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Special Issue IX



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Peer Review Articles

The correlation between CD200 expression and oxidant-antioxidant status in newly diagnosed B-chronic lymphocytic leukemia patients

 Hiba Muwafaq Saleem, Muthanna Mohammed Awad, Alaa Fadhil Alwan

 1-13

Abstract viewed: **123** PDF downloaded: **57**

DOI : [10.53730/ijhs.v6nS9.12163](https://doi.org/10.53730/ijhs.v6nS9.12163)

 PDF

Analysis of gestational trophoblastic disease in Baghdad Teaching Hospital

 Hanan Jawad Kadhim, Raghad Abdul-Haleem Aleessa

 14-30

Abstract viewed: **59** PDF downloaded: **51**

DOI : [10.53730/ijhs.v6nS9.12164](https://doi.org/10.53730/ijhs.v6nS9.12164)

 PDF

Seroprevalence of Toxoplasma gondii among ocular infection patient in Shahid Dr. Aso Hospital in Sulaimani City, Iraq

 Diyari Othman Karim, Kamaran Hasan Azeez, Younis Sabir Abdullah

 31-39

Abstract viewed: **81** PDF downloaded: **45**

DOI : [10.53730/ijhs.v6nS9.12165](https://doi.org/10.53730/ijhs.v6nS9.12165)

 PDF

Influence of Al₂O₃/NbO₂ Nanostructures on Optical Properties of PVA/PVP Blend for Biomedical Application

Assessment of narrow alveolar ridge expansion by ossiodensification vs. ridge splitting technique for dental implant placement

Clinical and radiographic study

 Omar El Farouk Mossad Sadek Ahmed, Ashraf Abdel Fattah Mahmoud, Hossam Eldin Mohammed Ali, Mohamed Mahgob AlAshmawy, Abdelaziz Baiomy Abdullah, Mohamed Ashraf Abdel Fattah, Ali Ali Tawfik Abdo  392-403



Abstract viewed: **73** PDF downloaded: **33**

[DOI : 10.53730/ijhs.v6nS9.12291](https://doi.org/10.53730/ijhs.v6nS9.12291)

 PDF

Yang's keyhole plate versus conventional plate for treatment of mandibular sub-condylar fractures

Clinical and radiographic evaluation

 Ali Ali Tawfik Abdo, Ashraf Abdel Fattah Mahmoud, Abdel Aziz Baiomy Abdullah, Mohamed Mahgob AlAshmawy, Hossam Eldin Mohammed Ali, Omar El Farouk Mossad Sadek Ahmed, Mohamed Ashraf Abdel Fattah  404-411


Abstract viewed: **67** PDF downloaded: **24**

[DOI : 10.53730/ijhs.v6nS9.12292](https://doi.org/10.53730/ijhs.v6nS9.12292)

 PDF

An Indonesian adolescent with delayed management of airway foreign body (straight pins) aspiration

A case report

 Lucia Miranti Hardianingwati, Rizka Fathoni Perdana  412-416

Abstract viewed: **56** PDF downloaded: **22**

[DOI : 10.53730/ijhs.v6nS9.12298](https://doi.org/10.53730/ijhs.v6nS9.12298)

 PDF

Reducing dependence on moneylenders with Mawar Emas Program



 Supiandi, Risky Angga Pramuja, Muhammad Azizurrohman  417-428

Abstract viewed: **246** PDF downloaded: **83**

[DOI : 10.53730/ijhs.v6nS9.12337](https://doi.org/10.53730/ijhs.v6nS9.12337)

 PDF

Hypocalcemic seizure caused by vitamin D deficiency in infant



 Fatimah Arief, Nur Rochmah, Muhammad Faizi, Prastiya Indra Gunawan, Riza Noviandi  429-433

Abstract viewed: **54** PDF downloaded: **34**

[DOI : 10.53730/ijhs.v6nS9.12342](https://doi.org/10.53730/ijhs.v6nS9.12342)

 PDF

Management of persistent hyperinsulinemia hypoglycemia of infancy

 Adkhiatul Muslihatin, Nur Rochmah, Muhammad Faizi, Qurrota Ayuni Novia Putri  434-438

Abstract viewed: **66** PDF downloaded: **32**

[DOI : 10.53730/ijhs.v6nS9.12350](https://doi.org/10.53730/ijhs.v6nS9.12350)

 PDF



Sociological approach through holistic pattern in solving the problem of homosexuality from the Qur'an perspective

 Ahmad Zain Sarnoto, Nurdin  439-448

Abstract viewed: **84** PDF downloaded: **32**



[DOI : 10.53730/ijhs.v6nS9.12353](https://doi.org/10.53730/ijhs.v6nS9.12353)

 PDF

 Yuni Hisbiyah, Anang Endaryanto, Bagoes Setyoboedi, Nur Rochmah, Muhammad Faizi, Citrawati Dyah Kencono Wungu, Qurrota Ayuni Novia Putri  1395-1410
Abstract viewed: **65** PDF downloaded: **30**
[DOI : 10.53730/ijhs.v6nS9.12765](https://doi.org/10.53730/ijhs.v6nS9.12765)



 PDF

A proposed model for amending tax controls in Iraq to achieve a better tax outcome

 Ali Abbas Kareem Al-Khafaji, Ali Mutashar Muhammad Al-Zuhairy  1411-1420
Abstract viewed: **57** PDF downloaded: **14**
[DOI : 10.53730/ijhs.v6nS9.12552](https://doi.org/10.53730/ijhs.v6nS9.12552)

 PDF

The role of forensic medicine in achieving criminal justice

 Mohamed Bounaanaa, Mohamed Karym  1421-1436
Abstract viewed: **57** PDF downloaded: **18**
[DOI : 10.53730/ijhs.v6nS9.12553](https://doi.org/10.53730/ijhs.v6nS9.12553)

 PDF

Independent isolation of infectious waste management in the COVID-19 era



A case study in Java-Bali Indonesia

 Endang Wahyuningsih, Sutaryono, Jaka Widada, Heru Subaris Kasjono  1437-1444
Abstract viewed: **80** PDF downloaded: **48**
[DOI : 10.53730/ijhs.v6nS9.12314](https://doi.org/10.53730/ijhs.v6nS9.12314)

