Score is highest for the combination SVM (RFE). AUC is highest for KNN (GA). The performance of SVM (RFE) is better on all evaluation parameters except AUC. Similarly performance of the combination Decision Tree (SFS) is good for all the parameters except AUC.

Table 5:

Performance Metrics for Top 10 Features Using Classification Algorithm

Classification Model	Accuracy	Precision	Recall	F1 Score	AUC
Decision Tree					
Decision Tree (RFE)	0.49	0.64	0.57	0.60	0.46
Decision Tree (SFS)	**0.65**	0.68	**0.89**	**0.77**	0.47
Decision Tree (SBS)	0.61	**0.72**	0.68	0.70	**0.57**
Decision Tree (PSO)	0.61	**0.72**	0.68	0.70	**0.57**
Decision Tree (GA)	0.57	**0.72**	0.58	0.65	**0.57**
Decision Tree (DE)	0.58	0.70	0.66	0.68	0.54
KNN					
KNN (RFE)	0.62	0.68	0.81	0.74	0.50
KNN (SFS)	0.58	0.65	0.83	0.73	0.45
KNN (SBS)	0.68	**0.73**	0.85	0.78	0.63
KNN (PSO)	0.62	0.67	0.85	0.75	0.55
KNN (GA)	0.66	0.69	**0.91**	0.78	**0.71**
KNN (DE)	**0.70**	**0.73**	0.87	**0.79**	0.60
Logistic Regression					
Logistic Regression (RFE)	0.66	0.68	**0.92**	**0.78**	0.57
Logistic Regression (SFS)	0.66	0.68	**0.92**	**0.78**	0.57
Logistic Regression (SBS)	0.65	0.68	0.91	0.77	**0.67**
Logistic Regression (PSO)	0.63	0.67	0.89	0.76	0.63
Logistic Regression (GA)	0.65	0.69	0.87	0.77	0.61
Logistic Regression (DE)	**0.67**	**0.70**	0.89	**0.78**	0.66
Random Forest					
Random Forest (RFE)	0.59	0.67	0.77	0.72	0.53
Random Forest (SFS)	0.63	0.68	**0.87**	0.76	0.54
Random Forest (SBS)	**0.71**	**0.77**	0.81	**0.79**	**0.61**
Random Forest (PSO)	0.61	0.68	0.79	0.73	0.59
Random Forest (GA)	0.62	0.69	0.79	0.74	0.64
Random Forest (DE)	0.66	0.70	0.87	0.77	0.60
SVM					
SVM (RFE)	**0.68**	**0.69**	**0.96**	**0.80**	0.54
SVM (SFS)	0.66	0.68	0.92	0.78	0.47

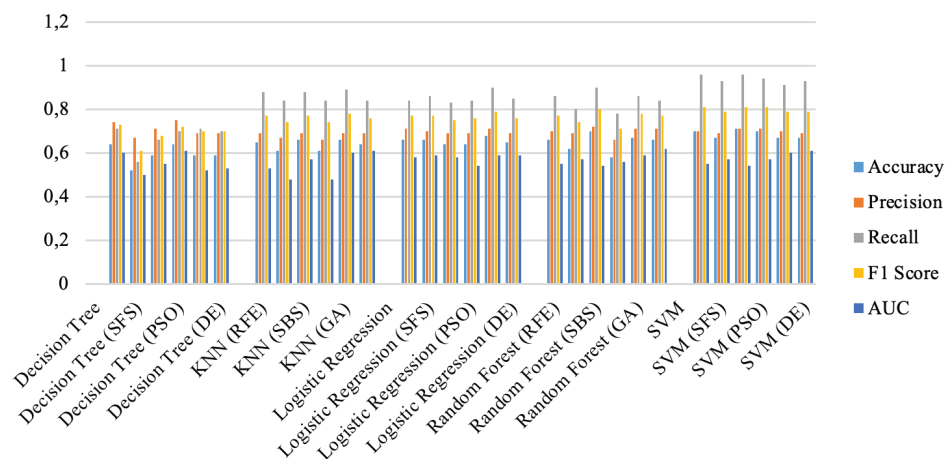
Classification Model	Accuracy	Precision	Recall	F1 Score	AUC
SVM (SBS)	0.67	**0.69**	0.92	0.79	0.58
SVM (PSO)	0.67	0.68	0.94	0.79	**0.61**
SVM (GA)	0.67	0.68	0.94	0.79	0.58
SVM (DE)	0.66	0.68	0.92	0.78	**0.61**

Comparative Analysis

The graphical visualization of the performance evaluation matrices of five classification algorithms in conjunction with wrapper algorithms is given in Figures 2 to 5.

Figure 2:

Performance Metrics of Classification Models based on All Features



In Figure 2, performance metrics are used to represent various classification algorithms. From the above, it can be seen that SVM performs better than other classification algorithms as per the values of recall, precision, and accuracy. In Figure 3, performance metrics are given for five classification algorithms in conjunction with six feature selection methods for the top 20 features. It can be seen from the bar charts that SVM performs better than other classification algorithms in combination with six algorithms based on the Recall values. For the remaining combination, there is no specific trend. However, the combination of Decision Tree (SBS) and Decision Tree (PSO) performs well based on all parameters except AUC.

Figure 3:

Performance Metrics of Classification Models based on Top 20 Features

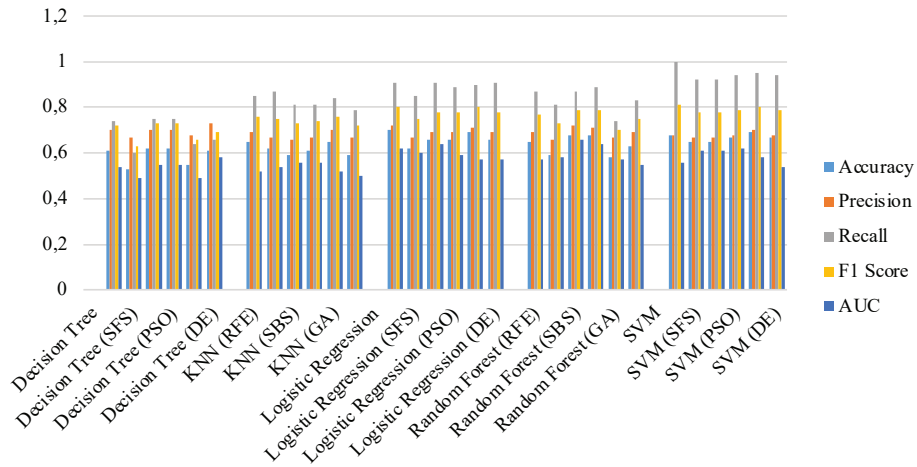


Figure 4:

Performance Metrics of Classification Models based on Top 15 Features

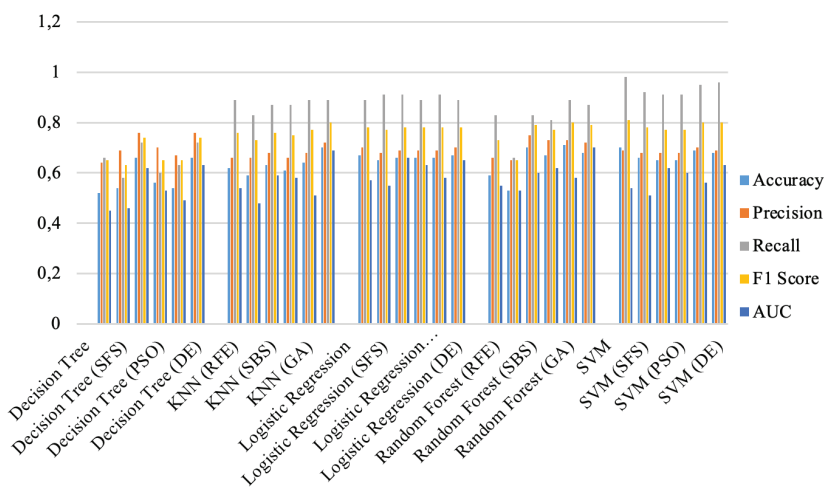


Figure 4 depicts the performance metrics of five classification algorithms in combination with wrapper methods. From the figure 4, it can be seen that SVM performs better than other classification algorithms based on recall. This graph is used to represent the Top 15 features.

Figure 5:

Performance Metrics of Classification Models based on Top 10 Features

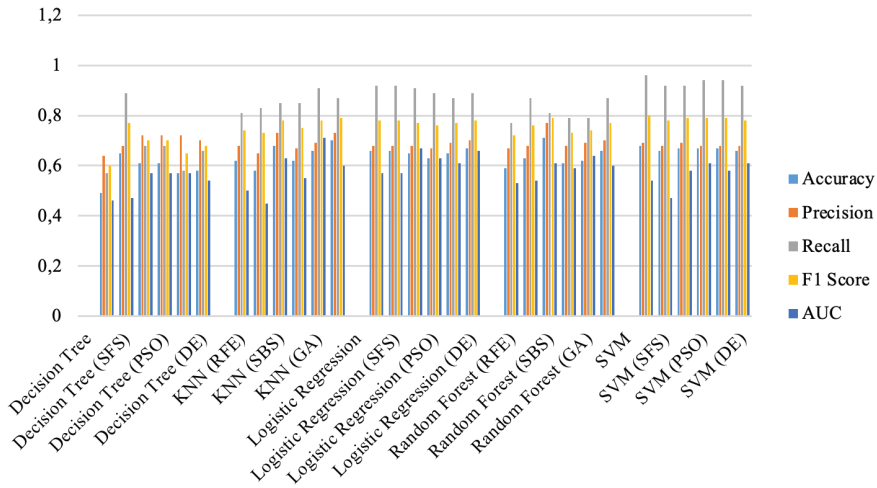


Figure 5 depicts the performance metrics for the five classification algorithms in conjunction with six wrapper algorithms. It is evident from Figure 5 that SVM performs better than other classification algorithms in conjunction with all six wrapper methods based on the recall. This graph is used to represent the Top 10 features.

Conclusion

It has been seen that from the data presented in Table 2-5 and Figures 2- 5 that the optimal combination of feature selection and classification algorithm varies with the evaluation matrix and number of features selected for fitting the classification algorithm. The summary of the best combinations is given in Table 6. To be specific, when all features are considered, SVM (PSO) is best based on Accuracy, and the decision tree (PSO) on the basis of precision. The SVM (RFE) and SVM (SBS) combination is best on recall and F1-score, demonstrating greater sensitivity in recognizing successful students. On the other hand, the highest AUC is observed for the combination random forest (DE), suggesting that this combination has better discriminating capabilities. Similar conclusions can be drawn for other combinations also.

In summary, these results indicate that SVM combined with RFE is the most consistent combination, particularly based on recall and F1-score**.** It shows that de-

terministic, greedy wrapper feature selection algorithms such as RFE are effective in combination with a margin-based classifier, i.e., SVM. On the other hand, evolutionary swarm-based wrapper (GA and DE) shows better strength in combination with ensemble or instance-based classifiers, i.e., random forest and KNN, based on AUC and accuracy.

Table 6:

Best Feature Selection and Classifier Combinations based on Evaluation Metrics

Feature Set Size	Metric	Best Combination (Wrapper + Classifier)	Value
All Features	Accuracy	SVM (PSO)	0.70
	Precision	Decision Tree (PSO)	0.75
	Recall	SVM (RFE) / SVM(SBS)	0.96
	F1-score	SVM (RFE) / SVM (SBS) / SVM (PSO)	0.81
	AUC	Random Forest (DE)	0.62
Top 20 Features	Accuracy	Logistic Regression (RFE)	0.70
	Precision	Random Forest (SBS)	0.72
	Recall	SVM (RFE)	1.00
	F1-score	SVM (RFE)	0.81
	AUC	Random Forest (SBS)	0.66
Top 15 Features	Accuracy	Random Forest (GA)	0.71
	Precision	Decision Tree (SBS / DE)	0.76
	Recall	SVM (RFE)	0.98
	F1-score	SVM (RFE)	0.81
	AUC	KNN + DE	0.69
Top 10 Features	Accuracy	Random Forest (SBS)	0.71
	Precision	Random Forest (SBS)	0.77
	Recall	SVM (RFE)	0.96
	F1-score	SVM (RFE)	0.80
	AUC	KNN (GA)	0.71

Note. The **Best Overall Combination** is determined based on consistency across feature subset sizes and balanced performance on recall and F1-score, which are critical metrics for educational outcome prediction.

In this research paper, the impact of Wrapper methods is studied with the help of a datasets and their performance is analyzed based on rank and quality parameters such as precision, accuracy, F1 Score, Recall, and AUC Curve. Based on the results presented in the earlier sections, the following can be inferred.

It is also observed that reducing the feature set to the top 10, 15, or 20 features did not significantly degrade model performance. In many cases, using fewer features led to even higher accuracy and generalization, supporting the practical utility of wrapper methods in reducing dimensionality without sacrificing predictive power. Future work can focus on combining wrapper methods with filter-based techniques to create hybrid approaches that are both computationally efficient and highly accurate. There is also scope for extending the framework to multiclass classification tasks and applying deep learning models in conjunction with feature selection.

References

Asif, R., Merceron, A., & Pathan, M. K. (2017). Predicting student academic performance at degree level: A case study. *International Journal of Intelligent Systems and Applications, 9*(12), 49–61. https://doi.org/10.5815/ijisa.2017.12.06

Bin Mat, U., Buniyamin, N., Arsad, P. M., & Kassim, R. A. (2014). An overview of using academic analytics to predict and improve students' achievement: A proposed proactive intelligent intervention. In *Proceedings of the 2013 IEEE 5th International Conference on Engineering Education (ICEED)* (pp. 126–130). IEEE.

Chandrashekar, G., & Sahin, F. (2014). A survey on feature selection methods. *Computers & Electrical Engineering, 40*(1), 16–28.

Das, S., & Suganthan, P. N. (2011). Differential evolution: A survey of the state-of-the-art. *IEEE Transactions on Evolutionary Computation, 15*(1), 4–31. https://doi.org/10.1109/TEVC.2010.2059031

Dash, M., & Liu, H. (1997). Feature selection for classification. *Intelligent Data Analysis, 1*(3), 131–156.

