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Kurdistan Institution for Strategic Studies and Scientific Research(KISSR)

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In This Issue:

Welcome to JKSNB

The Kurdistan Institution for Strategic Studies and Scientific Research (KISSR) of the Ministry of Higher Education and Scientific Research in Kurdistan, Iraq, published its research jointly with the Kurdish Non-governmental Academic Foundation under the name of the Kurdish Academia Journal, which was issued into seven issues. However, the research participation between the two institutions was terminated for administrative and technical reasons. Thanks for obtaining the approval letter no. 6497 on 82020/9/ by the Ministry of Higher Education in the Kurdistan Region to license the JKSNB to be recognized as a refereed academic journal to be issued by the Kurdistan Institution for Strategic Studies and Scientific Research. This independent scientific journal is affiliated with (KISSR) under the title «Kurdistani Journal of Specific Natural Science and Biomedicine(JKSNB)», and will begin issuing this No. 8 in December 2023.

JKSNB is a multidisciplinary journal publishing papers of high quality in the study of natural sciences, biology, biological processes and application in medicine. Examples of topic areas are Geology, environment, physics, agriculture, molecular medicine, cancer biology, immunobiology, pharmacogenomics, chemical biology, pharmacology, physiology, and basic or clinical research in various diseases.

JKSNB is a quarterly journal published in the English language. It publishes original, important, effective and influential research and strategic studies across various fields of study. We promote academic communication strive to achieve excellence, and stick to deadlines. The JKSNB keeps pace with current developments and keeps up with the aspirations of researchers and scholars inside and outside of the Kurdistan Region, in terms of scientific analyses and interpretations.

The journal aims to promote strategic scientific research in Kurdistan, creating vast areas of scientific analysis for academics and researchers in Biomedical and Natural Sciences, and supporting the public and private sectors to conduct scientific research.

We at KISSR have achieved great success in the Kurdistan Journal of Strategic Research (JKSS), we started in May 2021 and published five issues that year, and in 2022, seven issues. In 2023, 12 issues, to meet the needs of researchers, master›s and doctoral students who can publish their research in a short period to complete the degree enhancement and complete their thesis discussions. This is the result of the hard and continuous work of our employees, a great credit to researchers and participants in their scientific participation.

Therefore, we hope that we can make the same progress and participate in this scientific journal in cooperation with both editorial and advisory boards and reviewers to be in the trust of authors and researchers.

In addition, we are fully prepared for actual and serious participation in researchers works through profes-sors, scientific and experimental staff and our very modern laboratories, and with the talented elite of profes-sors and specialists in the fields of the JKSNB in the editorial and consulting boards, thank you to provide the best of serious and creative research.

Thank you all for your interest in scientific research, especially for your great scientific contribution in supporting this journal from now on.

Polla Khanaqa
Editor-in-Chief- JKSNB
President of The Kurdistan Institution for Strategic Studies and Scientific Research (KISSR)
Dec. 2023

Attitudes toward Nurses-Physicians' Collaboration among Undergraduate Nursing Students at University of Sulaimani

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Abstract

Background: Effective collaboration and teamwork between doctors and nurses play a vital role in patient care and maintaining a positive atmosphere. Each team member brings their unique viewpoint when it comes to evaluating and devising a care strategy for a patient. It is only by working together and sharing information that suitable treatment plans can be formulated. The main objective of this study was to assess attitudes of undergraduate nursing students towards nurse-physician collaboration at the College of Nursing, University of Sulaimani. A descriptive and cross-sectional study was conducted among 178 undergraduate nursing stratified random sampling to select the sample. Data were collected using self-administered questionnaires. Attitudes of students were measured using Jefferson scale of attitudes towards nurse-physician Collaboration. Results summarized using descriptive and inferential statistics using mean, standard deviation and t-test. $p < 0.05$ considered as significant. Nursing students exhibited favorable attitudes than with mean score of 51.9±3.53 and 47.49 and standard error of mean 0.474 and 0.931 respectively with $p = 0.043$. Students scored high on all subscales. However, statistically no significant differences were noted between students' gender with doctors' authority and caring versus curing, also between students' interest in nursing career and caring versus curing. This study identified that nursing students demonstrated favorable attitudes regarding internship between nurses and physicians.