 PDF


Changes in markers ferritin and c-reactive protein in patients with COVID-19 in Iraq

A case-control study

 Haider Abd Jabbar, Aqeel Raheem Hasan, Mohammed Ali Mones, Abdalkhaliq A. Abdalameer, May Jaleel Abed  1445-1453
Abstract viewed: **131** PDF downloaded: **9**
[DOI : 10.53730/ijhs.v6nS9.12284](https://doi.org/10.53730/ijhs.v6nS9.12284)

 PDF



Clinical comparison of antioxidant for pregnant women with iron deficiency anemia

 Zinah Naeem Mtele  1466-1473
Abstract viewed: **42** PDF downloaded: **14**
[DOI : 10.53730/ijhs.v6nS9.12286](https://doi.org/10.53730/ijhs.v6nS9.12286)

 PDF

To evaluate effect of three different reducing agents in recovery of bond strength to sodium hypochlorite treated dentin with composite resin



An in vitro study

 Abhishek Shelke, Aniket Jadhav, Anupam Sharma, Abhijit Jadhav, Shivani Chavan, Damini Bhagwat  1474-1480
Abstract viewed: **142** PDF downloaded: **20**
[DOI : 10.53730/ijhs.v6nS9.12331](https://doi.org/10.53730/ijhs.v6nS9.12331)

 PDF

Comparative evaluation of shear bond strength of universal bonding agent and total-etch bonding agent on superficial and deep dentin

An in vitro study

 Damini Bhagwat, Ashwini Gaikwad, Varsha Pandit, Mrunal Shinde, Ruchira Bhamare, Sailee Kalyankar  1481-1490
Abstract viewed: **134** PDF downloaded: **17**

**Role of RASSF1-A gene in early detection of colorectal carcinogenesis**

Thaer Ali Hussein, Ahmad Shandookh Hameed, Mohamed Hussein Amer

1908-1917

Abstract viewed: **52** PDF downloaded: **28**DOI : [10.53730/ijhs.v6nS9.12831](https://doi.org/10.53730/ijhs.v6nS9.12831)**Occupational contact dermatitis among healthcare workers in the COVID-19 Isolation ward**

Cita Rosita Sigit Prakoeswa, Damayanti, Sylvia Anggraeni, Menul Ayu

1918-1926

Umborowati, Sawitri, Astindari, Andre Yuindartanto

Abstract viewed: **50** PDF downloaded: **24**DOI : [10.53730/ijhs.v6nS9.12856](https://doi.org/10.53730/ijhs.v6nS9.12856)**Analysis of the ZNT8, GAD65, HLA-DQA1, HLA-DQB1, and C-peptides in Indonesian children with type 1 diabetes mellitus**

Nur Rochmah, Muhammad Faizi, Yuni Hisbiyah, Rayi Kurnia Perwitasari, Tyas

1927-1936

Maslakhathien Nuzula, Anang Endaryanto, Soetjipto Soetjipto

Abstract viewed: **51** PDF downloaded: **32**DOI : [10.53730/ijhs.v6nS9.12857](https://doi.org/10.53730/ijhs.v6nS9.12857)**Chemical castration with silver nitrate and potassium permanganate of local male bucks**

Dler Muwafaq Shukur, Qayes Taref Ali

1937-1955

Abstract viewed: **50** PDF downloaded: **29**DOI : [10.53730/ijhs.v6nS9.12836](https://doi.org/10.53730/ijhs.v6nS9.12836)**Preparation and identification of green polymers towards the production of sustainable latex from β -myrcene and some methacrylate monomers**

Fouad M. Hussein, Ali S. Ismail

1956-1973

Abstract viewed: **42** PDF downloaded: **21**DOI : [10.53730/ijhs.v6nS9.12837](https://doi.org/10.53730/ijhs.v6nS9.12837)**Study of spectroscopic analysis performed of laser produced Sn and Sn:Cr plasma**

Raghad T. Ahmed, Ala F. Ahmed, Kadhim A. Aadim

1974-1982

Abstract viewed: **36** PDF downloaded: **35**DOI : [10.53730/ijhs.v6nS9.12839](https://doi.org/10.53730/ijhs.v6nS9.12839)**Traumatic memory of Martyrs' Sons**

Zinah Ali Salih, Lena Adnan Khalaf Rashad

1983-1997



Abstract viewed: **38** PDF downloaded: **15**DOI : [10.53730/ijhs.v6nS9.12840](https://doi.org/10.53730/ijhs.v6nS9.12840)**Unrealistic optimism**

Zinah Ali Salih, Hanan Majiad Noor eabd

1998-2011

Abstract viewed: **30** PDF downloaded: **17**DOI : [10.53730/ijhs.v6nS9.12843](https://doi.org/10.53730/ijhs.v6nS9.12843)

Improving the quality of counseling services for students based on digital applications in line with the implementation of the Merdeka Curriculum


 B. M. A. S. Anaconda Bangkara, Irma Chmawati Maruf, Abdul Latif, Hasna Hashibah, Ikhsan  2100-2114

Abstract viewed: **72** PDF downloaded: **26**

[DOI : 10.53730/ijhs.v6nS9.12875](https://doi.org/10.53730/ijhs.v6nS9.12875)

 PDF

Knowledge, attitude and acceptance of amputation among amputee patients admitted in National Orthopaedic Hospital Enugu - Nigeria

 Justin Agorye Ingwu, Happiness Ogadi Anikwe, Chinenye Anehkhati, Hope Opara, Patricia Okpala, Mathias Agba  2115-2128

Abstract viewed: **50** PDF downloaded: **17**

[DOI : 10.53730/ijhs.v6nS9.12881](https://doi.org/10.53730/ijhs.v6nS9.12881)

 PDF

Study of endothelial cell loss in small incision cataract surgery (SICS)



 Sujata R. Charel, Hetaj K. Sheth, Astha Domadia, Jash Bavishi, Niyant N. Pandya  2129-2137

Abstract viewed: **73** PDF downloaded: **25**

[DOI : 10.53730/ijhs.v6nS9.12886](https://doi.org/10.53730/ijhs.v6nS9.12886)