Dubois, P. F. (2007). Python: Batteries included. *Computing in Science & Engineering, 9*(3), 7–9. https://doi.org/10.1109/MCSE.2007.58

Feng, C., & Xu, X. (2019). A hybrid feature selection method based on RFE and Relief for educational data mining. *Computers in Human Behavior, 101*, 228–237. https://doi.org/10.1016/j.chb.2019.07.002

Goldberg, D. E. (1989). *Genetic algorithms in search, optimization, and machine learning*. Addison-Wesley.

Guyon, I., & Elisseeff, A. (2003). An introduction to variable and feature selection. *Journal of Machine Learning Research, 3*, 1157–1182.

Guyon, I., Weston, J., Barnhill, S., & Vapnik, V. (2002). Gene selection for cancer classification using support vector machines. *Machine Learning, 46*(1–3), 389–422. https://doi.org/10.1023/A:1012487302797

Holland, J. H. (1992). *Adaptation in natural and artificial systems: An introductory analysis with applications to biology, control, and artificial intelligence*. MIT Press.

Islam, M. M., & Murase, K. (2018). A hybrid wrapper–filter approach for feature selection using mutual information and differential evolution. *Knowledge-Based Systems, 147*, 125–134. https://doi.org/10.1016/j.knosys.2018.01.002

Jain, A. K., & Zongker, D. (1997). Feature selection: Evaluation, application, and small sample performance. *IEEE Transactions on Pattern Analysis and Machine Intelligence, 19*(2), 153–158. https://doi.org/10.1109/34.574797

Kennedy, J., & Eberhart, R. (1995). Particle swarm optimization. In *Proceedings of the IEEE International Conference on Neural Networks* (pp. 1942–1948). IEEE.

Khan, M. J., & Jawaid, A. (2020). Prediction of at-risk students using genetic algorithm and random forest. *Education and Information Technologies, 25*(4), 2979–2997.

Kohavi, R., & John, G. H. (1997). Wrappers for feature subset selection. *Artificial Intelligence, 97*(1–2), 273–324.

Li, J., Cheng, K., Wang, S., Morstatter, F., Trevino, R. P., Tang, J., & Liu, H. (2017). Feature selection: A data perspective. *ACM Computing Surveys, 50*(6), 1–45.

Liu, H., & Yu, L. (2005). Toward integrating feature selection algorithms for classification and clustering. *IEEE Transactions on Knowledge and Data Engineering, 17*(4), 491–502. https://doi.org/10.1109/TKDE.2005.66

Maldonado, S., & Weber, R. (2009). A wrapper method for feature selection using support vector machines. *Information Sciences, 179*(13), 2208–2217.

Millman, K. J., & Aivazis, M. A. (2011). Python for scientists and engineers. *Computing in Science & Engineering, 13*(2), 9–12. https://doi.org/10.1109/MCSE.2011.36

Moslehi, F., & Haeri, A. (2020). A novel hybrid wrapper–filter approach based on genetic algorithm and particle swarm optimization for feature subset selection. *Journal of Ambient Intelligence and Humanized Computing, 11*, 1105–1127.

Patel, D., Saxena, A., & Wang, J. (2024). A machine-learning-based wrapper method for feature selection. *International Journal of Data Warehousing and Mining*. https://doi.org/10.4018/IJDWM.352041

Pedregosa, F., Varoquaux, G., Gramfort, A., Michel, V., Thirion, B., Grisel, O., … Duchesnay, E. (2011). Scikit-learn: Machine learning in Python. *Journal of Machine Learning Research, 12*, 2825–2830.

Pudil, P., Novovicová, J., & Kittler, J. (1994). Floating search methods in feature selection. *Pattern Recognition Letters, 15*(11), 1119–1125. https://doi.org/10.1016/0167-8655(94)90127-9

Saeys, Y., Inza, I., & Larrañaga, P. (2007). A review of feature selection techniques in bioinformatics. *Bioinformatics, 23*(19), 2507–2517.

Saxena, M., & Singh, N. P. (2025). Role of Hybrid Feature Selection Algorithms in Foreseeing Stu dent Performance. International Journal of Natural and Technical Sciences, 5(1), 43-75. https://doi.org/10.69648/NVAX8158

Sharma, M., & Kaur, P. (2020). An efficient differential evolution approach for feature selection in educational data mining. *International Journal of Cognitive Computing in Engineering, 1*, 33–43.

Siedlecki, W., & Sklansky, J. (1989). A note on genetic algorithms for large-scale feature selection. *Pattern Recognition Letters, 10*(5), 335–347.

Singh, A. K., & Karthikeyan, S. (2024). A wrapper feature selection method for predicting student dropout in higher education. *SSRN Electronic Journal*. https://doi.org/10.2139/ssrn.5002077

Sivanandam, S. N., & Deepa, S. N. (2007). *Introduction to genetic algorithms*. Springer. https://doi.org/10.1007/978-3-540-73190-0

Storn, R., & Price, K. (1997). Differential evolution – A simple and efficient heuristic for global optimization over continuous spaces. *Journal of Global Optimization, 11*(4), 341–359.

Tang, J., Alelyani, S., & Liu, H. (2014). Feature selection for classification: A review. In H. Liu & L. Yu (Eds.), *Data classification: Algorithms and applications* (pp. 37–64). CRC Press.

Tomasevic, N., Gvozdenovic, N., & Vranes, S. (2019). An overview and comparison of supervised data mining techniques for student exam performance prediction. *Computers & Education, 143*, 103676. https://doi.org/10.1016/j.compedu.2019.103676

Waheed, H., Goyal, M., & Khanum, A. (2020). Machine learning-based early prediction of students' performance using PSO. *Education and Information Technologies, 25*(6), 4693–4711.

Xue, B., Zhang, M., & Browne, W. N. (2015). A comprehensive comparison on evolutionary feature selection approaches to classification. *International Journal of Computational Intelligence and Applications, 14*(2), 1550008.

Xue, B., Zhang, M., & Browne, W. N. (2016). A survey on evolutionary computation approaches to feature selection. *IEEE Transactions on Evolutionary Computation, 20*(4), 606–626.

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Analysis of the Electroencephalographic and Clinical Phenotype of Epilepsy in Children With Small Chromosomal Changes

Learta Alili Ademi, Elena Sukarova-Angelovska

Abstract

Background/aim: To determine the prevalence of epilepsy, the type of clinical presentation and the electroencephalographic characteristics of epilepsy in small chromosomal changes in a population of patients with epilepsy in the Republic of North Macedonia.

Materials and methods: This retrospective observational study included 193 patients, of which 101 patients with epilepsy (SV = 4.28, SD = 3.68, min 0.04 years, max 14 years) separated into two groups, group A with epilepsy and detected Copy number variations (CNVs); and B1 group with epilepsy where no CNVs were detected. The remaining 92 patients are with detected CNVs and without epilepsy, group B2. For comparing the groups and evaluating the qualitative data, Pearson chi-square test and Fisher's Exact Test were used to determine the degree of dependence between categorical variables with significance $p < 0.05$, using STATA 12.0 software package.

Results: The correlation between epilepsy and CNVs as two dependent variables is statistically strong (Cramer's V = - 0.5567) and significant Pr = 0.000. Pearson's chi2 = 59.8215, Fishers exact = 0.000. The occurrence of epilepsy in the subjects is independent of the type of CNVs.

Conclusion: The expansion of genetic research in recent years has shown that a large part of epilepsy has a genetic basis. Determining the electroclinical features of epilepsy in relation to the occurrence of small chromosomal changes may, in the future, provide a more appropriate classification of epilepsies and key information in identifying the role of genes involved in the development of epilepsy.

Keywords: Epilepsy, small chromosomal changes, epileptic seizures, EEG, CNVs

Introduction

Epilepsy, as a common neurological disorder affecting over 70 million people worldwide, 1-2% of children, is characterized by recurrent seizures (Fisher et al., 2014). A seizure is defined as a sudden alteration of behavior caused by intermittent changes in the electrical functioning of the brain. The expansion of genetic research and technologies in recent years has shown that a large part of unexplained epilepsies has a genetic basis (Hunter et al., 2022; Chen et al., 2017)

New studies indicate that in patients with epilepsy, where chromosomal changes are found, refractory epilepsy most often occurs. Despite the recent introduction of new antiepileptic drugs (AEDs), about 1/3 of patients with epilepsy have refractory epilepsy (Watson et al., 2014).

Refractory epilepsy, according to the International League Against Epilepsy (ILAE), is defined as failure to control seizures when using therapy with two appropriately selected and tolerated AEDs for an appropriate period of time (Fattorusso et al., 2021).

In many patients with small chromosomal changes, seizures occur either as typical or specific, or as nonspecific or refractory (Weise et al., 2012). They also have specific/typical characteristics in terms of EEG findings and response to AED (Pucci et al., 2008).

The latest classification of epilepsies according to ILAE 2017 is a multi-level classification, namely: first level (type of epileptic seizure), second level (type of epilepsy), third level (type of epileptic syndrome) (Scheffer et al., 2017; Hirsch et al., 2022; Specchio et al., 2022).

Copy number variations (CNVs) are defined as the duplication or loss of a portion of a DNA molecule (Battaglia et al., 2005). Many authors believe that submicroscopic CNVs (microdeletions, microduplications) are an important cause of epilepsy (Elia et al., 2001).

Studies show that 5-30% of patients with epilepsy have CNVs in the genome (Nagamani et al., 2013), and this is the case in about 3% of patients with idiopathic generalized epilepsy (IGE) and 1% of patients with focal epilepsies. This percentage is significantly higher when there is early-onset epilepsy in childhood, with alterations found in a large number of chromosomal regions, such as 14q12, 7q11.23, 15q13.3, 16p11.2, and 16p13.11 (Spreiz et al., 2014), 2q24.2-q24.3, 16p13.11-p13.2, 7q11.22, 15q11.2-q13.3 loci, as well as the 6q16.3q22.31 mi-

crodeletion (Szafranski et al., 2015). A European study involving 222 patients with epilepsy reported the detection of CNVs in 31.9% (Helbig et al., 2014), in 7.9% of 315 patients with epileptic encephalopathy (Mefford HC et al.,2011). In 1p36 deletion, epilepsy was reported in 68% of 86 patients (Verrotti et al., 2018; Greco et al., 2018). In a study of 87 patients with Wolf-Hirschhorn syndrome (del4p16.3), epilepsy was reported in 93%, of whom 81% had well-controlled epilepsy (Battaglia et al., 2009). The electroclinical phenotype of epilepsy in 15q13.3 microdeletion is characterized by absence seizures and focal EEG bursts (Whitney et al., 2021).

The main tool in the identification of genes for susceptibility to epilepsy is considered to be the analysis of electro-clinical features of epilepsy associated with CNVs. (Kurosawa et al., 2005)

Refractory epilepsy is common in Angelman syndrome, microdeletion 15q11-13, Prader-Willi syndrome, microdeletion 15q11.2-q13; 22q11.2 deletion (DiGeorge Syndrome), and 2p deletion. Rare cases of Williams syndrome (del7q11.23) have been reported to be associated with infantile spasms (Schinzel et al., 2001; Sorge et al., 2013).

Materials and Methods

In this retrospective nine-year observational study, the clinical and electroencephalographic characteristics of epilepsy, including the type of epilepsy, the specific EEG findings, and the response to AED in patients with small chromosomal changes, were analyzed. The above characteristics were compared with those of patients with epilepsy in whom CNVs were not detected. Data on clinical, genetic, and neurological examinations of 193 patients were collected from the files (hospital and/or outpatient) as well as the HIS system of the PHI University Clinic for Children's Diseases, in Skopje, including those from January 2015 to December 2023, in whom genetic analysis of array comparative genomic hybridization (arrayCGH) was performed.

This study was approved by the institutional ethics committee for human research of the Medical faculty, University St. Cyril and Methodius, in Skopje, North Macedonia, No 03-525/8, on February 7th 2024. The committee operates in accordance with the directive 2001/20/EC of the European Parliament and the Council since 04.04.2001. An informed consent form for participation in the study was prepared and signed by the parent, guardian, or legal representative of all participants prior to data inclusion in the study. The study was conducted in accordance with the

ethical principles of the Declaration of Helsinki and applicable national guidelines. Patient confidentiality was strictly maintained throughout the study.

The type of epilepsy, seizure types, and the response to AED are monitored in all subjects until the year 2023. All patients with CNVs and/or epilepsy, in whom aCGH was performed, were included. Inclusion and exclusion criteria include the following:

Inclusion criteria:

- Age of 0-14 years
- Diagnosis of epilepsy
- Diagnosis of microdeletions and/or microduplications

Exclusion criteria:

- Neurodegenerative diseases
- Cerebral palsy of non-genetic cause with no diagnosed epilepsy
- Neurological single gene disorders (e.g., neurofibromatosis, tuberous sclerosis)
- Known neurogenetic syndromes (Down syndrome and fragile X syndrome)

Based on the established diagnosis of epilepsy, a classification of epilepsy and seizures was performed. According to the 2017 ILAE Classification of Epilepsies, they were classified into focal and generalized epilepsy types. The type of seizure was determined due to hetero/anamnesis, classifying into focal with/without generalization and generalized according to the 2017 ILAE Classification of Seizures. The efficacy of the prescribed AED was also evaluated, and was classified into refractory (RE) and controlled epilepsy (CE) (2014 ILAE Definition).

EEG findings were re/evaluated for the diagnosis of epilepsy and determination of the type of epileptiform discharges. EEG findings were classified into generalized discharges, focal/multifocal discharges, hypsarrhythmia, and normal findings. Regarding the CNVs due to aCGH testing, microdeletion or/and/or microduplication was determined. Data on clinical characteristics were collected due to the presence/absence of dysmorphic features, motoric deficiency, and mental deficiency.

The results of neuroimaging analyses (brain MRI) were evaluated. For detection of the pathogenicity of the obtained changes, the aDGV (array Database of Genomic Variants) database was used, and other databases were included as needed – ClinVar, OMIM, Decipher, etc.