Keywords: Attitudes, Collaboration, Nurse, Physician.

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Introduction

Teamwork plays a crucial role in ensuring safe and high-quality care (Mahdizadeh et al., 2015). Given the complexity of caring for critically ill patients, fostering a teamwork attitude becomes essential (Georgiou et al., 2017). Collaboration, as defined by Jasemi et al. (2013), involves the exchange of perspectives between two or more individuals on a common theme. Elsous et al. (2017) describe collaboration between nurses and physicians as a cooperative effort where responsibilities are shared to address challenges and make decisions for patient care plans. The objective of interprofessional collaboration is to create an environment of mutual trust and respect, providing equal opportunities for team members to share their knowledge and expertise (Hamlan et al., 2015). Nurse-physician collaboration necessitates a shared goal and a reciprocal obligation to deliver high-quality care to address patient needs (Sharifiyana et al., 2016). Communication between physicians and nurses is a complex process and is considered a crucial aspect of delivering high-quality patient care (Al-Jabri, et al, 2023). Their relationship goes beyond a mere exchange of information; it involves a professional and collaborative decision-making process aimed at achieving the best possible patient care (Alibakhshikenari, 2018). Effective communication is instrumental in reaching collaborative decisions that lead to positive patient outcomes (Elsous et al., 2017). Evidence suggests that nurse-physician collaboration significantly contributes to improved disease outcomes, including reduced mortality rates, readmissions, complications, as well as prevention of ventilator-associated pneumonia and bedsores (Wheelan et al., 2003). Conversely, a lack of a proper professional relationship between physicians and nurses can lead to burnout and stress among nurses (Jasemi et al., 2013). In genuine interprofessional collaboration, both parties must possess the ability to make independent decisions and have the authority to implement those decisions. Additionally, both parties should actively participate in the decision-making process based on their respective expertise to achieve optimal healthcare outcomes for patients (Mahmoodian et al., 2014). The specialized responsibilities of nurses and physicians necessitate autonomy (Valizadeh et al., 2017). Professional autonomy in nursing refers to nurses' right and responsibility to make decisions about patient care and have the freedom to act accordingly. According to Mohamed (2018), an important aspect of professional autonomy is nurses' ability to make decisions, which forms the foundation of their professional knowledge. Research conducted on ICU nurses in Greece found that while they had moderate levels of autonomy in technical tasks, their autonomy in the decision-making process was low (Papathanassoglou et al., 2005). Similarly, Dorgham and Al-Mahmoud's study revealed a low level of professional autonomy among Egyptian nurses. This lack of autonomy is a significant reason why nurses express a desire to move to nonclinical roles (Karra et al., 2014). Additionally, nurses' ability to independently perform nursing services plays a vital role in their job satisfaction (Motamed-Jahromi et al., 2015).

Although there are differences in attitudes, most nursing students emphasize the value of working as a team in the healthcare settings. They recognize the value of open communication, respect for one another, and teamwork in patient care (Tschannen et al., 2018). However, some students can worry about hierarchical dynamics because they think that doctors might ignore their suggestions. In order to prepare students for successful nurse-physician interaction, contemporary nursing education places a strong emphasis on interprofessional collaboration (Nielsen et al, 2013).

Aim of the study: To examine the attitudes of undergraduate nursing students toward nurse–physician collaboration and to find out the association between the attitudes subscales with students' gender, academic stage and interest in nursing career of the students.