 PDF

Screening of catharanthus roseus stem extract for anti-ulcer potential in wistar rat

 Suraj Mandal, Sweta Goel, Monika Saxena, Polly Gupta, Jyoti Kumari, Prashant Kumar, Mukesh Kumar, Rahul Kumar, Km. Shiva  2138-2170

Abstract viewed: **53** PDF downloaded: **8**

[DOI : 10.53730/ijhs.v6nS9.12889](https://doi.org/10.53730/ijhs.v6nS9.12889)

 PDF

The glycemic control among type 1 diabetes children pre and during COVID-19 pandemic

A systematic review and meta-analysis



 Wika Y. Deakandi, Farahdina Farahdina, Qurrota A. N. Putri, Tyas M. Nuzula, Katherine Fedora, Qorri Aina, Nur Rochmah, Muhammad Faizi  2171-2182

Abstract viewed: **79** PDF downloaded: **38**

[DOI : 10.53730/ijhs.v6nS9.12893](https://doi.org/10.53730/ijhs.v6nS9.12893)

 PDF

Isolation, Screening and molecular characterization of bio surfactant producing microbes from hydrocarbon polluted soil

 Putha Deepika Sai Lakshmi, Beesa Hrushik Samyukth, Golamari Siva Reddy, Boddu Sumalatha  2183-2192

Abstract viewed: **33** PDF downloaded: **18**

[DOI : 10.53730/ijhs.v6nS9.12899](https://doi.org/10.53730/ijhs.v6nS9.12899)

 PDF

Postoperative bone defects' reconstruction in patients with focal form of chronic post-traumatic osteomyelitis

 Aleksandr G. Sonis, Evgeniy A. Stolyarov, Jean Geringer, Denis G. Alekseev, Sergey V. Ladonin, Maria Yu. Sefedinova, Maria A. Bezrukova  2193-2202

Abstract viewed: **72** PDF downloaded: **36**

[DOI : 10.53730/ijhs.v6nS9.12901](https://doi.org/10.53730/ijhs.v6nS9.12901)

 PDF

Clinic, diagnosis and treatment of dunbar syndrome in children

 Andrey V. Andreev, Mikhail V. Krasnov, Larisa P. Nikitina  2203-2212



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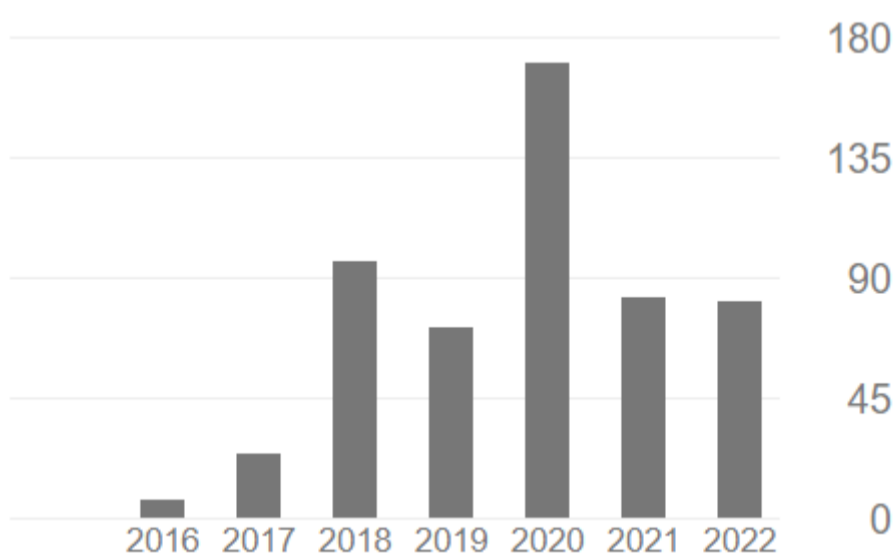
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h-index	14	14
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Effectiveness of selenium supplementation in children with autoimmune thyroiditis: A systematic review and meta-analysis

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Abstract---Deficiencies of Selenium (Se) is correlated with the risk and onset of autoimmune thyroid disease (AITD). The aim of this study

was to determine the association between Se supplementation and AITD. Methods: Electronic data searches of 4 databases were performed. We assessed the included studies using PRISMA for protocol assurance. Five studies met our inclusion criteria and were analyzed. The predictor covariate in the present study was Se administration. The outcome measures were levels of thyroid peroxidase antibodies (TPO-Ab), thyroglobulin antibody (Tg-Ab), free thyroxine (fT4), and thyroid volume. Results: Of the five studies that met the inclusion criteria, one randomized controlled trial (RCT) was included in the qualitative review, whereas four quasi-experimental studies were included in the meta-analysis. The results showed that Se supplementation significantly reduced TPO-Ab levels (MD 90.85; 95% CI 61.71–120.00; $p < 0.00001$) and fT4 levels (MD 1.52; 95% CI, 0.55–2.50; $p = 0.002$), while the RCT showed that Se supplementation significantly reduced Tg-Ab levels. Conclusions: Se supplementation significantly reduces TPO-Ab and fT4 levels in children and adolescents with AITD. The limited number of studies and population sizes emergence of further studies especially RCTS are needed to make a better meta-analysis.

Keywords---autoimmune thyroid disease, Selenium, Thyroid antibody.

Introduction

Autoimmune thyroid disease (AITD) is an autoimmune condition of the thyroid gland. The two primary types of AITD are Grave's Disease (GD) and Hashimoto's Thyroiditis (HT), which are characterized by T and B cell infiltration of the thyroid and the production of antibodies specific to thyroid antigens (Tomer et al., 2009). The peak onset of AITD occurs between 30 and 50 years of age, but in the early onset of AITD, symptoms manifest between puberty and 20 years of age (Shin et al., 2019). In spite of genetics, environment, and lifestyle as risk factors, deficiencies of micronutrients, including Selenium (Se) is correlated with the risk and onset of AITD (Giovinazzo et al., 2016).