The following data were collected from the subjects: gender; age of first seizure; motor deficit; mental deficit, CNVs; type of EEG trace; type of epileptic seizure; types of epilepsy; dysmorphic features; changes in neuroimaging, number of AEDs used, and type of epilepsy in relation to refractoriness.

The classification of patients into groups was performed due to the presence/absence of epilepsy and/or the presence/absence of CNVs. Therefore, classified into: Group A (A) included those with epilepsy and CNVs, Group B1 (B1) with detected CNVs without epilepsy, and Group B2 (B2) with epilepsy without CNVs.

The classification of patients into groups is shown in Table 1.

Table 1:

Classification of patients into groups due to the presence/absence of epilepsy and/or the presence/absence of CNVs

Group A	**Group B**	
	B1	**B2**
Epilepsy and small chromosomal changes	CNVs without epilepsy	Epilepsy without CNVs

STATISTICAL ANALYSIS: Continuous data were interpreted using standard descriptive statistics: mean, standard deviation, maximum value, minimum value (SD; min-max), and number of observations. Categorical variables were summarized as absolute frequencies and percentages. Group comparisons were performed using Pearson's chi-square test to evaluate associations between categorical variables when expected cell counts were adequate. Fisher's exact test was applied in parallel for contingency tables with small cell counts to ensure the robustness of significance testing. Effect sizes were quantified using Cramér's V to complement *p*-values and to assess the magnitude of observed associations, with values >0.1 interpreted as clinically meaningful. A two-sided *p-value* <0.05 was considered statistically significant. All analyses were conducted using the STATA statistical software package (version 12.0), ensuring standardized and reproducible statistical evaluation of group differences.

Results

Genetic analysis (arrayCGH) was performed in 193 subjects. In 144 (74.6%) subjects, small chromosomal changes (CNVs) were detected, of which 69 (47.92%)

were female, and 75 (52.08%) were male. Of these, 84 (58.33%) had a microdeletion, 43 (29.86%) had a microduplication, while 17 (11.81%) had both a microdeletion and a microduplication. In the groups, microdeletion was the most common, detected in 30/52 (57.69%) of group A and in 54/92 (58.70%) of group B1. Microduplication was detected in 16/52 (30.77%) in Group A, and in 27/92 (29.35%) of Group B1 (as shown in Table 2). Microdeletions were the predominant CNV type in both groups. The distribution of CNV types between Group A and Group B1 did not differ significantly, as demonstrated by the chi-square test (p = 0.984), with a negligible effect size (Cramer's V = 0.015), indicating no meaningful association between CNV type and group allocation.

Table 2:

Analysis of the types of small chromosomal changes in each group of patients with CNVs (group A and group B1)

	Group A	Group B1	Total	Chi-square test	(P- value) *	Fisher's exact test	Cramers V
	N = 52	N = 92	N =144				
CNVs							
Microdeletion	30 (57.6%)	54 (58.7%)	84 (58.3%)	0.0331	0.984	1.000	0.0152
Microduplication	16 (30.8%)	27 (29.4%)	43 (29.9%				
Both	6 (11.5%)	11 (11.9%)	17 (11.8%)				

Statistical analysis was performed using STATA version 12.0. Statistical significance was assessed using the chi-square test ($p < 0.05$), with effect size estimated by Cramer's V (significant if > 0.1). Fisher's exact test was applied where appropriate.

Characteristics of the Patients

Out of 144 patients with small chromosomal changes, 108 (75%) had motor deficiency. Motor deficiency was present in 78/101 with epilepsy. Motor impairment was most frequently associated with microdeletions, occurring in 61 of 84 patients (72.6%), with a statistically significant association between CNV type and motor

deficit (Pearson's chi square (1) = 12.57, $p < 0.001$; Fisher's exact test $p < 0.001$), and a moderate effect size (Cramér's V = 0.35), indicating a meaningful relationship between microdeletions and motor dysfunction.

Mental deficit was noted in 133/144 subjects (92.36%) with small chromosomal changes, most prevalent in the microdeletion type of small chromosomal change, and in 79/84 (94.05%) subjects. However, due to the uniformly high prevalence, statistical discrimination between CNV types was limited, suggesting that mental deficit represents a core phenotype associated with CNVs irrespective of subtype.

Dysmorphia was noted in 137/144 (95.14%) of the patients with small chromosomal changes. In patients with epilepsy, it was noted in 87/101 (86.1%), group A = 49, group B2 = 38. However, no statistically significant association was found between epilepsy status and dysmorphia (Pearson's chi-square (1) = 0.16, p = 0.689; Fisher's exact test, $p = 0.776$; Cramér's V = 0.04), indicating a negligible effect size. (see Table 3 and Figure 1).

Table 3:

Analysis of demographic and clinical characteristics of patients in each group separately.

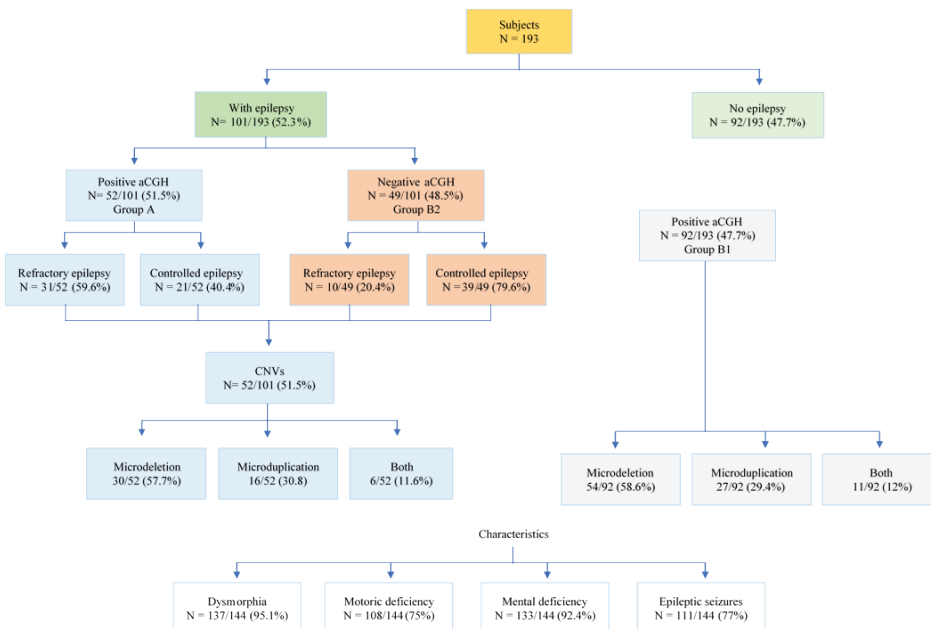
Statistical analysis using the statistical software package STATA 12.0, chi-square test, Fisher's exact test, and Cramer's V test were evaluated *significant at p<0.05, Cramer's V > 0.1

	Group A	Group B1	Group B2	Total	Chi square test	(P - value)*	Fishers exact test	Cramer's V
	N = 52	N = 92	N = 49	N =193				
Female	24 (46.2%)	45 (48.9%)	19 (38.8%)	88 (45.6%)	1.3335	0.513	0.538	0.0831
Male	28 (53.9%)	47 (51.1%)	30 (61.2%)	105 (54.4%)				
Dysmorphia	49 (94.2%)	88 (95.6%)	38 (77.6%)	175 (90.7%)	13.452	0.001**	0.003	0.2640
Motor deficit	48 (92.3%)	60 (65.2%)	30 (61.2%)	138 (71.5%)	15.370	0.000**	0.000	0.2822
Mental deficit	50 (96.2%)	83 (90.2%)	49 (100%)	182 (94.3%)	6.1477	0.046*	0.039	0.1785
*significant at 0.05 level, ** significant at 0.01 level								

When clinical characteristics were compared across all three groups (A, B1, and B2), significant differences were observed for dysmorphia (p = 0.001; Cramér's V = 0.26), motor deficit (p < 0.001; Cramér's V = 0.28), and mental deficit (p = 0.046; Cramér's V = 0.18), while sex distribution did not differ significantly between groups (p = 0.513). Demonstrated in Table 3. These findings support the presence of distinct clinical profiles across the patient groups.

Figure 1:

Graphical representation of the classification of patient groups and respondent clinical characteristics of patients in groups, and results of the survey analysis presented in percentage



Among patients with epilepsy, 39/60 (65%) were patients with the controlled type of epilepsy (group A = 18, group B2 = 21), while 39/41 (95.12%) were patients with the refractory type of epilepsy (group A = 30, group B2 = 9). Mental deficit was detected in controlled epilepsy 60/60 patients (group A = 21, group B2 = 39), while in refractory epilepsy 39/41 patients (group A = 29, group B2 = 10) (see Table 4). Despite these high prevalences, the association between epilepsy type (controlled vs. refractory) and clinical features did not reach statistical significance (Pearson's chi-square (1) = 2.99, p = 0.084; Fisher's exact test, p = 0.162), with a small-to-moderate effect size (Cramer's V = 0.17).

Table 4:

Prevalence of motor deficit, mental deficit, and dysmorphia in groups of patients with epilepsy (Group A and Group B2) in relation to types of epilepsy

Epilepsy type	**Motor deficiency**			
	Group A		Group B2	
	Absent (N)	Present (N)	Absent (N)	Present (N)
Refractory	1	30	1	9
Controlled	3	18	18	21
Total	4	48	19	30
Epilepsy type	**Mental deficiency**			
	Group A		Group B2	
	Absent (N)	Present (N)	Absent (N)	Present (N)
Refractory	2	29	0	10
Controlled	0	21	0	39
Total	2	50	0	49
Epilepsy type	Dysmorphia			
	Group A		Group B2	
	Absent(N)	Present (N)	Absent (N)	Present (N)
Refractory	2	29	3	7
Controlled	1	20	8	31
Total	3	49	11	38
N = number of patients				

Table 5:

Prevalence of epilepsy and types of epilepsy in patients with small chromosomal changes (group A + group B1).

*Statistical analysis using statistical software package STATA 12.0, chi square test, Fishers exact test and Cramers V test were evaluated *significant at p<0.05, Cramers V > 0.1*

CNVs	**Epilepsy**		**Total**
	No	Yes	
None	0	49 (48.5%)1	49 (25.39%)
Present	92 (100.00%)	52 (51.49%)	144 (74.61%)
Total	**92 (**100.00%)	**101 (**100.00%)	**193 (**100.00%)
Pearson's chi2(1) = 59.8215 Pr = 0.000, Cramer's V = - 0.5567, Fishers exact = 0.000			

CNVs	Epilepsy type		Total
	Refractory	Controlled	
None	10 (9.90%)	39 (38.61%)	49 (48.51%)
Present	31 (30.69%)	21 (20.79%)	52 (51.49%)
Total	41 (40.59%)	60(59.41%)	101
Pearson's chi2(1) = 16.0812 Pr = 0.000, Cramer's V = 0.3990, Fishers exact = 0.000			

Epilepsy was diagnosed in 101 of the patients, of which 43/101 (42.57%) were female, 24 (55.8%) from group A and 19 (44.2%) from group B2, while 58/101 (57.43%) were male, Group A = 28 (48.2%) and Group B2 = 30 (51.8%) (see Table 5). N = 41 (40.59%) had refractory epilepsy, of whom 19 (46.34%) were female, and 22 (53.66%) were male, while N = 60 (59.41%) had controlled epilepsy, of whom 24 were female (40%) and 36 were male (60%) (see Table 5). Sex distribution did not show a marked imbalance between refractory and controlled epilepsy, suggesting that sex was not a major determinant of epilepsy severity in this cohort.

A highly significant association was observed between the presence of CNVs and epilepsy (Pearson's chi-square (1) = 59.82, $p < 0.001$; Fisher's exact test, $p < 0.001$). All patients without CNVs were non-epileptic, whereas epilepsy was present in 52 of 144 patients (51.5%) with CNVs. The effect size was large (Cramer's V = 0.56), indicating a strong and clinically meaningful association between CNVs and the occurrence of epilepsy.

Furthermore, CNV status was significantly associated with epilepsy type (refractory versus controlled) (Pearson's chi-square (1) = 16.08, $p < 0.001$; Fisher's exact test, $p < 0.001$). Refractory epilepsy was more frequent among patients with CNVs (31/52, 59.6%) compared with those without CNVs (10/49, 20.4%), whereas controlled epilepsy predominated in patients without CNVs. The corresponding Cramer's V value (0.40) indicates a moderate-to-strong effect size, suggesting that the presence of CNVs is not only associated with epilepsy occurrence but also with increased epilepsy severity. Overall, Table 5 demonstrates a robust statistical relationship between small chromosomal changes and both the presence and refractoriness of epilepsy, supported by highly significant *p*-values and moderate-to-large effect sizes, underscoring the clinical relevance of CNVs in epilepsy risk stratification.

Neuroimaging was performed in 135/193 of patients from the groups, namely group A = 52, group B1 = 60, and group B2 = 49. The most common finding was normal findings, in 83 patients, compared to 52 patients with abnormalities in

brain structures. When neuroimaging findings were compared across Groups A, B1, and B2, normal imaging remained the predominant finding in all groups. Statistical comparison did not demonstrate a significant difference in the distribution of normal versus abnormal neuroimaging findings between groups (chi-square test, $p > 0.05$), indicating that the presence of structural brain abnormalities was not strongly associated with group allocation.

Clinical Characteristics of Epileptic Seizures and Epilepsy

Epileptic seizures were detected in a total of 107 patients, i.e., in 101 patients with epilepsy, group A = 52, group B1 = 6, and group B2 = 49, of which the generalized type occurs in a total of 50/107 patients, namely in 25/52 subjects from group A, 2/92 patients from group B1 and 23/49 patients from group B2. In group A, the most common type of epileptic seizure is generalized, in group B1, the most common type of epileptic seizure is focal (4/6), while in group B2, the most common type of epileptic seizure is focal with/without generalization (26/49). Due to the small number of epileptic patients in Group B1, statistical comparisons of seizure types were performed between Group A (patients with CNVs) and Group B2 (patients without CNVs) (see Table 6).