Justification of the study: Nurses and physicians, who bear significant responsibility for patient care, face challenges in effective communication. Studies have highlighted differences in the attitudes of nurses and physicians toward

collaboration. The current healthcare system suggests that nurses are not fully exercising their autonomy when working with physicians, while physicians tend to hold a dominant role in various aspects of patient care. This situation diminishes the contribution of nurses to the healthcare delivery system. Additionally, literature has identified a gap in educating associate degree nursing students on effective communication with physicians. Notably, there is a lack of published research specifically addressing nurse-physician collaboration in Kurdistan, particularly in Sulaimani city.

Materials and Methods:

Design of the study: Quantitative design with descriptive study was carried out at the College of Nursing / University of Sulaimani from the period of 11/1/2022 to 25/4/2022 to find out the attitudes of nursing students toward nurses-physicians collaboration.

Administrative Arrangements and ethical consideration: the scientific committee of the College of Nursing / University of sulaimani approved the study. Consequently, an official letter submitted to the presidency of university of Sulaimani to obtain their permission for data collection. Accordingly, an official letter addressed to the College of Nursing at the university to gain cooperation with the nursing college students for data collection.

Setting of the study: College of Nursing / University of Sulaimani, which was established in 2001 and is located in the old camp in Sulaimani city center, was involved in the current study. Actually, 375 students are studying in the college in four stages (first, second, third, and fourth).

Sampling of the study: A stratified random sampling was used to select the sample size of the study according to inclusion criteria included students in the second, third, and fourth stages from college of nursing who has willing to participate in the study. First stage students excluded from the study that was because they have not exposed to the clinical areas in the health care settings and communication with healthcare providers particularly with the nurses and physicians. Therefore, second, third, and fourth stage students were included in the study. First, the number of the students from each stage obtained from the registration unit of the college. Consequently, random sampling technique was used to select half of the students in each stage separately as (68, 59, and 51) students were selected from (second, third, and fourth) stages respectively, so that the total number of the participants was (178) students.

Methods of data collection: Interview was held with each stage to get their verbal consent, in addition is to ensure them that the obtained information will be kept confidential and for scientific purpose only. Consequently, the data collected using self-administered questionnaire.

Tool of data collection (Study instrument): In order to collect the proper information and relevant to the present study, a questionnaire was constructed by the researcher which consisted of (2) parts. The first part was the socio-demographic attributes composed of eight items concerned with demographic characteristics of the students which are:(age, gender, economic status, academic stage, residency, father's occupation, mother's occupation, and interest in nursing career). The second set of questions was the "Jefferson Scale of Attitudes toward Physician–Nurse Collaboration" (JSAPNC). The JSAPNC comprises a 15-question survey designed to explore participants' viewpoints regarding collaboration between physicians and nurses. The survey focuses on four main aspects: 'shared education and teamwork' (7 questions), 'emphasizing care over cure' (3 questions), 'nurse autonomy' (3 questions), and 'physician authority' (2 questions). Respondents provide their opinions using a 4-point Likert scale, where

responses range from 'strongly disagree' (1) to 'strongly agree' (4). A higher overall score on the survey indicates a more favorable attitude towards collaborative relationships between physicians and nurses. Completing the JSAP-NC typically takes around five to ten minutes. The English version of the questionnaire translated into Kurdish language in order to be understandable to the students and facilitate the data collection.

Scoring: The responses measured on 4-point Likert scale. Items (8, 10) were reversely scored as: (4) = strongly disagree; (3) = disagree; (2) = agree; and (1) = strongly agree, while the rest (13) statements were scored as: (1) = strongly disagree; (2) = disagree; (3) = agree; (4) = strongly agree. It is worthwhile to mention that the higher mean of score of the statements, between minimum15 and maximum 60, indicates the more favorable pattern of attitude toward nurses-physicians' collaborations.

Data analysis (statistical analysis): The obtained responses were coded and inserted into an excel sheet, then analyzed with the statistical package of social sciences (SPSS) version 25, including descriptive statistics (frequency, percentage, mean of score, and standard deviation), and inferential statistics using t-test.