The highest concentration of Se in the thyroid gland indicates the importance of Se for thyroid metabolism (Duntas et al., 2015). In addition to its regulatory role in thyroid hormone deiodination, Se preserves the integrity of thyrocytes via glutathione peroxidase (GPx)1 and GPx3 activities (Duntas et al., 2015). Moreover, Se triggers an oxidative burst in response to T-cell receptor stimulation (Huang Z et al., 2012), suppresses human leukocyte antigen-DR isotype molecule development (Balázs et al., 2012), increases the activity of T regulatory cells and suppresses cytokine secretion. Thus, it prevents follicular cell apoptosis and protects against AITD development (Duntas et al., 2015). Several studies have been conducted, particularly in areas with low Se levels, to determine whether Se supplementation affects the development of thyroid-related immunologic disorders (Ibrahim et al., 2019). However, the results were inconsistent (Schomburg et al., 2021). Systematic reviews have shown that Se supplementation

in adults improves thyroid function and stabilizes thyroid antibody levels at different durations of administration (Zhang et al, 2019).

The development of early-onset AITD in pediatric age is largely genetic, while nongenetic factors such as Se deficiency account for up to 20% (Brix et al., 2001), making it a stronger genetic predisposing risk factor than late-onset AITD in adults (Shin et al, 2019). However, there is an increased prescribing of Se for AITD patients (Negro et al., 2016) and the pediatric population (Filipowicz et al., 2021). While many published reviews highlight the importance of Se supplementation in adults, we found no specific review of Se supplementation in children and adolescents. Therefore, we conducted a systematic review and meta-analysis to provide a strong basis for the clinical application of Se supplementation in children and adolescents with early-onset AITD.

Method

This study was conducted in accordance with the Preferred Reporting Items for Systematic Examination and Meta-Analysis (PRISMA) guidelines. The study protocol was registered in the PROSPERO database (CRD42022309983). <https://www.crd.york.ac.uk/PROSPERO/#myprospero>

Search Strategy and selection of studies

The PubMed, ScienceDirect, Directory of Open Access Journals, and Wiley databases were searched electronically for studies published until February 2022 that assessed the effectiveness of Se supplementation in children and adolescents with autoimmune thyroiditis. The search was conducted using keywords constructed on Medical Subject Heading (MeSH) and other additional keywords: ["Selenium" or "Selenite"] and ["Thyroiditis" or "Autoimmune thyroiditis (AIT)" or "Graves' Disease" or "Hashimoto Thyroiditis" "Hypothyroidism" or "Hyperthyroidism"] and ["Children" or "Pediatric"]. Studies with larger sample sizes were included in the review if duplications were found. We imported all search results into the Mendeley reference manager to remove duplicates and screen. Three researchers (YH, BS, and QA) examined the titles and abstracts of the papers to identify eligible studies and then screened the full texts of all articles separately. Disagreements between the three authors were discussed until a consensus was reached. The PRISMA flow diagram shows the excluded studies and the reasons for their exclusion (Figure 1).

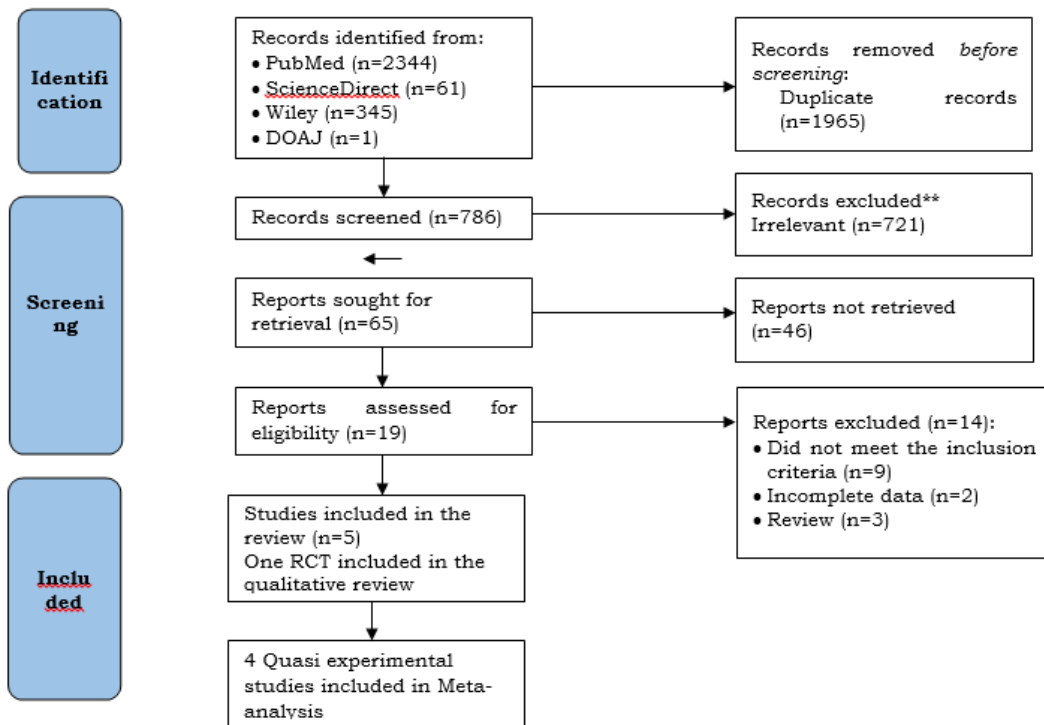


Figure 1. Flow Diagram of the literature search (PRISMA flow-chart) (DOAJ, Directory of Open Access Journals; RCT, randomized controlled trial)

Eligibility and inclusion criteria

The study design included in this review was a clinical trial. The authors independently assessed the titles and abstracts of papers to find relevant research according to the following criteria: (1) Studies assessing the effectiveness of Se supplementation in children with autoimmune thyroiditis (including HT, GD, or both); (2) The study population was children and adolescent (age <19 years) with AITD (HT was diagnosed based on the presence of Thyroglobulin antibodies (Tg-Ab) and/or Thyroid peroxidase (TPO)-Ab seropositivity, as well as at least one of the following: abnormal thyroid function, enlarged thyroid gland, or thyroid morphological alterations; GD was diagnosed based on finding seropositivity of Thyroid stimulating hormone receptor (TR)-Ab, TPO-Ab, Tg-Ab accompanied by abnormal thyroid function, diffuse goiter, morphological changes, and signs and symptoms related to GD); (3) The full text of the article was accessible; (4) Considering the limited number of similar studies in the pediatric and adolescent population, we included all randomized controlled trials and also quasi-controlled studies which is assigned subjects to groups based on non-random criteria.