As shown in Table 6, seizure-type distribution differed significantly between Groups A and B2 when stratified by epilepsy type (refractory vs. controlled). The overall comparison demonstrated a statistically significant association between seizure type and group allocation (Pearson's chi-square (2) = 27.83, $p < 0.001$; Fisher's exact test, $p < 0.001$), with a large effect size (Cramer's V = 0.53), indicating a strong and clinically meaningful relationship.

In patients with refractory epilepsy, polymorphic seizures were the most common seizure type, occurring in 26 of 41 patients (63.4%), including 18 patients from Group A and 8 patients from Group B2. Conversely, in patients with controlled epilepsy, generalized seizures predominated, affecting 36 of 60 patients (60.0%), with 15 patients from Group A and 21 patients from Group B2 (see Table 6).

Table 6:

Analysis of seizure types in types of epilepsy in patients with (group A) and without CNVs (group B2) using the statistical software package STATA 12.0.

*Statistical analysis using the statistical software package STATA 12.0, chi-square test, Fisher's exact test, and Cramér's V test were evaluated *significant at p<0.05, Cramér's V > 0.1*

Epileptic seizure type	Group A		Group B2		
	Refractory epilepsy	Controlled epilepsy	Refractory epilepsy	Controlled epilepsy	Total
Focal with/without generalization	3 (9.7%)	4 (19.1%)	0	12 (30.7%)	19
Generalized	10 (32.3%)	15 (71.4%)	2 (20%)	21 (53.9%)	48
Polymorphic	18 (58%)	2 (9.5%)	8 (80%)	6 (15.4%)	34
Total	31	21	10	39	101
(Pearson chi2 (2) = 27.8349. Pr = 0.000, Cramer's V = 0.5250, Fishers exact = 0.000).					

Polymorphic seizures were strongly associated with refractory epilepsy, especially in patients with CNVs, suggesting a more complex and severe epileptic phenotype in this group. In contrast, generalized seizures were more common in controlled epilepsy, regardless of CNV status, indicating a comparatively more favorable seizure profile. Overall, these findings indicate that seizure semiology differs between patients with and without CNVs and between refractory and controlled epilepsy, with CNV-positive patients showing a higher burden of complex and polymorphic seizure types.

The age of onset of the first seizure in the subjects is equally distributed under 1 year of age and over 1 year of age. In 52/107 (48.6%) subjects with epileptic seizures, the first seizure occurred under 1 year of age, while in 55/107 (51.4%) subjects, it occurred over 1 year of age. However, group-wise analysis revealed a significant difference in the timing of seizure onset. In Group A, seizure onset most frequently occurred before 1 year of age, affecting 32 of 52 patients (61.5%), whereas in Group B2, seizure onset was more commonly observed after 1 year of age, occurring in 31 of 49 patients (63.3%). Statistical analysis demonstrated a significant association between group allocation and age at seizure onset (Pearson's chi-square (2) = 6.81, p = 0.033; Fisher's exact test, p = 0.038). Furthermore, the Cramer's V value exceeding 0.1 (Cramer's V = 0.25) indicates that the association is not only statistically significant but also of potential clinical relevance, suggesting that CNV-positive epilepsy is associated with an earlier onset of seizures.

Regarding antiepileptic therapy, the most common is the use of monotherapy, in 59 subjects, while polytherapy is used in 42 subjects. However, in group A, polytherapy with AED is used in most subjects, in 28 subjects, compared to group B2, in which polytherapy is used in 14 subjects. Statistical analysis demonstrated a significant association between group allocation and AED treatment strategy (Pearson's chi-square = 6.63, p = 0.010; Fisher's exact test, p = 0.009–0.015). The effect size was small to moderate (Cramer's V = 0.26), indicating a clinically relevant difference in treatment complexity between groups. Although monotherapy predominated in the overall cohort, patients in Group A were significantly more likely to require AED polytherapy than patients in Group B2. The statistically significant p-values confirm that this difference is unlikely to be due to chance, while the Cramer's V value exceeding 0.1 indicates a meaningful association. These findings suggest that patients in Group A exhibit a more severe or treatment-resistant epilepsy phenotype, necessitating more complex pharmacological management.

Among the 101 patients diagnosed with epilepsy, generalized epilepsy was the most frequent epilepsy type, occurring in 57 patients (56.4%). Generalized epilepsy was more common in Group A, affecting 33 of 52 patients (63.5%), compared with Group B2, where it was observed in 24 of 49 patients (49.0%). Focal epilepsy with or without secondary generalization was identified in 32 patients (31.7%), with a higher prevalence in Group B2 (22 patients) than in Group A (10 patients). Epileptic encephalopathies were less frequent: West syndrome was diagnosed in 11 patients (10.9%), while Dravet syndrome was observed in only one patient (1.0%). Statistical analysis demonstrated a significant association between epilepsy type and group allocation (Pearson's chi-square (3) = 9.11, p = 0.028; Fisher's exact test p = 0.019), with a moderate effect size (Cramer's V = 0.30), indicating that epilepsy classification differed meaningfully between patients with and without CNVs.

Within the CNV-positive group (Group A), generalized epilepsy was most commonly associated with microdeletions (15/33), followed by microduplications (14/33) and combined CNVs (4/33). Focal epilepsy was less frequent and was observed across all CNV subtypes. West syndrome was identified predominantly in patients with microdeletions. However, the overall association between CNV subtype and epilepsy type did not reach statistical significance (Pearson's chi-square (6) = 9.93, p = 0.128; Fisher's exact test, p = 0.080), despite a moderate effect size (Cramer's V = 0.31). More detailed analysis of types of epilepsy due to types of small chromosomal changes is shown in Table 7.

Table 7:

Analysis of prevalence of the type of epilepsy in relation to the type of small chromosomal change in patients with CNVs (group A)

*Statistical analysis using the statistical software package STATA 12.0, chi-square test, Fisher's exact test, and Cramér's V test were evaluated *significant at p<0.05, Cramér's V > 0.1*

CNVs type	Focal	Generalized	Dravet sy	West sy	Total
Microdeletion	6 (60.00%)	15 (45.45%)	1 (100.00%)	8 (10.00%)	30 (57.69%)
Microduplication	2 (20.00%)	14 (42.42%)	0 (0.00%)	0 (0.00%)	16 (30.77%)
Both	2 (20.00%)	4 (12.12%)	0 (0.00%)	0 (0.00%)	6 (11.54%)
Total	10 (100.00%)	33 (100.00%)	1 (100.00%)	8 (100.00%)	52 (100.00%)
Pearson chi2 (6) = 9.9299. Pr = 0.128, Cramer's V = 0.3090, Fishers exact = 0.080					

Regarding epilepsy severity, refractory epilepsy was markedly more prevalent in Group A, affecting 31 of 41 patients with refractory epilepsy (75.6%), compared with 10 of 41 patients (24.4%) in Group B2, indicating a strong association between CNV presence and treatment-resistant epilepsy. Overall, statistically significant findings support the conclusion that CNV status is associated with both epilepsy type and severity. Within Group A, refractory epilepsy was more common than controlled epilepsy (31/52, 59.6% vs. 21/52, 40.4%), indicating a higher burden of treatment-resistant epilepsy among patients with CNVs.

Electroencephalographic Characteristics

Electroencephalography (EEG) was performed in 74/144 subjects with small chromosomal changes, of which focal/multifocal epileptiform seizures were noted in 33/73 (44.59%) subjects (group A = 20, group B1 = 13), generalized seizures in 27/73 (36.49%) subjects (group A = 22, group B1 = 5). Statistical analysis demonstrated a significant difference in EEG epileptiform patterns between groups (Pearson's chi-square (6) = 13.01, p = 0.043; Fisher's exact test p = 0.045), with a moderate effect size (Cramer's V = 0.30), indicating a meaningful association between group allocation and EEG seizure pattern (see Table 8).

The most common EEG finding in the groups is focal/multifocal bursts in 63/123 subjects; however, in group A, the most common finding is generalized bursts in 22 subjects. Hypsarrhythmia occurred in 9 (12.16%) subjects (9 from group A), while normal findings were noted in 5 patients. The distribution of EEG findings differed significantly among the three groups (Pearson's chi-square (6) = 23.91, p = 0.001; Fisher's exact test, *p* = 0.002), with a moderate effect size (Cramer's V = 0.31). These results indicate that EEG patterns vary significantly according to group, with more severe and generalized EEG abnormalities, including hypsarrhythmia, clustering in patients with CNVs, particularly in Group A (Table 8).

Table 8:

Analysis of clinical and electroencephalographic characteristics of epilepsy in each group of patients (group A, group B1, and group B2).

*Statistical analysis using the statistical software package STATA 12.0, chi-square test, Fisher's exact test, and Cramer's V test were evaluated *significant at p<0.05, Cramer's V > 0.1*

N = 52		Group A	Group B1	Group B2	Total	Chi square test	(P - value) *	Fisher`s exact test	Cramer's V
	N = 92	N = 49	N =193						
Epilepsy		52		49	101				
Type						16.0812	0.000**	0.000	0.3990
Refractory		31 (59.62%)		10 (20.41%)	41 (40.59%)				
Controlled		21 (40.38%)		39 (79.59%)	60 (59.41%)				
Type						9.1127	0.028*	0.019	0.3004
Focal		10 (19.23%)		22 (44.90%)	32 (31.68%)				
Generalized		33 (63.46%)		24 (48.98%)	57 (56.44%)				
Dravet sy		1 (1.92%)			1 (0.99%				
West sy		8 (15.38%)		3 (6.12%)	11 (10.89%)				

Epileptic seizure	52	6	49	107	10.5087	0.033*	0.048	0.2216
Focal	7 (13.46%)	4 (66.67%)	12 (24.49%)	23 (21.5%)				
Generalized	25 (48.08%)	2 (33.33%)	23 (46.94%)	50 (46.73%)				
Polymorphic	20 (38.46%)		14 (28.57%)	34 (31.78%)				
EEG findings	52	21	49	122	23.9085	0.001**	0.002	0.3118
Focal/multifocal	20 (38.46%)	13 (59.09%)	30 (61.22%)	63 (51.22%)				
Generalized	22 (42.31%)	5 (22.73%)	16 (32.65%)	43 (34.96%)				
Hypsarrhythmia	9 (17.31%)		3 (6.12%)	12 (9.76%)				
Normal	1 (1.92%)	4 (18.18%)		5 (4.07%)				
Neuroimaging	52	60	49	161	0.8957	0.639	0.650	0.0746
Normal	32 (61.54%)	42 (70%)	32 (65.31%)	106 (65.84%)				
Abnormal	20 (38.46%)	18 (30%)	17 (34.69%)	55 (34.16%)				
AET	52		49	101	6.6343	0.010**	0.015	-0.2563
Monotherapy	24 (46.15%)		35 (71.43%)	59 (58.42%)				
Polytherapy	28 (53.85%)		14 (28.57%)	42 (41.58%)				
Age of first seizure					6.8061	0.033*	0.038	0.2522
< 1 year old	32 (61.54%)	2 (33.33%)	18 (36.73%)	52 (48.60%)				
>1year old	20 (38.46%)	4 (66.67%)	31 (63.27%)	55 (51.40%)				
*significant at 0.05 level, ** significant at 0.01 level								

Electroclinical Characteristics of Small Chromosomal Changes

The most common type of epileptic seizure in subjects with small chromosomal changes is a generalized epileptic seizure, which occurs in 27 subjects out of a total of 58 subjects who experienced an epileptic seizure (see Table 8). Epileptic seizures most often occur in a microdeletion type of chromosomal change, in 33/58 (56.90%) subjects. Statistical analysis did not demonstrate a significant association between seizure type and CNV subtype (microdeletion, microduplication, or combined changes) (Pearson's chi-square (4) = 5.06, p = 0.281; Fisher's exact test, p = 0.23). The effect size was small to moderate (Cramer's V = 0.21), suggesting a possible trend without reaching statistical significance, likely influenced by the limited sample size within CNV subgroups.

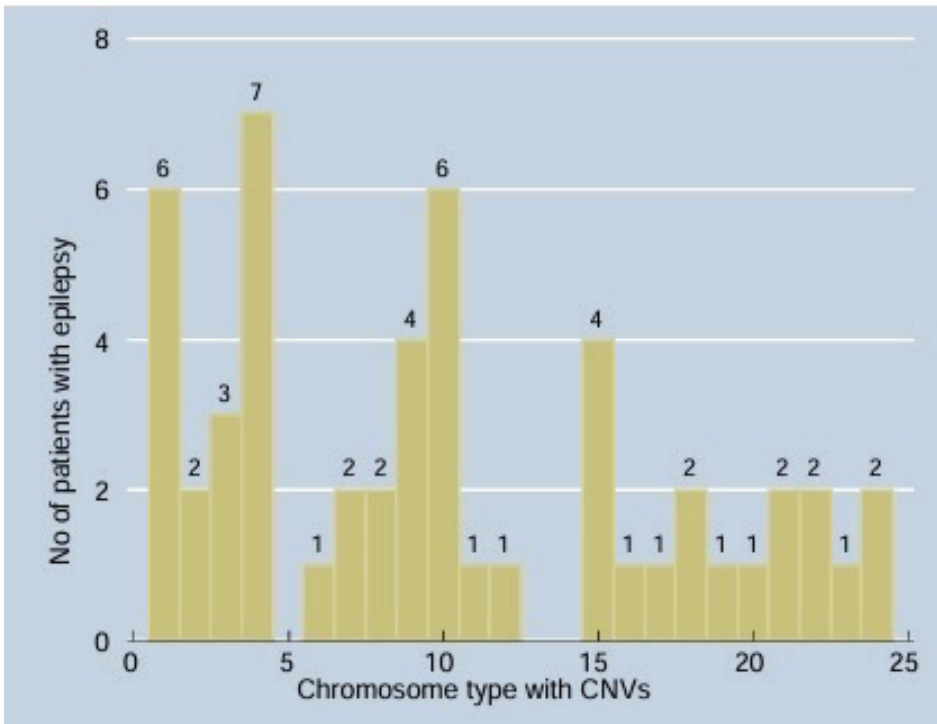
Although generalized seizures and microdeletions predominated among patients with CNVs and epilepsy, the lack of statistical significance indicates that seizure type is not strongly determined by CNV subtype alone. The modest effect size suggests a potential association that may become evident in larger cohorts.