Results:

Table (1) Distribution of the study sample according to their

Socio-demographic characteristics by frequency and percentage.

Students' Characteristics		Frequency	Percentage
Age Groups	Years 20 <	32	18.0
	Years 22 – 20	139	78.1
	Years 23 ≤	7	3.9
	Mean ± SD	1.19 ± 20.42	
Gender	Male	50	28.1
	Female	128	71.9
Economic status	Sufficient	65	36.5
	Barely sufficient	113	63.5
Academic level	2nd stage	68	38.2
	3rd stage	59	33.1
	4th stage	51	28.7
Residency	Urban	61	34.3
	Rural	117	65.7
Father's employment status	Governmental employee	62	34.8
	Private sector employee	25	14.0
	Self-employee	63	35.4
	Jobless	28	15.7
Mother's employment status	Governmental employee	23	12.9
	Private sector employee	6	3.4
	Self-employee	2	1.1
	Housekeeper	147	82.6
Interest in Nursing career	Not interested	31	17.4
	Little interested	55	30.9
	Very interested	92	51.7
Total		178	100

The highest number of the participants (78.1%) were between 20-22 years old with mean age of 20.42 ± 1.19 of standard deviation. Female participants comprise (71.9%) of the sample, (63.5%) reported having a barely sufficient economic status, (38.2%, 33.1%, and 28.7%) were distributed on second, third, and fourth stage respectively, (65.7%) resided in rural areas, while, (35.4%) of the fathers were self-employed, (82.6%) of the mothers were housekeeper, more than half of the participants (51.7%) are very interested in the nursing profession, 30.9% were little interested, and 17.4% not interested.

Top of FormTable (2) The response of the participants to the JSANPC

(attitude towards collaborative care with physicians) items.

#	Attitude items	strongly agree .Fr %	agree .Fr %	disagree .Fr %	Strongly disagree .Fr %	Mean SD ±
1	A nurse should be viewed as a collaborator and colleague with a physician rather than his/her assistant.	104 58.4	70 39.3	4 2.2	0 0	3.56 0.541 ±
2	Nurses are qualified to assess and respond to psychological aspects of patient's needs.	107 60.1	66 37.1	5 2.8	0 0	3.57 0.550 ±
3	During their education, medical and nursing students should be involved in teamwork in order to understand their respective roles.	140 78.7	36 20.1	1 0.6	1 0.6	3.76 0.472 ±
4	Nurses should be involved in making policy decisions affecting their working conditions.	61 34.3	103 57.9	11 6.2	3 1.7	3.24 0.642 ±
5	Nurses should be accountable to patients for the nursing care they provide.	124 69.7	51 28.7	3 1.7	0 0	3.67 0.502 ±
6	There are many overlapping areas of responsibility between physicians and nurses.	107 60.1	56 31.5	14 7.9	1 0.6	3.51 0.665 ±
7	Nurses have special expertise in patient education and psychological counseling.	103 57.9	65 36.5	9 5.1	1 0.6	3.51 0.621 ±
8	Doctors should be the dominant authority in all health care matters.	6 3.4	34 19.1	54 30.3	84 47.2	3.21 0.869 ±
9	Physicians and nurses should contribute to decisions regarding the hospital discharge of patients.	76 42.7	74 41.6	24 13.5	4 2.2	3.24 0.770 ±
10	The primary function of the nurse is to carry out the physician's orders.	7 3.9	13 7.3	53 29.8	105 59.0	3.43 0.794 ±
11	Nurses should be involved in making policy decisions concerning the hospital support services upon which their work depends.	126 70.8	44 24.7	7 3.9	1 0.6	3.65 0.582 ±