Exclusion criteria

The exclusion criteria were as follows: (1) unrelated titles and abstracts; (2) case reports, case series, reviews, and commentaries; (3) incomplete or ungeneralized data; (4) duplicate studies; and (5) unavailable full text.

Data extraction

To provide high-validity data and avoid human errors, three authors (YH, NR, and QA) independently performed data extraction. Any discrepancies, including the lack of concordance in study selection, were resolved by discussion with other investigators (AE, BS, MF, and CD) until a consensus was reached. The following information was extracted from each paper: (1) first author name, (2) year of publication, (3) sample sizes of the case and control groups, (4) age of participants, (5) country, (6) main findings, (7) TPO-Ab levels in the case and control groups, (8) Tg-Ab levels in the case and control groups, (9) free thyroxine (fT4) levels in the case and control groups, and (10) thyroid volume in the case and control groups.

Quality assessment

Three independent reviewers (YH, NR, and QA) assessed the quality of each study based on the checklist of the Joanna Briggs Institute, and if a discrepancy was found, senior researchers (AE, BS, MF, and CD) were consulted. The RCT and quasi-experimental study checklists featured 13 and 9 items, respectively.

Outcome measures

The predicting covariate in this study was Se administration. The outcome measures were TPO-Ab, Tg-Ab, fT4 levels, and thyroid volume. They were determined after an initial search for covariate screening for inclusion in the meta-analysis.

Statistical analysis

The pooled mean difference (MD) and 95% confidence interval of TPO-Ab, Tg-Ab, and fT4 levels and thyroid volume among children with AITD between the intervention and control groups were assessed using a forest plot. The data were checked for heterogeneity and potential publication bias before identifying significant factors. The chi-square and I² tests were used to measure study heterogeneity.; if p heterogeneity >0.05, and I² > 50%, a random-effects model was used. The Review Manager version 5.3 was used to analyze the data (Cochrane Collaboration, London, UK). Two authors (YH and QA) conducted statistical analyses independently to avoid methodological errors.

Results**Characteristics of included studies**

Our search found 2,751 potentially relevant papers. Among them, 1,965 and 721 papers were excluded because of duplicate records and irrelevant titles, respectively. The abstracts of 65 papers were analyzed, and the full texts of 46 articles were not retrieved because of irrelevant abstracts. Full texts of the remaining 19 papers were assessed for inclusion. Of these, we excluded papers that did not meet the inclusion criteria (n=9), had incomplete data (n=2), or were reviews (n=3). Finally, five studies were included in this review; one RCT was included in the qualitative review, and four studies were included in the meta-analysis. Figure 1 illustrates the paper selection process utilized in our study, while Table 1 highlights the baseline characteristics of the included paper.

Table 1. Characteristics of included studies

Study	Design	Participants' Age, years (mean \pm SD)	Country	Sample Size	Selenium level before supplementation	Case Setting
Bonfig et al 2010 ¹⁹	Quasi experimental	12.2 \pm 2.2	Germany	Control Group (levothyroxine alone): 18 Intervention Group I (levothyroxine + Selenium 100 μ g): 13 Intervention Group II (levothyroxine + Selenium 200 μ g): 18	Not available	Autoimmune Thyroiditis (newly diagnosed autoimmune Thyroiditis and hypothyroidism, with or without a goiter)
Onal et al 2012 ²⁹	Quasi experimental	12.2 \pm 2.1	Turkey	Control Group (Placebo): 23 Intervention Group (50 μ g L-selenomethionine): 30	88.5 \pm 17.5 μ g/L	Newly diagnosed with Hashimoto Thyroiditis
Gabulov et al 2019 ³²	Quasi experimental	11.0 \pm 0.83	Azerbaijan	Control Group (L-Thyroxin): 14 Intervention Group (L-Thyroxin + 100 μ g L-selenomethionine): 17	Group I: 68.7 \pm 2.34 μ g/L Group II: 69.8 \pm 1.9 μ g/L	Autoimmune Thyroiditis in a state of drug-induced euthyroidism
Zhang et al 2022 ¹⁸	Quasi experimental	7.67 \pm 1.22	China	Control Group (15–20 mg methimazole): 50 Intervention Group (15–20 mg methimazole + 50 μ g selenium (2 times/d): 53	Not available	Grave's disease
Kyrgios et al 2018 ²¹	RCT	11.3 \pm 0.3	Greece	Control Group (Placebo):33 Intervention Group (200 μ g L-selenomethionine): 39	Not available	Autoimmune Thyroiditis (euthyroidism or treated hypothyroidism and goiter in thyroid gland ultrasonography)

RCT, randomized controlled trial

The outcome of Se supplementation

Thyroid peroxidase antibodies

In the data synthesis, we included four studies that assessed the association between Se administration and TPO-Ab levels. Pooled analysis revealed that patients receiving Se supplementation had lower TPO-Ab levels than controls (MD, 90.85; 95% CI, 61.71–120.00; $p < 0.00001$) (Figure 2).

Thyroglobulin antibodies

Four studies assessing the correlation between Se administration and Tg-Ab levels were included in the data synthesis. Pooled analysis revealed that Tg-Ab levels were not significantly different between the Se administration group and controls (MD, 9.41; 95% CI, -38.74–57.56; $p = 0.7$) (Figure 2).