The largest number of subjects with epilepsy were noted in small chromosomal changes on chromosome 4. Also shown for the other chromosomes in Figure 2. The clustering of epileptic cases in CNVs involving chromosome 4 points toward chromosome-specific susceptibility, warranting further investigation of gene content and dosage-sensitive regions on this chromosome.

Figure 2:

Graphical presentation of the number of patients with small chromosomal changes who have epilepsy, shown for each chromosome separately.

*(*No 23 = Chromosome Y, **No 24 = Chromosome X)*



The most common microdeletion syndromes in which epilepsy was diagnosed are Wolf-Hirschhorn syndrome (6/7 subjects), Angelman syndrome (3/5 subjects), and Williams syndrome (1/9 subjects). More detailed information regarding these syndromes and their electroclinical characteristics is given in Table 9.

Table 9:

Prevalence of epilepsy and the electroclinical phenotype in most common microdeletion syndromes with their genotype in our group of patients (group A+ group B1)

Syndrome	Microdeletion	No. of patients with epilepsy	Epilepsy type	Type of epilepsy	Type of epilep-tic seizure	Type of EEG finding	Age of first seizure	AET	Neuroimaging findings
Wolf-Hirschhorn syndrome	del4p	6/7	REF (4)	GEN (4)	PS (5)	HA (2)	< 1: 6	POLYTH (4)	NORM(5)
			CONT (2)	WS (2)	FwG (1)	F/MF (2)		MON-OTH (2)	ABN (1)
						G (2)			
Angelman syndrome	del15q 11-13	3/5	REF (3)	GEN (2)	GS (2)	G (2)	< 1: 2	POLYTH (3)	NORM (3)
				WS (1)	PS (1)	HA (1)	>1: 1		
Williams syndrome	del7q 11.23	1/9	REF (1)	WS (1)	PS IS (1)	HA (1)	< 1	POLYTH (1)	ABN (1)

The remaining small chromosomal changes in which epilepsy is more frequent are microCNVs on chromosome 4, chromosome 1, chromosome 10, chromosome 9, and chromosome 15. More details about the types of CNVs and their electroclinical characteristics are shown in Table 10.

Table 10:

Type of epilepsy, type of epileptic seizure and type of EEG findings found in number of patients with types (deletion/duplication) of small chromosomal changes shown for each chromosome number separately.

Chr	**Microdeletion/ microduplication**	**N**	**Epilepsy type (N)**	**Epilepsy type (N)**	**Epileptic seizure type (N)**	**EEG findings (N)**
1	Del1p	6	REF (3)	GEN (3)	FwG (3)	F/MF (2)
	Del1q21 (3)					G (2)
	dup1p13.3		CONT (3)	F (2)	GS (3)	HA (2)
	dup1q21.2			WS (1)		

2	Del2p	2	REF (2)	F (1)	FwG (1)	F/MF (1)
	del2q24.3			DS (1)	GS (1)	G (1)
3	del3p25.3	3	REF (1)	GEN (3)	FwG (1)	F/MF (2)
	del3q29		CONT (1)		GS (2)	HA (1)
	dup3p11.1					
4	Del4p (6)	8	REF (4)	GEN (6)	GS (2)	Norm (1)
	del4q13.1		CONT (4)	WS (2)	PS (5)	HA (2)
						F/MF (2)
	dup4q24				FwG (1)	G (3)
6	del6q22.1	1	CONT (1)	GEN (1)	GS (1)	MF (1)
7	Del7q11.23	3	REF (1)	GEN (2)	GS (2)	MF (1)
	dup7q31.1		CONT (2)	WS (1)	PS (1)	G (1)
	dup7q35					HA (1)
8	dup8p11.21p11.1x3	2	REF (2)	GEN (2)	GS (2)	G (1)
	dup8p23.3					
9	del9p24.3	4	REF (4)	GEN (2)	GS (1)	MF (2)
	dup9p del9p dup9q			F (2)	FwG (3)	G (1)
	del9q31.1 (2)					F (1)
10	del10q26.3	5	REF (3)	GEN (2)	FwG (2)	F/MF (2)
	dup10q25.3					
	del10q11.22			F (2)		G (2)
	dup10p13		CONT (2)		GS (3)	
	dup10q11.22			WS (1)		HA (1)
	dup10q22.2					
11	dup11p11.12	1	REF (1)	GEN (1)	GS (1)	G (1)
14	del14q11.2	1	REF (1)	GEN (1)	GS (1)	MF (1)
15	del15q11.2q13.1 (3)	5	REF (4)	GEN (3)	FwG (1)	F/MF (1)
	dup15q11.2q13.3		CONT (1)	F (1)	GS (3)	MF (1)
	del15q24.1			WS (1)	PS (1)	G (2)
						HA (1)

16	del16p11.2	3	REF (2)	GEN (1)	GS (2)	G (1)
	Del16p13.11		CONT (1)	WS (2)	PS (1)	HA (2)
	dup16q22.2					
17	dup17p11.2	1	REF (1)	GEN (1)	GS (1)	G (1)
18	del18p	2	REF (2)	GEN (1)	GS (2)	MF (1)
	dup18p11.32x4			WS (1)		HA (1)
19	del19p13.13	1	REF (1)	GEN (1)	GS (1)	G (1)
20	del20p11.1q11.1	2	REF (1)	GEN (1)	FwG (1)	F/MF (1)
	del20q		CONT (1)	F (1)	GS (1)	G (1)
21	del21q22.3	1	CONT (1)	GEN (1)	GS (1)	G (1)
22	dup22q11.21	1	CONT (1)	GEN (1)	GS (1)	G (1)
X	delXp22.12	3	REF (3)	GEN (2)	FwG (1)	F/MF (2)
	dupXp22.33x3			F (1)	PS (2)	G (1)
	dupXp11.23					
Y	dupYp11.2q11.22	2	REF (2)	F (1)	FwG (1)	F/MF (2)
	dupYq11.22.3			GEN (1)	GS (1)	

Abbreviations for Table 9 and Table 10					
Refractory	REF	Focal with generalization	FwG	Monotherapy	MONOTH
Controlled	CONT	Infantile spasms	IS	Normal finding	NORM
Generalized	GEN	Focal/ multifocal discharges	F/MF	With abnormalities	ABN
West syndrome	WS	Generalized discharges	G	Generalized seizure	GS
Polymorphic seizures	PS	Polytherapy	POLYTH	Hypsarrhyth-mia	HA
Focal epilepsy	F	Dravet sy	DS	Number of patients	N

Discussion

In this study, aCGH analysis identified CNVs in approximately three-quarters of the tested children, supporting its diagnostic utility in the evaluation of neurodevelopmental disorders and epilepsy. Although microdeletions were more frequently observed, the CNV subtype alone did not appear sufficient to account for the range of phenotypes seen. Moreover, epilepsy risk may be influenced by CNV size, gene content, and involvement of recurrent pathogenic loci, rather than by deletion versus duplication status alone (Miller et al., 2010; Cooper et al., 2011; Mefford et al., 2011; Coe et al., 2014; Olson et al., 2014).

Neurodevelopmental impairment was a prominent feature in CNV-positive patients. Motor deficit occurred more often in patients with microdeletions, consistent with the stronger impact of haploinsufficiency on dosage-sensitive genes involved in development (Miller et al., 2010; Cooper et al., 2011; Coe et al., 2014). Regardless of CNVs subtype, cognitive impairment was present in nearly all patients, reflecting a common outcome of diverse genomic rearrangements (Girirajan et al., 2012; Olson et al., 2014). Dysmorphic features were frequent but showed no meaningful association with epilepsy status, in keeping with evidence that dysmorphia reflects genomic imbalance rather than epileptogenic mechanisms.

Epilepsy affected more than half of the patients and was strongly associated with CNV presence, showing a markedly higher prevalence of refractory epilepsy. These results support earlier reports demonstrating a higher prevalence of rare CNVs in drug-resistant epilepsy and developmental and epileptic encephalopathies (Mefford et al., 2010; Berg et al., 2010; Mefford et al., 2011; Møller et al., 2015). Seizure phenotype appeared to vary with genetic status, with polymorphic seizures more often observed in refractory epilepsy and generalized seizures more commonly seen in controlled epilepsy. These observations may be consistent with underlying network involvement in genetic epilepsy, although alternative explanations are considered as possible (Mefford et al., 2011; Coppola et al., 2019).

Patients with CNVs more often experienced seizure onset within the first year of life and more frequently required polytherapy, features commonly associated with greater epilepsy severity and less favorable prognosis (Berg et al., 2010; Mefford et al., 2011). The predominance of normal neuroimaging findings suggests that genetic epilepsies are mainly characterized by molecular and circuit-level disturbances, rather than by gross structural abnormalities (Gaillard et al., 2009; Barkovich et al., 2015). EEG abnormalities were prevalent and varied across groups;

meanwhile, CNV-positive patients, especially those with refractory epilepsy, more often showed generalized discharges and severe EEG patterns such as hypsarrhythmia, suggesting widespread network disruption (Mefford et al., 2011; Olson et al., 2014; Scheffer et al., 2017).

Significant electroclinical heterogeneity was evident at the chromosome level, with clustering of epilepsy in CNVs affecting chromosomes 4, 15, 1, 9, and 10. The prominence of chromosome 4, especially the 4p and 4q regions, aligns with known genotype–phenotype correlations, including Wolf–Hirschhorn syndrome (Zollino et al., 2014; Paprocka et al., 2024). CNVs at 15q11–q13 and other recurrent loci showed similarly diverse epilepsy phenotypes, showing the importance of locus-specific interpretation (Helbig et al., 2009; Ben-Shachar et al., 2009; Mefford et al., 2011).

Our findings suggest that CNVs may be associated with epilepsy through disruption of neurodevelopmental networks, with potential effects on seizure type and severity, EEG features, and treatment response. Broad CNV classification alone may not be sufficient to consistently predict clinical outcomes, and more detailed locus and gene-level interpretation could be helpful in improving clinical interpretation.

The strengths of this study include the integration of genomic data with detailed electroclinical phenotyping and the direct comparison between CNV-positive and CNV-negative epilepsy, which permits clinically relevant, locus-oriented observations. However, the findings should be interpreted with caution, particularly in light of the relatively small sample sizes for individual CNV loci. Future multicenter studies with larger cohorts, systematic gene-level CNV interpretation, and longitudinal electroclinical follow-up will be important to further clarify genotype–phenotype relationships and support the development of precision medicine approaches in epilepsy.

Conclusion

Determining the electroclinical features of epilepsy in relation to the occurrence of small chromosomal changes may, in the future, provide a more appropriate classification of epilepsies and provide key information in identifying the role of genes involved in the development of epilepsy. Knowledge about the outcome of epilepsy may be enriched, and the application of targeted antiepileptic therapy based on specific chromosomal changes may be possible.

Declaration of Originality and Authorship

All authors state that the submission of this article is based on original work and neither has been published elsewhere in whole or in part, in any print or electronic media, nor is it under consideration by another journal for publication.

The manuscript has been read and approved by all authors, the requirements for authorship have been met, and each author believes that the manuscript represents honest work.

Informed Consent

The authors declare that written informed consent was obtained from the patients for publication of this original research work. This study was approved by the institutional ethics committee of the Medical faculty, University St. Cyril and Methodius, in Skopje, North Macedonia (Ethics Committee for human research). The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and applicable national guidelines. Patient confidentiality was strictly maintained throughout the study.

Ethics committee for human research of the Medical faculty, University Street, Cyril and Methodius, in Skopje, North Macedonia, issued approval no. 03-525/8, for the conduct of the study.

References

Battaglia, A., Filippi, T., South, S. T., & Carey, J. C. (2009). Spectrum of epilepsy and electroencephalogram patterns in Wolf–Hirschhorn syndrome: Experience with 87 patients. *Developmental Medicine & Child Neurology, 51*(5), 373–380. https://doi.org/10.1111/j.1469-8749.2009.03294.x

Battaglia, A., & Guerrini, R. (2005). Chromosomal disorders associated with epilepsy. *Epileptic Disorders, 7*(3), 181–192. https://doi.org/10.1684/epd.2005.0078

Chen, T., Giri, M., Xia, Z., Subedi, Y. N., & Li, Y. (2017). Genetic and epigenetic mechanisms of epilepsy: A review. *Neuropsychiatric Disease and Treatment, 13*, 1841–1859. https://doi.org/10.2147/NDT.S141456

Elia, M., Musumeci, S. A., Ferri, R., & Ayala, G. F. (2001). Chromosome abnormalities and epilepsy. *Epilepsia, 42*(Suppl. 1), 24–27. https://doi.org/10.1046/j.1528-1157.42.s.1.6.x

Fattorusso, A., Matricardi, S., Mencaroni, E., Dell'Isola, G. B., Di Cara, G., Striano, P., & Verrotti, A. (2021). Pharmacoresistant epilepsy: An overview on existing and emerging therapies. *Frontiers in Neurology, 12*, Article 674483. https://doi.org/10.3389/fneur.2021.674483

Fisher, R. S., Acevedo, C., Arzimanoglou, A., Bogacz, A., Cross, J. H., Elger, C. E., Engel, J., Forsgren, L., French, J. A., Glynn, M., Hesdorffer, D. C., Lee, B. I., Mathern, G. W., Moshé, S. L., Perucca, E., Scheffer, I. E., Tomson, T., Watanabe, M., … Wiebe, S. (2014). ILAE official report: A practical clinical definition of epilepsy. *Epilepsia, 55*(4), 475–482. https://doi.org/10.1111/epi.12550

Greco, M., Ferrara, P., Farello, G., Striano, P., & Verrotti, A. (2018). Electroclinical features of epilepsy associated with 1p36 deletion syndrome: A review. *Epilepsy Research, 139*, 92–101. https://doi.org/10.1016/j.eplepsyres.2017.12.020

Helbig, I., Swinkels, M. E. M., Aten, E., Caliebe, A., van 't Slot, R., Boor, R., von Spiczak, S., Muhle, H., Jähn, J. A., van Binsbergen, E., van Nieuwenhuizen, O., Jansen, F. E., Braun, K. P. J., de Haan, G.-J., Tommerup, N., Stephani, U., Hjalgrim, H., Poot, M., Lindhout, D., … Koeleman, B. P. C. (2014). Structural genomic variation in childhood epilepsies with complex phenotypes. *European Journal of Human Genetics, 22*(7), 896–901. https://doi.org/10.1038/ejhg.2013.262