12	Nurses should also have responsibility for monitoring .the effects of medical treatment	77	77	21	3	3.28
		43.3	43.3	11.8	1.7	0.736 ±
13	Nurses should clarify a physician's order when they feel that it might have the potential for detrimental effects .on the patient	99	68	7	4	3.47
		55.6	38.2	3.9	2.2	0.682 ±
14	Physicians should be educated to establish collaborative relationship with nurse	99	68	40	1	3.4
		55.8	38.2	5.6	0.6	0.631 ±
15	Interprofessional relationships between physicians and nurses should be included in their educational pro- .grams	81	77	17	3	3.32
		45.5	43.3	9.6	1.7	0.717 ±
SD ± Total mean of score						۵۱.۹±۳,۵۳

Table 2 reveals that a significant majority of participants strongly agreed or agreed to all the attitude statements and strongly disagreed or disagreed to the statements (8 and 10) which scored reversely, and all the JSANPC items got high mean of scores falling between 3.21±0.869 and 3.76±0.472 with the total of 51.9±3.53 which indicates favorable pattern of attitudes toward collaboration between nurses and physicians.

Table (3) JSANPC subscales' mean with standard deviation.

JSANPC subscales	Mean	Std. Deviation
(Shared education and collaboration (7items	24.15	2.60 ±
(Doctors' authority (2 items	6.64	1.11 ±
(Nurses' autonomy (3 items	10.38	1.15 ±
(Caring vs. curing (3 items	10.73	1.22 ±
(Total score (15- 60	51.9	4.44 ±

The participants' responses on JSANPC subscales got high mean of scores. Shared education and collaboration got mean (24.15±2.6), doctor's authority (6.64±1.11), nurses' autonomy (10.38±1.15), and caring versus curing (10.73±1.22) that show favorable attitudes of the nursing students toward collaboration between nurses and physicians in all subscales.

Table (3) Comparison of Jefferson Scale of Attitudes toward Physician-Nurse Collaboration and its Subscales Results with gender of Nursing Students.

Variables	Male N=50 Mean ± SD	Female N=128 Mean ± SD	p. value t-test
Shared education and collaboration	23.4±3.51	24.4±2.32	H. Sig 0.001>
Doctors' authority	6.60±1.56	6.67±1.49	N. Sig 0.159
Nurses' autonomy	10.1±0.97	10.4±1.21	Sig 0.008
Caring vs. curing	10.7±1.22	10.8±1.24	N. Sig 0.464

The table presents a comparison between genders regarding nurses'-physicians' collaboration on different variables. Concerning Shared education and collaboration, the p-value is less than 0.001, indicating a highly significant difference between genders, and regarding nurses' autonomy, the p-value is 0.008 indicating a statistically significant association between both genders. However, for doctors' authority and caring versus curing, the p-values are more than 0.05, which indicate no significant differences between male and female students.

Table (5) Comparison of Jefferson Scale of Attitudes toward Physician-Nurse Collaboration and its Subscales Results with academic stages of Nursing Students.

Variables	2nd stage N=68 Mean ± SD	3rd stage N=59 Mean ± SD	4th stage N= 51 Mean ± SD	p. value t-test
Shared education and collaboration	24.2±2.10	24.0±2.33	23.7±3.40	H. Sig 0.001>
Doctors' authority	7.1±•,٩٧	6.67±1.12	6.58±1.21	H. Sig 0.001>
Nurses' autonomy	10.3±1.29	10.5±1.05	10.3±1.07	Sig 0.010>
Caring vs. curing	10.7±1.26	10.6±1.16	10.5±1.25	H. Sig 0.001>

The table 5 presents that there is highly significant association between academic stages and JSANPC subscales of shared education and collaboration, doctors' authority and caring versus curing (P<0.001), and significant association between students' academic stages with nurses' autonomy (P<0.010).

Table 6: Comparison of Jefferson Scale of Attitudes toward Physician-Nurse Collaboration and its Subscales Results with are interested in nursing career of Nursing Students.