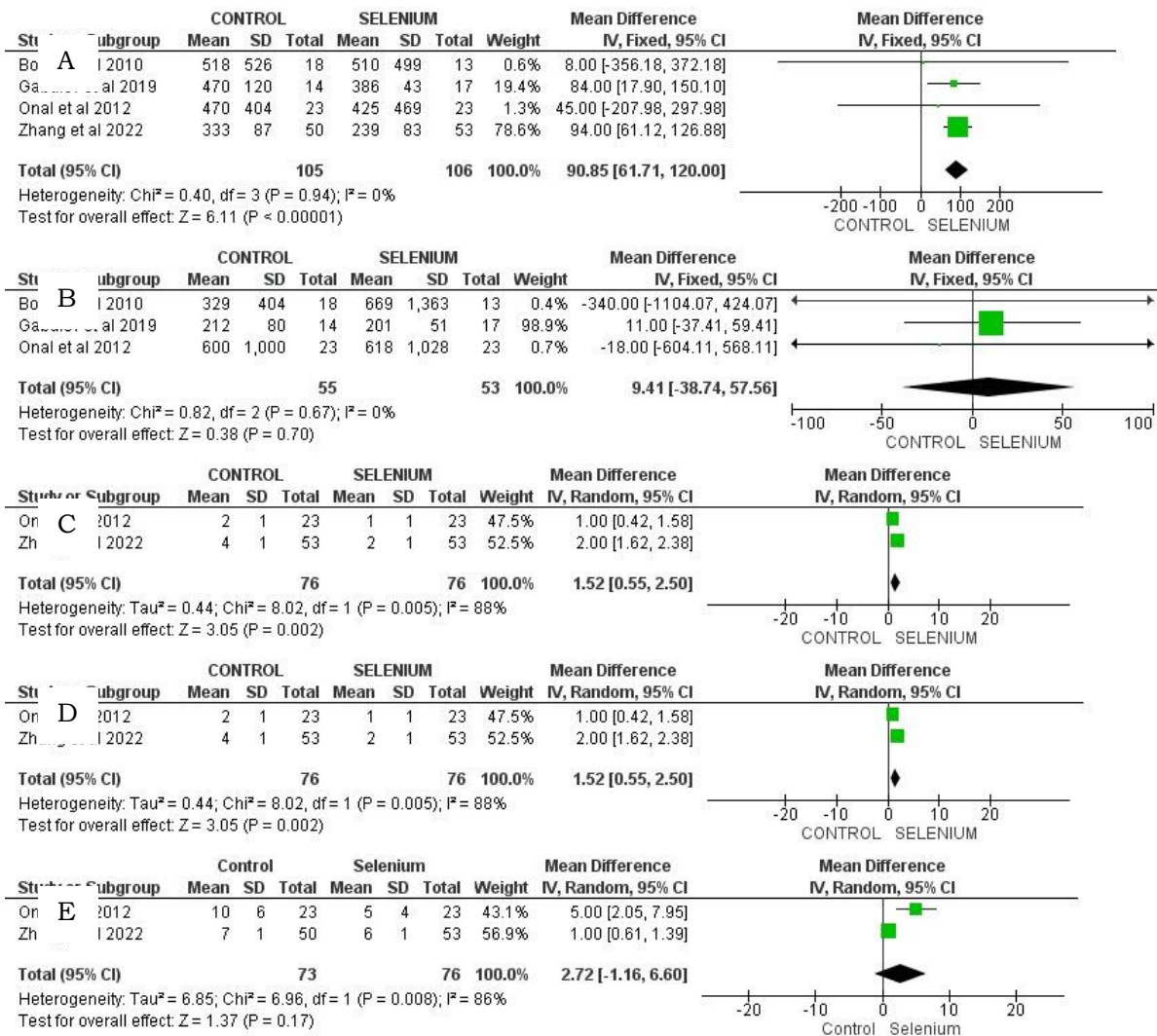


Figure 2. Forest Plot of the association between Se supplementation and outcome parameters among children with autoimmune thyroiditis. A. TPO-Ab, B. Tg-Ab, C. fT4, D. Thyroid Volume. TPO-Ab, thyroid peroxidase antibodies; Tg-Ab, thyroglobulin antibody; fT4, free thyroxine

Free thyroxin

Two quasi-experimental studies assessed the association between Se administration and fT4 levels. Pooled analysis revealed that patients receiving Se supplementation had higher fT4 levels than controls (MD, 1.52; 95% CI, 0.55–2.50; $p=0.002$) (Table 2).

Thyroid volume

Two quasi-experimental studies assessed the association between Se administration and thyroid volume. Pooled analysis revealed that thyroid volume was not significantly different between the Se administration group and controls (MD, 2.72; 95% CI, -1.16–6.6; $p=0.17$) (Table 2).

Table 2. Summary of the Association Between Selenium Administration and Outcome Parameters among Children with Autoimmune Thyroid Disease

Parameters	Outcome measure		Mean Difference	95% CI	I2	p
	Control	Selenium Supplementation				
TPO-Ab (U/m)	447.5 ± 284.3	390 ± 273.5	90.85	61.71, 120.00	0%	<0.0001*
Tg-Ab (U/m)	380.3 ± 494.7	496. ± 814	9.41	- 38.74, 57.56	0%	0.7
fT4 (ng/dL)	3 ± 1	1.5 ± 1	1.52	0.55- 2.50	88%	0.002*
Thyroid Volume (cm ³)	9 ± 3.5	5.5 ± 2.5	2.72	-1.16- 6.60	86%	0.17

* $p < 0.05$ indicates statistical significance. Note — data are presented using mean ± SD; CI, confidence interval; TPO-Ab, thyroid peroxidase antibodies; Tg-Ab, thyroglobulin antibody; fT4, free thyroxine

A Randomized Controlled Trial

A single-center, double-blind, placebo-controlled RCT with 71 children with AITD (14 boys, 57 girls) was included in this study. Participants were randomized into two groups to receive 200 µg daily of organic Se (as L-selenomethionine) (intervention group) or placebo (control group) blindly daily for 6 months. Se supplementation significantly reduced Tg-Ab levels ($p=0.021$). Although TPO-Ab levels decreased in the intervention group, the difference between the groups was not statistically significant ($p=0.219$). There was no significant difference in the thyroid volume between the groups ($p=0.485$).

Source of heterogeneity and publication bias

Heterogeneity among studies

TPO-Ab and Tg-Ab were assessed using a fixed-effects model ($I^2 \leq 50\%$), while fT4 and thyroid volume were assessed using a random-effects model ($I^2 > 50\%$). Evidence of heterogeneity among the studies in our meta-analysis is presented in Table 3.