Hunter, S. E., Jalazo, E., Felton, T. R., Smith, J. D., & Lopez, A. R. (2022). Epilepsy genetics: Advancements in the field and impact on clinical practice. In S. J. Czuczwar (Ed.), *Epilepsy*. Exon Publications. DOI not available

Kurosawa, K., Kawame, H., Okamoto, N., Shimada, A., Hamada, N., & Nagashima, K. (2002). Epilepsy and neurological findings in chromosomal disorders. *Brain & Development, 24*(5), 378–382. https://doi.org/10.1016/S0387-7604(02)00157-4

Mefford, H. C., Yendle, S. C., Hsu, C., Cook, J., Khan, M., de Kovel, C. G. F., … Scheffer, I. E. (2011). Rare copy number variants are an important cause of epileptic encephalopathies. *Annals of Neurology, 70*(6), 974–985. https://doi.org/10.1002/ana.22645

Nagamani, S. C., Erez, A., Ben-Zeev, B., Frydman, M., Winter, S., Zilberberg, E., … Lerman-Sagie, T. (2013). Detection of copy-number variation in AUTS2 by targeted exonic array CGH in patients with developmental delay and autism spectrum disorders. *European Journal of Human Genetics, 21*(3), 343–346. https://doi.org/10.1038/ejhg.2012.207

Pucci, L., Curatolo, P., Coppola, G., Vigevano, F., & Specchio, N. (2008). Epilepsy and electroencephalographic anomalies in chromosome 2 aberrations: A review. *Epilepsy Research, 79*(1), 63–70. https://doi.org/10.1016/j.eplepsyres.2007.11.008

Scheffer, I. E., Berkovic, S., Capovilla, G., Connolly, M. B., French, J., Guilhoto, L., Hirsch, E., Jain, S., Mathern, G. W., Moshé, S. L., Nordli, D. R., Perucca, E., Tomson, T., Wiebe, S., Zhang, Y.-H., & Zuberi, S. M. (2017). ILAE classification of the epilepsies: Position paper of the ILAE Commission for Classification and Terminology. *Epilepsia, 58*(4), 512–521. https://doi.org/10.1111/epi.13671

Schinzel, A., & Niedrist, D. (2001). Chromosome imbalances associated with epilepsy. *American Journal of Medical Genetics, 106*(2), 119–124. https://doi.org/10.1002/1096-8628(20010108)106:2<119::AID-AJMG1012>3.0.CO;2-F

Sorge, G., & Sorge, A. (2013). Epilepsy and chromosomal abnormalities. *Italian Journal of Pediatrics, 39*, 36. https://doi.org/10.1186/1824-7288-39-36

Specchio, N., Wirrell, E. C., Scheffer, I. E., Nabbout, R., Riney, K., Samia, P., … Auvin, S. (2022). ILAE classification and definition of epilepsy syndromes with onset in childhood. *Epilepsia, 63*(6), 1398–1442. DOI not readily available (not all consortium papers have a single unique DOI)

Spreiz, A., Haberlandt, E., Baumann, M., Knaus, A., & Kastner, J. (2014). Chromosomal microaberrations in patients with epilepsy, intellectual disability, and congenital anomalies. *Clinical Genetics, 86*(4), 361–366. https://doi.org/10.1111/cge.12238

Szafranski, P., Von Allmen, G. K., Graham, B. H., Stankiewicz, P., Beaudet, A. L., Cheung, S. W., … Lupski, J. R. (2015). 6q22.1 microdeletion and susceptibility to pediatric epilepsy. *European Journal of Human Genetics, 23*(2), 173–179. https://doi.org/10.1038/ejhg.2014.92

Verrotti, A., Greco, M., Varriale, G., Coppola, G., Signoriello, G., & Pignataro, V. (2018). Electroclinical features of epilepsy in monosomy 1p36 syndrome and their implications. *Acta Neurologica Scandinavica, 138*(6), 523–530. https://doi.org/10.1111/ane.12934

Watson, C. T., Marques-Bonet, T., Sharp, A. J., & Mefford, H. C. (2014). The genetics of microdeletion and microduplication syndromes: An update. *Annual Review of Genomics and Human Genetics, 15*, 215–244. https://doi.org/10.1146/annurev-genom-091212-153408

Weise, A., Mrasek, K., Klein, E., Fröhlich, H., & Liehr, T. (2012). Microdeletion and microduplication syndromes. *Journal of Histochemistry & Cytochemistry, 60*(5), 346–358. https://doi.org/10.1369/0022155412440000

Whitney, R., Nair, A., McCready, E., Robinson, R., & Welch, M. (2021). The spectrum of epilepsy in children with 15q13.3 microdeletion syndrome. *Seizure, 92*, 221–222. https://doi.org/10.1016/j.seizure.2021.07.025

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Dietary Acidity and Dental Erosion: Effects of Frequent Acidic Food and Beverage Intake

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Abstract

Dental erosion is a multifactorial issue that is becoming increasingly related to modern eating patterns, and particularly due to the increased amount of acidic food and drinks being consumed. Although some studies have demonstrated that dietary acids can erode teeth in a laboratory setting, very few clinical studies have examined the habits of adult individuals who consume these types of foods. Therefore, the purpose of this study was to evaluate the relationship between the frequency of consumption of various acidic dietary products (lemons, carbonated beverages, sour candies, vinegar, pickled vegetables, and sports drinks) and the presence of dental erosion in adult patients. The primary hypothesis of this study is that adult patients diagnosed with clinical dental erosion consume acid-containing dietary products more frequently than adults without erosive lesions. To test the hypothesis, a quantitative, cross-sectional study was conducted with a sample of 60 adult patients aged 18-40 years who presented at a dental clinic in a university environment. Each participant underwent a clinical examination performed by trained and calibrated dental examiners. The participants were divided into two groups: a study group of patients with clinically confirmed erosive lesions (n = 30) and a control group of patients without erosive lesions (n = 30). A standardized questionnaire was used to ascertain the dietary patterns of each group of participants by determining how much the participants consumed weekly and monthly of the acidic foods (lemon juice, carbonated drinks, etc.) under study. Comparison of means between groups was accomplished using an independent samples t-test with $p<0.05$ set as statistically significant. Participants with dental erosive lesions indicated significantly greater frequency of consumption on both a weekly and monthly basis than participants without erosive lesions for all foods assessed except for pickled vegetables.

Keywords: dental erosion, dietary acids, eating behavior, enamel demineralization, adults

Introduction

Tooth erosion represents the progressive loss of hard dental tissues and has been a feature throughout human history. Teeth in prehistoric populations were often used in the processing of abrasive foods, to which structural wear naturally occurred. However, due to changes in lifestyle and dietary habits, dental erosions still appear as a common and clinically significant feature. Currently, dental erosion is understood to be a chronic, localized, and pathological dental hard tissue due to the action of chemical dissolution brought about by acids, independent of bacterial activity. In contrast to the acids produced by oral microbiota, dental erosion is primarily associated with exogenous dietary acids, as well as endogenous acid exposure resulting from gastric reflux or recurrent vomiting, often linked to systemic or psychosomatic conditions**.** Exogenous acids are derived from dietary intake and are commonly found in citrus fruits, carbonated soft drinks, sour candies, vinegar, and sports drinks. In contrast, endogenous (intrinsic) acids originate from gastric sources such as reflux or vomiting, resulting in repeated acid exposure of the enamel surface.

This research was intended to explore the possible association between adults' eating habits and the development of dental erosion. To accomplish a systematic and accurate analysis, research participants were divided into two groups based on clinical assessments of their teeth: (1) included adults with erosive lesions, and (2) included adults without any clinically detectable signs of erosion. A comparison of both groups will create an opportunity for the researchers to test for links between specific types of eating patterns and the progression of dental erosion.

Literature Review

The lesions in adults, such as erosion, are increasingly recognized as significant oral health issues, and numerous studies emphasize the role of acids, primarily from dietary sources, in the development of dental erosion (Chow & Brown, 1973; Samet et al., 2025). The mineral component of human teeth is composed of calcium and phosphorus apatite crystals, [$Ca_{10}(PO_4)_6(OH)_2$], a hard, water-insoluble salt essential for the formation of both bone and teeth. (Zhubi et al., 2024)**.**

According to Cheng et al. (2009), acidic drinks have a direct effect on causing demineralization to the enamel, and how often a person drinks acidic drinks can be related to what their teeth look like after they have lost some enamel due to having an acidic drink frequently. Many studies in which a dentist examines a person for ero-

sive lesions after their first visit show that most of the time, erosive lesions will be visible during a person's first dental examination (Elton et al., 2009). There are two ways a person can prevent erosive lesions on their teeth from progressing. Dentists help to educate patients and prevent further damage from other causes (i.e., using fluoride), and patients use individual preventive measures (i.e., brushing their teeth twice a day, reducing intake of sugar-containing food items). Many biochemical processes in organisms use buffer solutions that keep the pH approximately the same throughout most of these processes, being about 7.3 and 7.4 (Samet, 2025; Samet & Ristovska, 2016). Many studies have found that low salivary pH increases enamel breakdown risk and thereby links the environments of the mouth to both caries and erosive lesions (Pattem et al., 2022; Samet et al., 2025).

Food and drink that are high in citric acid or phosphoric acid can sap our dental enamel of calcium, weakening it. When enamel becomes weaker than before it was consumed, the ability to properly bond to restoration materials can also diminish (Dina et al., 2025). For example, researchers were able to assess the effect soda beverages (cola) have on enamel using in vitro studies and also determined how acidic (pH) citrus drinks erode an individual's dental enamel by measuring the changes in pH level, as well as measuring any enamel loss during the experiment. According to Kessler and Türp, soda beverages weaken enamel. This finding was confirmed by a study by Lussi et al. in their recent publication. (Kessler & Türp, 2020; Lussi et al., 2023).

The article also cites that numerous studies (Alcázar-Hernández et al., 2024; Ganss et al., 2001; Venables et al., 2005) show how systemic factors and lifestyle choices affect dental erosion. Sports drinks may cause dental damage in individuals participating in sports due to their acidity; however, recent research indicates that adding nano hydroxyapatite to these beverages may protect teeth from damage caused by the acidity of the pH. In addition, gastric reflux and psychological disorders such as bulimia cause adults to expose their teeth to the same acidic substance in the stomach repeatedly; the level of exposure increases as a result of exacerbated vomiting and nausea associated with bulimia (Bahal & Djemal, 2014; Beresescu et al., 2025; Min et al., 2011).

Research has shown that certain medications and other pharmacological agents, including aspirin, aspirin-like substances (i.e., salicylates), and particularly vitamin C, any antihypertensives (medications used to lower high blood pressure), and tricyclic antidepressants (which are commonly used to treat depression), may negatively affect the quality of saliva (i.e., pH and/or flow) and can contribute to enamel demineralization (Kaidonis et al., 2017; Venables et al., 2005; Zimmer et

al., 2015). The erosive potential of acidic beverages, specifically sports drinks such as those sold under the Gatorade name, has been discussed in many studies using in vitro methods, with the findings showing frequent use of these products can increase enamel demineralization, as well as providing insight into lifestyle choices and current eating/drinking patterns. Studies that assess erosive potential, however, generally have relied on in vitro experimental methods and general dietary patterns (González-Aragón Pineda et al., 2016; Willershausen et al., 2014). When producing powdered or ready-to-drink versions of sports drinks, acacia gum (or gum arabic) has been identified as a sugar replacement that is stable against salivary breakdown and oral bacteria, and therefore represents a non-cariogenic additive (Blazevska et al., 2025).

In summary, the studies reviewed provide overwhelming evidence for the impact of dietary acids on dental erosion but highlight the limited research on the pattern of consumption of those dietary acids in adults' natural eating habits. Therefore, the purpose of this study will be to evaluate how intake of the following food and drink products correlates with the presence of clinically diagnosed erosive lesions to better understand this issue (Chow & Brown, 1973; Samet et al., 2025; Zhubi et al., 2024).

Methods and Materials

The present study was designed as a quantitative cross-sectional study to assess the association between the consumption of acidic dietary products and the occurrence of dental erosions in adults. The patients in this study were adults of both sexes, aged 18–40 years, attending the Clinic for Dental Diseases and Endodontics at the University Clinical Center "Prof. Dr. Bojo Andreski" in Skopje. A sample of 60 participants was selected, and they were clinically examined and separated into two groups by calibrated dental professionals based on the presence or absence of erosive lesions**:** a study group of 30 participants diagnosed with clinically confirmed erosive lesions of the teeth, and a control group of 30 participants without erosive lesions. Exclusion criteria for the study were as follows: (1) Presence of chronic diseases, and (2) Certain physiological conditions.

Dietary habits data were collected using a standardized questionnaire (Table 1), and participants were surveyed regarding their weekly and monthly intake of lemon juice, carbonated drinks, sour candies, vinegar (acetic acid), pickled vegetables, and sports drinks. The collected data were subjected to statistical analysis by using

statistical programs of STATISTICA 7.1; SPSS 17.0; Mean values of consumption frequencies between the study and control groups were compared using the independent samples t-test.

Table 1:

Questionnaire for consumption of specific acidic foods and beverages.

	Consumption per week	Consumption per month
Lemon juice		
Carbonated drinks		
Sour candies		
Acetic acid (vinegar)		
Pickled vegetables		
Sports drinks		

Results and Discussion

From the analysis of the result of the present study it is evident that those participants who have been diagnosed as having erosive lesions, consume a considerably greater amount of food and/or drink that contain an acid content than those individuals diagnosed as not having erosive lesions; which supports our hypothesis of this research and also corresponds with other currently available literature that has established that the presence of exogenous dietary acids contributes significantly to the aetiology of dental erosion.

Lemon Juice Consumption

In Table 2, the results of weekly lemon juice consumption are presented. In the study group, the mean value was 8.2 ± 5.5, whereas in the control group it was lower, 2.5 ± 1.3. The difference in lemon juice consumption, in the t-test, was statistically significant ($p = 0.001051$).