Variables	Not interested N=31 Mean ± SD	Little interested N=55 Mean ± SD	Very interested N= 92 Mean ± SD	p. value t-test
Shared education and collaboration	3.34 24.1±	23.8±2.95	24.4±2.36	H. Sig 0.000

Doctors' authority	**6.61±1.55**	**6.78±1.27**	**6.72±1.47**	**H. Sig 0.000**
Nurses' autonomy	**10.3±1.15**	**10.3±1.26**	**10.4±1.15**	**H. Sig 0.000**
Caring vs. curing	**10.7±1.22**	**10.7±1.23**	**10.8±1.20**	**N. Sig 0.359**

Table 6 reveals that there is high significant association between shared education, doctors' authority, and nurses' autonomy with the students interests in nursing career (P<0.001), but no significant differences found between interest in nursing career and caring versus curing (P>0.05).

Discussion

The interaction and cooperation between medical personnel, specifically staff nurses and physicians, have been subject to variations over time. Numerous studies have unveiled a diminished degree of teamwork between staff nurses and physicians (Elham & El-Hanafy, 2018). Conversely, some research has indicated that staff nurses tend to exhibit a higher level of collaboration (Melkamu et al., 2020). The objective of the present study is to assess the viewpoints of the nursing students regarding the partnership between nurses and physicians.

The results shows that about three quarters of the students are female, fall within the age of 20-22 years, highest portion of them have barely sufficient economic status, highest percentage are in second academic stage, about two third come from rural areas, most of the fathers are self-employed and majority of the mothers are housekeepers, and a significant portion of the students are very interested in nursing career. These results match the results of a similar study conducted on 270 undergraduate nursing students in 2015 at Tehran University of Medical Sciences, which approximately show similar proportions of the mentioned characteristics.

According to the Jefferson Scale (JSAPNC), the obtained total mean of scores (51.9) indicates that nursing students exhibited a favorable positive outlook regarding the teamwork and partnership between doctors and nurses and recognition of the importance of collaboration and a departure from traditional hierarchical roles. A study conducted in Tehran University of Medical Sciences on nursing students, the total mean of scores of (JSAPNC) was (51.06) which is similar to the current study (Zakerimoghadam, 2015). In addition, another study conducted in Egypt by Karima, et al, (2011) on medical surgical nurses showed nurses had a positive attitude towards collaboration between physicians and nurses. The mean and standard deviation of the obtained Jefferson Scale scores by nurses indicated their positive attitude towards collaboration between physicians and nurses (51.21). Garber et al. (2009) from the United States (52.31) and Hansson et al. (2010) from Sweden (51.7) also reported nurses' positive attitudes toward collaboration between physicians and nurses. In contrast, a research done by Ardahan et al., nursing students' average attitude score (26.11) was less than half of the Jefferson Scale's total score, indicating a less positive or even unfavorable attitude toward physician and nurse collaboration. The status of nurses within the care and treatment team appears to be the cause of this disparity. According to Ardahan et al., despite the theoretical and practical education that emphasizes teamwork, the attitude toward the nursing profession has not yet altered in Turkey. It appears that common sense in Turkish care and medical facilities; it appears that nurses viewed as physicians' assistants rather than as coworkers.

The researcher asserts that modifications in nursing education are responsible for the optimistic outlook found in the current study and other investigations. In the contemporary university curriculum, nursing students undergo instruction in effective interaction with fellow care and treatment team members, with a specific focus on physicians, in order to deliver suitable medical care.

Overall, the interpretation of the JSANPC subscales suggests a generally positive attitude towards shared education and collaboration, nurses' autonomy, and the balance between caring and curing. However, there is some variability in attitudes towards the doctor's authority. These findings highlight the importance of promoting collaborative practices, recognizing nurses' autonomy, and maintaining a balance between caring and curing in healthcare settings, but the subscale, doctors' authority, got least mean (6.64±1.11), that is potentially some of the students may have faced tough treatment by some of the physicians in clinical environment.