Table 3. Characteristics of Selenium Supplementation in the included studies

Study	Selenium Supplementation			
	Dose	Duration	Type of selenium	Output
Bonfig et al. 2010 ¹⁹	Control Group: L-Thyroxin Intervention Group I: L-Thyroxin + 100 µg sodium-selenite Intervention Group II: L-Thyroxin + 200 µg sodium-selenite	12 Months	Sodium-selenite (non-organic Se)	Tg-Ab levels significantly decreased after 12 months of treatment in control group and intervention group II ($p=0.03$ and $p=0.01$, respectively). The decrease in TPO-Ab concentration was the greatest in intervention group II (reduction of 40.5%) after 12 months of treatment; however, the difference was not significantly significant.
Onal et al 2012 ²⁹	Control Group (Placebo) Intervention Group (50 µg L-selenomethionine)	3 months	L-selenomethionine (Organic Se)	Significant thyroid volume regression. Thyroid echogenicity, TPO-Ab, and Tg-Ab levels were unchanged after Se treatment
Gabulov et al 2019 ³²	Control Group (L-Thyroxin): Intervention Group (L-Thyroxin + 100 µg L-selenomethionine)	6 months	L-selenomethionine (Organic Se)	TPO-Ab levels significantly decreased after treatment in the intervention group ($p=0.044$). Tg-Ab levels decreased after treatment but not significantly in the intervention group ($p=0.75$)
Zhang et al 2022 ¹⁸	Control Group (15–20 mg methimazole): 50 Intervention Group (15–20 mg methimazole + 50 µg selenium (2 times/d): 53	4-5 weeks	Selenium (not explained in detail)	TPO-Ab and fT4 levels and Thyroid volume significantly decreased after treatment in the intervention group ($p<0.05$)
Kyrgios et al 2018 (RCT) ²¹	Control Group (Placebo): 33 Intervention Group (200 µg L-selenomethionine): 39	6 months	L-selenomethionine (Organic Se)	Tg-Ab levels significantly reduced after treatment in the intervention group, but TPO-Ab levels did not decrease significantly. There was no change in thyroid volume in both groups

TPO-Ab, thyroid peroxidase antibodies; Tg-Ab, thyroglobulin antibody; fT4, free thyroxine; RCT, randomized controlled trial

Subgroup analysis showed that Se supplementation at a dose of 100 µg significantly reduced TPO-Ab levels (Figure 3, Table 4), while Se administration for ≥ 6 months significantly reduced TPO-Ab levels but not Tg-Ab levels (Figure 4, Table 5).

Figure 3. Subgroup Analyses according to doses of Selenium Supplementation

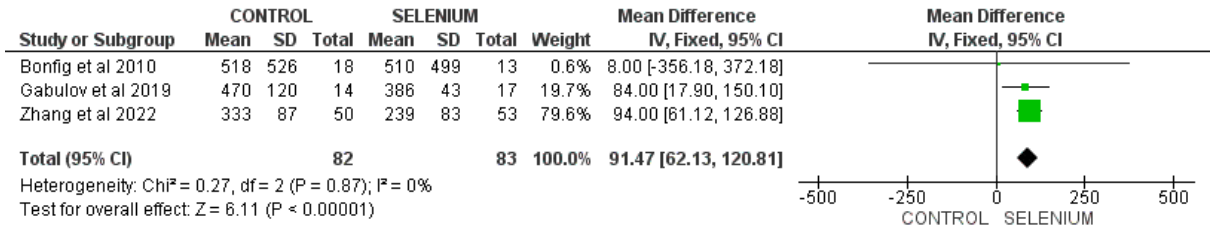


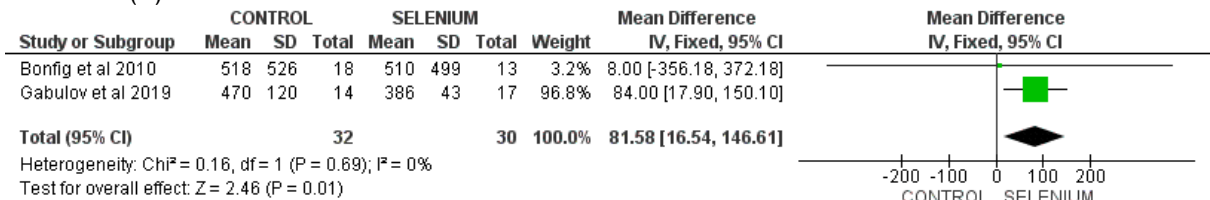
Table 4. Subgroup Analyses according to doses of Selenium Supplementation

Selenium Dose	Number of Studies	Parameters	Outcome Measures		Mean Difference	95% CI	I ²	p
			Control	Selenium Supplementation				
100 µg	3	TPO-Ab	440±244	378 ± 208	91.47	62.13, 120.81	0%	<0.0001

CI, confidence interval; TPO-Ab, thyroid peroxidase antibodies

Figure 4. Forest plot sub group analysis duration treatment for ≥6 months

(A) TPOAb



(B) TGAAb

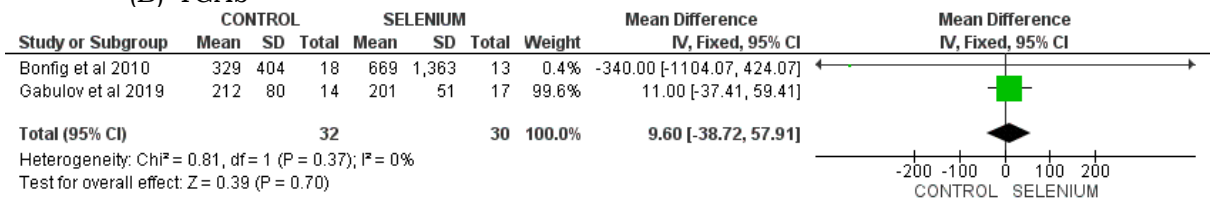


Table 5. Subgroup Analyses according to Duration of Supplementation (≥ 6 months)

Duration of Selenium supplementation	Number of Studies	Parameters	Outcome Measures		Mean Difference	95% CI	I ²	p
			Control	Selenium Supplementation				
≥ 6 months	2	TPO-Ab	494 \pm 323	448 \pm 271	81.58	16.54, 146.6	0%	0.01*
		Tg-Ab	270.5 \pm 242	435 \pm 707	9.6	-38.72, 57.91	0%	0.70

* p <0.05 indicates statistical significance

CI, confidence interval; TPO-Ab, thyroid peroxidase antibodies; Tg-Ab, thyroglobulin antibody

Potential publication bias

According to the Cochrane Handbook of Systematic Review, the funnel-plot asymmetry test should be used only when there are at least 10 studies in the meta-analysis due to the insufficient power of the test to distinguish the probability of true asymmetry. Therefore, we did not perform funnel-plot analysis to estimate publication bias, as the number of included studies was <10 (Higgins et al., 2019).