Table 2: Presentation of patients from both groups regarding the consumption of lemon juice weekly and monthly, and the t-test

Lemon juice	Average 1*	Average 2*	t-test	df	p	N–1*	N–2*	St.Dev 1*	St.Dev 2*
Weekly	8,24138	2,500000	3,540863	39	0,001051	29	12	5,51398	1,314257
Monthly	32,13333	6,533333	7,298063	58	0,000000	30	30	18,57832	4,897102

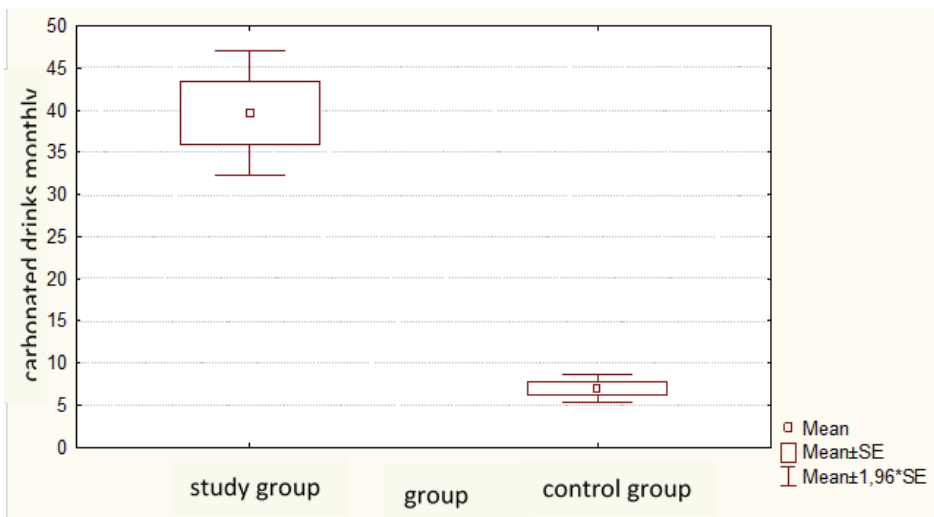
1*-study group

2* - control group

On average, the monthly lemon juice consumption in the study group was 32.1 ± 18.6, while in the control group it was much lower, 6.5 ± 4.9. The results are presented in Table 2 and Figure 1**.** The difference in monthly lemon juice consumption, according to the t-test, was statistically significant ($p < 0.000001$)**.**

Figure 1:

Presentation of patients from both groups regarding monthly lemon juice consumption.



The results indicate that the use of citric acid, which is found naturally in many different citrus fruits, is likely to contribute to the demineralization of the outer layers of tooth enamel due to its chelating properties. The highest amount of citric acid was found in lemon juice, which produced the greatest erosive effect in this study. These results support the findings by Zimmer et al. 2015 where it was determined that repeated exposure to citrus foods and drinks leads to an increase

in the amount of erosion found on tooth surfaces. González-Aragón Pineda et al. (2016) noted that 31.7% of adolescents developed erosive lesions because of the consumption of acidic drinks; thus showing that the intake of lemon juice is one of the major factors leading to the development of erosive lesions.

Carbonated Drink Consumption

In Table 3, the results of weekly carbonated drink consumption are presented. In the study group, the mean value was 14.4 ± 6.1, whereas in the control group it was 2.2 ± 1.1. The difference in carbonated drink consumption, in the t-test, was statistically significant ($p < 0.000001$).

Table 3:

Presentation of patients from both groups regarding the consumption of carbonated drinks weekly and monthly, and the t-test

Carbonated drinks	Average 1*	Average 2*	t-test	df	p	N–1*	N–2*	St.Dev.1*	St.Dev.2*
Weekly	14,43333	2,166667	6,845149	40	0,000000	30	12	6,12335	1,114641
Monthly	39,66667	6,962963	8,033182	55	0,000000	30	27	20,73367	4,327741

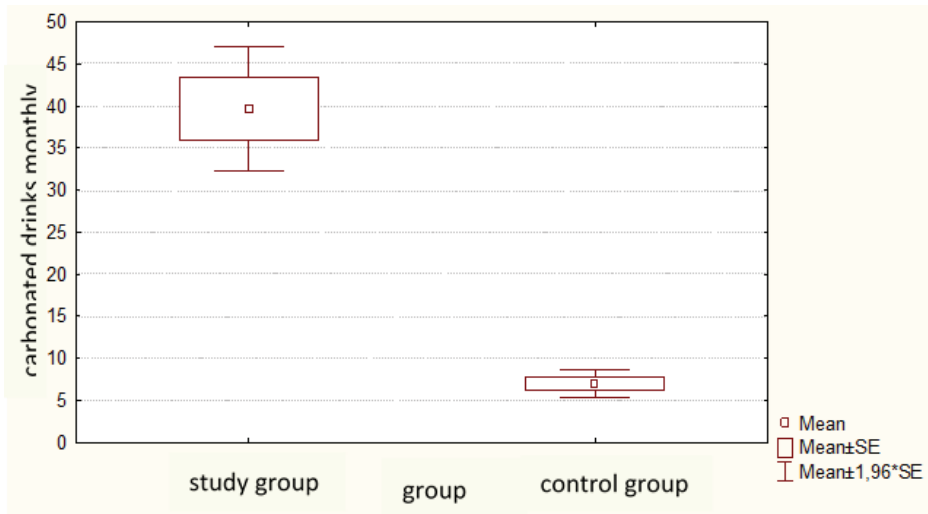
1*-study group

2* - control group

The average monthly consumption of carbonated drinks in the study group was 39.6 ± 20.7, while in the control group, it was lower, 7.0 ± 4.3. These results are shown in Table 3 and Figure 2. The difference in monthly carbonated drink consumption, in the t-test, was statistically significant ($p < 0.000001$).

Figure 2:

Presentation of patients from both groups regarding monthly carbonated drinks consumption.



Carbonated beverages contain a combination of a high level of phosphoric and citric acids, low pH, and high levels of titratable acid. Studies in vitro have shown that drinking these products frequently and extensively can result in very large amounts of enamel surface loss over time (Zimmer et al., 2015). These types of studies from the public health perspective should be alarming, considering the large number of adolescents and young adults consuming carbonated drinks regularly.

Sour Candy Consumption

Results in Table 4, weekly consumption of sour candies in the study group, where the mean value was 20.4 ± 11.3, while consumption of sour candies was lower in the control group, 2.0 ± 0.0. According to the t-test, the difference in sour candy consumption between the two groups was statistically significant (p = 0.000981).

Table 4: Presentation of patients from both groups regarding the consumption of sour candies weekly and monthly, and the t-test.

Sour candies	Average 1*	Average 2*	t-test	df	p	N–1*	N–2*	St.Dev.1*	St.Dev.2*
Weekly	20,43333	2,000000	3,617917	33	0,000981	30	5	11,25162	0,000000
Monthly	49,30000	5,100000	8,751087	48	0,000000	30	20	22,21082	4,517801

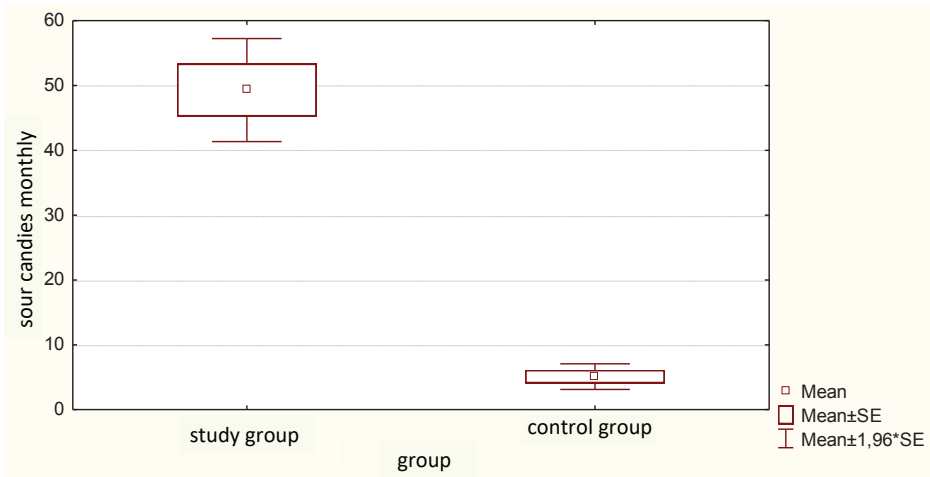
1*-study group

2* - control group

For monthly consumption, the study group had a mean of 49.3 ± 22.2, while the control group's consumption was lower, 5.1 ± 4.5. These results are shown in Table 4 and Figure 3. The difference in monthly sour candy consumption, according to the t-test, was statistically significant ($p < 0.000001$).

Figure 3:

Presentation of patients from both groups regarding monthly sour candies consumption.



These findings also correlate with an in vitro study by Lussi et al. (2023), where it was shown that sour candies had the highest erosive potential because of the very low pH level, high acid concentration, and long duration of retention in the mouth. They concluded from their research that frequent use of sour candies is going to present a risk to tooth enamel, as even chewing gum, which contains some amount of calcium bicarbonate, will not provide sufficient protective effects from this acidic material when used frequently (i.e., daily) for an extended period. Therefore, the likelihood of enamel erosion is very high for children and adolescents who are the primary target group for marketing sour candies.

Vinegar Consumption

In Table 5, the results of weekly vinegar (acetic acid) consumption are presented. In the study group, the mean value was 20.6 ± 20.4, while consumption was lower in the control group, 4.6 ± 2.4 per week. The difference in vinegar consumption in the t-test was statistically significant ($p = 0.018821$).

Table 5:

Presentation of patients from both groups regarding the consumption of vinegar weekly and monthly, and the t-test

Vinegar	Average 1*	Average 2*	t-test	df	p	N–1*	N–2*	St.Dev.1*	St.Dev.2*
Weekly	20,63333	4,600000	2,454051	38	0,018821	30	10	20,43912	2,366432
Monthly	34,40000	8,379310	7,454129	57	0,000000	30	29	17,83371	6,032139

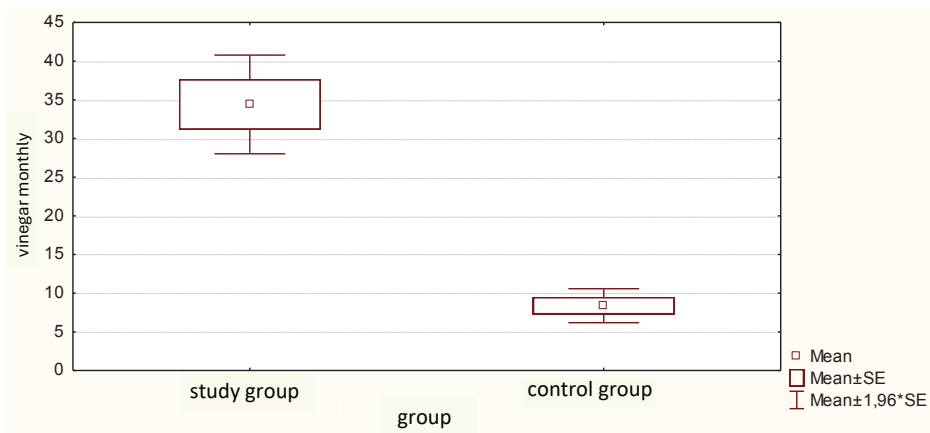
1*-study group

2* - control group

The average monthly vinegar consumption in the study group was 34.4 ± 17.8, while it was lower in the control group, 8.4 ± 6.0 per month. These results are shown in Table 5 and Figure 4. The difference in monthly vinegar consumption, in the t-test, was statistically significant (p = 0.018821).

Figure 4:

Monthly vinegar consumption of both patient groups.



The similarities found in this study are consistent with those from Willershausen et al. (2014), where they report that a variety of vinegar types (e.g., balsamic, raspberry, white, wine) exert the same erosive effects with only minor differences in terms of pH levels. Acetic acid, the active ingredient in vinegar, lowers the pH level of the mouth and also creates damage to the tooth enamel with frequent use (Samet et al., 2025). The findings from the current study provide additional evidence to support the classification of various types of vinegar as a high-risk food group when used consistently over time.

Pickled Vegetable Consumption

In Table 6, the results of the average weekly consumption of pickled vegetables are presented. In the study group, the mean value was 11.7 ± 8.9, while in the control group it was 5.4 ± 3.1. The difference in weekly pickled vegetable consumption, in the t-test, was not statistically significant (p = 0.132214).

Table 6:

Presentation of patients from both groups regarding the consumption of pickled vegetables weekly and monthly, and the t-test

Pickled vegetables	Average 1*	Average 2*	t-test	df	p	N–1*	N–2*	St.Dev.1*	St.Dev.2*
Weekly	11,72414	5,400000	1,544858	32	0,132214	29	5	8,95982	3,130495
Monthly	28,44828	4,454545	7,456750	49	0,000000	29	22	14,73719	3,555375

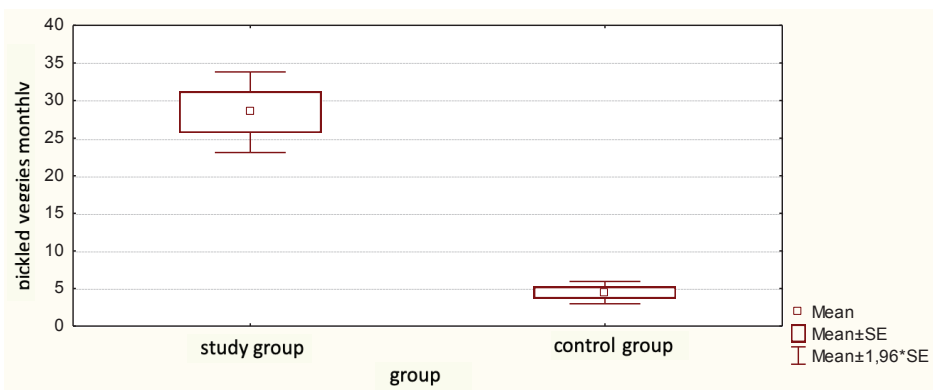
1*-study group

2* - control group

In Table 6 and Figure 5, the results of monthly pickled vegetable consumption are presented. In the study group, the mean value was 28.4 ± 14.7, while consumption was lower in the control group, 4.5 ± 3.6. The difference in monthly pickled vegetable consumption, was statistically significant (p < 0.000001).

Figure 5:

Presentation of patients from both groups regarding monthly pickled vegetable consumption.



The differences in consumption patterns may be contributing to this discrepancy, as infrequent use probably does not have a large effect on enamel erosive potential

(Beresescu et al., 2025). In addition to pH, major factors influencing the erosive potential of acidic foods are the frequency and duration of exposure, and salivary buffer capacity (Samet et al., 2025). Although it may seem that weekly consumption is too insignificant to be a factor, monthly consumption better represents food choices that have an effect on enamel erosion.

Sports Drink Consumption

In Table 7**,** the weekly consumption of sports drinks is presented, where the study group mean value was between 5.4 ± 4.4, whereas no patients in the control group consumed sports drinks.

Table 7: Weekly and monthly consumption of sports drinks of both groups.

Sports drinks	Average 1*	Average 2*	t-test	df	p	N–1*	N–2*	St.Dev.1*	St.Dev.2*
Weekly	5,35000			18		20	0	4,40424	
Monthly	18,72414	2,000000	4,849739	37	0,000022	29	10	10,78324	1,333333

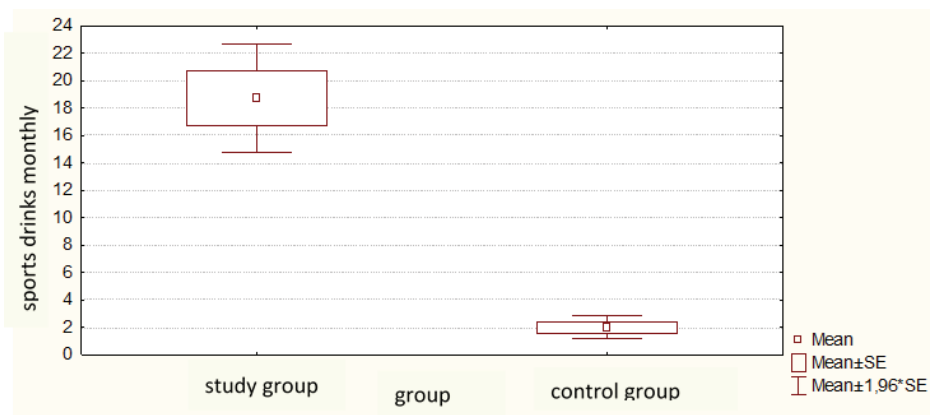
1*-study group

2* - control group

The average monthly consumption of sports drinks in the study group was 18.7 ± 10.8**,** while it was lower in the control group, 2.0 ± 1.3. These results are shown in Table 7 and Figure 6**,** where the difference in sports drink consumption, according to the t-test, was statistically significant (p = 0.000022).

Figure 6:

Monthly sports drinks consumption of patients from both groups.



Sports drinks have an erosive potential due to their ingredients, which usually contain both citric acid and high levels of carbohydrates. Young adults often consume sports drinks in large quantities and multiple times a day; therefore, they may consume them immediately before or just after physical activity, at which time the body's production of saliva is reduced. Lack of saliva increases the risk of demineralization of teeth through food and drink sources, including sports drinks.

Interpretation and Preventive Implications

The results of the present study indicate that adults with clinically confirmed dental erosion consume acidic foods and beverages significantly more frequently than individuals without erosive lesions. The results support previous studies, which have shown that regular consumption of acid (dietary acids) relates strongly to a significantly increased incidence of erosive tooth wear. As previously cited, developing dental erosion is directly influenced, not only by the particular type of acidic product consumed, but also by the amount, frequency, and consistency with which it is consumed. Consumption of acidic dietary products can result in regular exposure to dietary acids that can exceed the buffering ability of saliva and cause enamel demineralisation and a reduced potential for natural processes of enamel remineralisation. In comparison, infrequent or sporadic consumption of acidic dietary products does not appear to affect enamel strength as significantly.

These findings suggest that dietary modification, or the adjustment of dietary content, can be a key factor in managing dental erosion from a preventive perspective. Decreasing the occurrence of consumption of food or drink that is acidic, and decreasing the length of time foods and drinks remain in contact with each other in the mouth, as well as providing neutralization solutions after consuming acidic foods/drinks, may reduce the likelihood of dental erosion. Additional studies suggest that dairy foods, particularly milk, yogurt and cheese, are helpful to reduce enamel demineralization when consumed at the end of a meal, as these foods contain calcium and phosphate which increase the remineralization capacity of teeth; they also contain casein phosphopeptide–amorphous calcium phosphate complexes which help with remineralization and the buffering of acids (Beresescu et al., 2025; Chaudhary et al., 2017; Reynolds, 2008; SAMET et al., 2025).

The importance of clinical assessment and education of patients in determining who is at greater risk of dental erosion is also highlighted by these findings. The inclusion of a dietary assessment during routine dental check-ups for patients may allow dentists to detect dental erosion-related issues early and put into place meth-

ods for preventative treatment for adult patients with frequent consumption of acidic food products.

Study Limitations

Nonetheless, there were several restrictions in this investigation that have yet to be resolved. As the study has a cross-sectional design, it is hard to know whether acidic food consumption caused any erosion of teeth. Also, as food consumption data were collected through self-reports, they may not accurately reflect actual behaviour. Furthermore, this research had a small sample size and was only performed in one location. Factors other than acidic food consumption that may have affected the results, such as salivary flow rate, buffering capacity, and/or social class, weren't evaluated either. Regardless, this research has provided great detail about the impact of an individual's diet on their dental health.

Conclusion

The consensus emerging from this research study is that there is a significant relationship between consuming acidic foods frequently and having dental erosions. Analysis of dental erosive lesions found that individuals aged 18 to 40 years with dental erosion consumed lemon juice, carbonated beverages, sour candies, vinegar, and sports drinks more frequently than those without erosive lesions. Other analyses have revealed that not only the types of acidic products consumed are significant, but the amount and regularity of consumption also play an important role. Occasional use of these products may have little or no effect on the development of erosive lesions. This insight demonstrates the necessity of implementing preventive strategies that involve both behavioural modification and protective interventions. Future investigations may be warranted to establish specific individual differences in sensitivity to erosive lesions, taking into account the composition of their saliva, genetics, and lifestyle.

References

Alcázar-Hernández, J. M., Pecci-Lloret, M. R., & Guerrero-Gironés, J. (2024). Oral Manifestations in Patients in Treatment with Antidepressants: A Systematic Review. In *Journal of Clinical Medicine* (Vol. 13, Issue 22). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/jcm13226945

Bahal, P., & Djemal, S. (2014). Dental Erosion from an Excess of Vitamin C. *Case Reports in Dentistry*, *2014*(1), 485387. https://doi.org/10.1155/2014/485387

Beresescu, G., Beresescu, L., & Beresescu, G. (2025). *Erosive Impact of Acidic "Healthy" Beverages on Dental Enamel: A Systematic Review (2013–2025)*. https://doi.org/10.20944/PREPRINTS202505.1016.V1

Blazevska, Z., Pavlova, V., & Samet, A. (2025). *APPLICATION OF ACACIA GUM, A PLANT DIETARY FIBER, IN THE PRODUCTION OF POWDERED DRINKS*. www.globalscientificjournal.com

Chaudhary, I., Tripathi, A. M., Yadav, G., & Saha, S. (2017). Effect of Casein Phosphopeptide-amorphous Calcium Phosphate and Calcium Sodium Phosphosilicate on Artificial Carious Lesions: An in vitro Study. *International Journal of Clinical Pediatric Dentistry*, *10*(3), 261. https://doi.org/10.5005/JP-JOURNALS-10005-1447

Chow, L. C., & Brown, W. E. (1973). Phosphoric Acid Conditioning of Teeth for Pit and Fissure Sealants. *Journal of Dental Research*, *52*(5), 1158. https://doi.org/10.1177/00220345730520053501;ISSUE:ISSUE:DOI

Dina, K., Velkovski, V., & Samet, A. (2025). Determination of Hematological and Biochemical Parameters in Blood Samples from Oncological Patients in Kosovo. *International Journal of Technical and Natural Sciences*, *5*(1), 25–41. https://doi.org/10.69648/NDDD5147

Dina, K., Velkovski, V., Samet, A., & Blazevska, Z. (2025). *IMPACT OF DIETARY SUPPLEMENT RECOMMENDATIONS ON HEMATO-LOGICAL AND BIOCHEMICAL PARAMETERS IN CANCER PATIENTS FROM KOSOVO: A SIX-MONTH CLINICAL EVALUATION*. www.globalscientificjournal.com

Elton, V., Cooper, L., Higham, S. M., & Pender, N. (2009). Validation of enamel erosion in vitro. *Journal of Dentistry*, *37*(5), 336–341. https://doi.org/10.1016/J.JDENT.2009.01.006

Ganss, C., Klimek, J., & Giese, K. (2001). Dental erosion in children and adolescents - A cross-sectional and longitudinal investigation using study models. *Community Dentistry and Oral Epidemiology*, *29*(4), 264–271. https://doi.org/10.1034/J.1600-0528.2001.290405.X;SUBPAGE:STRING:ABSTRACT;WEBSITE:WEBSITE:PERICLES;WGROUP:STRING:PUBLICATION

González-Aragón Pineda, Á. E., Borges-Yáñez, S. A., Lussi, A., Irigoyen-Camacho, M. E., & Angeles Medina, F. (2016). Prevalence of erosive tooth wear and associated factors in a group of Mexican adolescents. *Journal of the American Dental Association*, *147*(2), 92–97. https://doi.org/10.1016/j.adaj.2015.07.016

Kaidonis, J. A., Anastassiadis, P. M., Lekkas, D., Ranjitkar, S., Townsend, G. C., & Amaechi, B. T. (2017). Prevention and control of dental erosion by professionally applied treatment. *Clinical Dentistry Reviewed 2017 2:1*, *2*(1), 1–9. https://doi.org/10.1007/S41894-017-0018-9

Kessler, P., & Türp, J. C. (2020). Influence of Coca-Cola on orthodontic materials. A systematic review. *Swiss Dental Journal*, *130*(12), 983–993. https://doi.org/10.61872/sdj-2020-12-02

Lussi, A., Lussi, A., Megert, B., & Shellis, R. P. (2023). The erosive effect of various drinks, foods, stimulants, medications and mouthwashes on human tooth enamel. *SWISS DENTAL JOURNAL SSO – Science and Clinical Topics*, *133*(7/8), 440–455. https://doi.org/10.61872/sdj-2023-07-08-01

Min, J. H., Kwon, H. K., & Kim, B. I. (2011). The addition of nano-sized hydroxyapatite to a sports drink to inhibit dental erosion—In vitro study using bovine enamel. *Journal of Dentistry*, *39*(9), 629–635. https://doi.org/10.1016/J.JDENT.2011.07.001

Pattem, J., Field, J., Waterhouse, P. J., & German, M. J. (2022). The dynamic interplay of dietary acid pH and concentration during early-stage human enamel and dentine erosion. *Frontiers in Dental Medicine*, *3*, 1040565. https://doi.org/10.3389/FDMED.2022.1040565/FULL

Reynolds, E. C. (2008). Calcium phosphate-based remineralization systems: Scientific evidence? *Australian Dental Journal*, *53*(3), 268–273. https://doi.org/10.1111/J.1834-7819.2008.00061.X

Samet Aksu. (2025). SPECTROPHOTOMETRIC MONITORING OF THE OXIDATION OF THIOBENZAMIDE AT VARYING OXIDANT CONCENTRATIONS | KNOWLEDGE - International Journal. *Knowledge International Journal*, *73*(3), 583–588. https://ojs.ikm.mk/index.php/kij/article/view/7943

Samet, A., Omeragic, A., Velkovski, V., & Samet, S. (2025). Determination of the ph value of unstimulated and stimulated saliva in a group of patients with and without dental erosion. *JNSM - Journal of Natural Sciences and Mathematics of UT*, *10*(19–20), 17–30. https://doi.org/10.62792/ut.jnsm.v10.i19-20.p3106

Samet, A., & Ristovska, N. (2016). Effect of pH to the Oxidation of Thiobenzamide with Cr(-VI) Reagent. *Bulletin of the Chemists & Technologists of Bosnia & Herzegovina / Glasnik Hemičara i Tehnologa Bosne i Hercegovine*, 102. https://openurl.ebsco.com/contentitem/gcd:133917508?sid=ebsco:plink:crawler&id=ebsco:gcd:133917508

Venables, M. C., Shaw, L., Jeukendrup, A. E., Roedig-Penman, A., Finke, M., Newcombe, R. G., Parry, J., & Smith, A. J. (2005). Erosive effect of a new sports drink on dental enamel during exercise. *Medicine and Science in Sports and Exercise*, *37*(1), 39–44. https://doi.org/10.1249/01.MSS.0000150017.74892.F5

Willershausen, I., Weyer, V., Schulte, D., Lampe, F., Buhre, S., & Willershausen, B. (2014). In vitro study on dental erosion caused by different vinegar varieties using an electron microprobe. *Clinical Laboratory*, *60*(5), 783–790. https://doi.org/10.7754/CLIN.LAB.2013.130528

Zhubi, D., Pavlova, V., Samet, A., & Author, ©. (2024). Blood Analysis of Patients with Rheumatoid Disease from Kosovo Using Non-Steroidal Anti-Inflammatory Drug-Aspirin. *International Journal of Natural and Technical Sciences (IJTNS)*, *4*(2), 57–80. https://doi.org/10.69648/WYSO8796

Zimmer, S., Kirchner, G., Bizhang, M., & Benedix, M. (2015). Influence of Various Acidic Beverages on Tooth Erosion. Evaluation by a New Method. *PLOS ONE*, *10*(6), e0129462. https://doi.org/10.1371/JOURNAL.PONE.0129462

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