Sensitivity Analysis

In this meta-analysis, no single study affected the statistical significance of the results after deleting a particular study.

Discussion

In this review, we included five studies with a total of 307 children and adolescents with AITD. The meta-analysis was performed on four quasi-experimental studies, and we included one RCT to compare the data. Pooled analysis revealed that Se supplementation significantly reduced TPO-Ab levels. However, in RCT study, TPO-Ab levels decreased but not in significant value. Several meta-analyses on Se supplementation in adults with AITD have reported a significant decrease in TPO-Ab levels (Zhang et al., 2019 and Fang et al., 2016). Analysis of our two subgroups showed a significant reduction in TPO-Ab levels following the administration of 100 μ g Se for ≥ 6 months. A review of adults reported a significantly steeper decline in TPO-Ab levels at a dose of 200 μ g Se over 6 months (Duntas et al., 2003). A contrasting result was reported by Anastakilasis et al (2012). Selenium deficiency disrupts the balance of T cells, prompts B lymphocytes to synthesize more anti-thyroid antibodies, induces thyroid peroxidase activity and, as a result, thyroid tissue destruction (Zhang et al., 2022). Se supplementation can effectively improve these pathological and physiological alterations by increasing antioxidant capacity, decreasing thyrocyte damage, and inhibiting TPO-Ab and Tg-Ab expression (Zhang et al., 2022).

Our pooled data showed no significant effect of Se supplementation on the Tg-Ab levels. The RCT included in this study reported a significant reduction in Tg-Ab levels with 200 µg L-selenomethionine for 6 months, which is similar to the findings in an adult study (Anastasilakis, 2012). However, several meta-analyses on adults have shown that Se supplementation has no effect on Tg-Ab levels (Zhang et al., 2019). Thyroglobulin is a physiological circulating antigen. As a result, Tg-Ab concentrations are typically less specific for AITD diagnosis and prognosis (Bonfig et al., 2010).

The fT4 level is commonly used as an indicator of thyroid function. Se supplementation improved fT4 levels in the pooled analyses in this study. Since fT4 was not the primary outcome in the RCT included in this review, there was no data for comparison. A previous study in adults showed that Se supplementation for 3–6 months had a significant effect on serum fT4 levels, indicating higher deiodinase activity induced by L-selenomethionine (Esposito et al., 2017). In this study, thyroid volume decreased after Se supplementation, but not significantly, similar to that in the RCT. 21 In the study of adults with AITD, 12 months of Se supplementation resulted in a moderate decrease in thyroid volume (Balázs et al., 2012). However, the two trials reported no difference (de Farias et al., 2015). In autoimmune diseases, thyroid gland enlargement is caused by the infiltration of lymphocytes, plasma cells, and macrophages (Balázs et al., 2008). As a result, immune-system Se-dependent enzymes can assist avoid tissue damage and induce thyroid volume reductions (Chistiakov et al., 2005).

There was some variation in the patient's conditions and types of interventions between the studies included in this meta-analysis, which may have led to different results. The dosage of Se used in the included studies varied from 50 to 200 µg/day with a duration of 4-5 weeks to 12 months. However, in the RCT by Kyrgios et al. (2021) participants received 200 µg L-selenomethionine daily for 6 months. Turker et al. discovered that selenomethionine doses more than 100 g daily for 9 months were necessary to reduce TPO-Ab concentrations in adults (Turker, 2006), while our subgroup analyses showed a significant reduction of TPO-Ab with 100 µg of Se for ≥ 6 months. The type of Se administered affects the therapeutic outcomes. In our meta-analysis, three studies used organic Se (L-selenomethionine), and one study used inorganic Se (selenite). Although both organic and inorganic Se can equally increase GPx levels, the storage of organic Se in the body is longer than that of inorganic Se (Alfthan, 2000).

Several studies in adults demonstrated a significant role of Se supplementation in Se deficient subjects in areas that are geographically proven to be low Se status areas, such as Poland (Kryczyk-koziół, 2021) and Germany (Gärtner et al., 2002). Unfortunately, not all studies in this meta-analysis examined baseline Se levels before supplementation, although the author stated that Se deficiencies had been reported in their region (Bonfig et al., 2010 and Onal et al., 2012).

The safety window between toxicity and the required dose of Se is narrow, and the inorganic forms of Se are more toxic than the organic (Gorini et al., 2021). In this systematic review, no side effects were reported at any dose. Although Kyrgios et al (2019) (200 µg l-selenomethionine daily for 6 months) reported a rash during the treatment in one patient, further investigation revealed no correlation. The

recommended Se dosage and duration in children remain unclear. However, the tolerable upper intake level of Se for healthy children aged 1–18 years ranges between 90 and 400 µg/day (Institute of Medicine (US) Panel on Dietary Antioxidants and Related Compounds, 2000). The strength of this study is that it is the first meta-analysis to evaluate the effectiveness of Se supplementation in AITD patients with pediatric and adolescent subjects who have a different predisposition to AITD than adult subjects.

Nevertheless, this study had several limitations: first, the power of analysis may be limited due to the limited number of studies and population sizes.; second, there were few RCTs on Se supplementation in children with AITD; third, the heterogeneity in the type, dose, and duration of Se supplementation, and variable data on Se levels prior to supplementation led to differences in results between the meta-analysis and RCT.

Conclusion

Our meta-analysis showed that Se supplementation at a dose of 100 µg/day for ≥6 months significantly reduced the TPO-Ab and improved ft4 levels in children and adolescents with AITD. However, these results differ from those reported in RCTs conducted in children. Thus, the emergence of further studies especially RCTS with more complete data are needed to make a better meta-analysis with bigger data to confirm the effects of Se supplementation in children with AITD.

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