The results indicate that there are significant differences between genders in terms of shared education and collaboration, as well as nurses' autonomy. Females tend to exhibit higher favorable attitudes in all subscales compared to males. However, no significant differences found between genders regarding attitudes towards the doctor's authority and the balance between caring and curing. The cooperation between physicians and nurses is probably influenced by various prohibiting factors, including variations in income, gender-based disparities in conventional perceptions about the two vocations, the prevailing authority of physicians, and the constrained scope of nursing's professional responsibilities (Karima, et al, 2011; Ramezani-Badr, et al, 2009).

The results reveal the mean values for shared education and collaboration, decrease slightly from the second stage (24.2) to the fourth stage (23.7). In addition, for doctors' authority from (7.10) decreasing to (6.58), and for caring versus curing decreasing from (10.7) to (10.5), and p-value for the t-test is less than 0.001indicating a highly significant difference in the mean values between the stages. The mean values for nurses' autonomy appear to be relatively consistent across the second (10.3), third (10.5), and fourth (10.3) stages, showing no significant trend in this variable over time. The standard deviation remains relatively stable, indicating consistent responses for this variable as well, and the p-value for the t-test is 0.010, indicating a statistically significant difference in the mean values between the stages indicating significant differences in attitudes toward physician-nurse collaboration and its subscales among nursing students in different academic stages. Students in the second stage tend to have more favorable pattern of attitudes in shared education and collaboration, recognizing the doctor's authority, and balancing caring and curing. However, students in the third stage tend to have a higher mean score in recognizing nurses' autonomy compared to those in the second and fourth stages. These findings suggest that attitudes and perceptions towards collaboration and professional roles may evolve throughout the nursing education program.. In a study conducted in Iran, the outcomes indicate the absence of statistically significant variances when examining the attitudes of nursing students across their first to fourth academic years. The test outcomes for the average disparity in attitude scores related to shared education and teamwork, the balance between caring and curing, and nurses' autonomy similarly revealed a lack of substantial distinction over time (Zakerimoghadam, et al, 2015).

The results suggest that attitudes toward collaboration between nurses and physicians, as well as perspectives on doctors' authority and nurses' autonomy vary significantly based on students' levels of interest in a nursing career. However, differences in attitudes related to "Caring vs. Curing" were not found to be statistically significant across the interest groups.

For the subscales of "Shared education and collaboration," "Doctors' authority," and "Nurses' autonomy," the p-values are all (0.000), indicating that the differences in mean scores between the groups are statistically highly significant. This suggests that there are meaningful differences in attitudes toward these aspects of collaboration among nursing students with varying levels of interest in the nursing profession.

For the subscale of "Caring vs. curing," the p-value is (0.359), which is greater than the conventional threshold

of (0.05) for statistical significance. This means that the differences in mean scores for this subscale between the groups are not statistically significant. In other words, the attitudes related to "Caring vs. curing" do not appear to differ significantly among the three groups of nursing students.

In summary, the table indicates that there are significant differences in attitudes toward shared education, collaboration, doctors' authority, and nurses' autonomy among nursing students with different levels of interest in the nursing profession. However, there are no significant differences in attitudes related to "Caring vs. curing" among nursing students in Sulaimani city.

Limitation of the study:

The study conducted in one area and from only nursing students that may limit its generalizability, also data should be collected from undergraduate medical students to explore the differences between both groups. Another point, there was no conducted studies in Iraq to compare them with the current study.

Conclusions:

It is noted in this study that undergraduate nursing students have favorable opinions toward collaboration between nurses and physicians in all the four subscales, however their opinions regarding doctors' authority is lower than shared education and collaboration, nurses autonomy, and caring versus curing. Furthermore, the female students' perspectives are higher than the male students in all subscales. The study results show a significant association between the students' academic stages and the subscales. In addition, there is a highly significant association between students' interest in nursing career with shared education and collaboration, doctors' authority, and nurses' autonomy, while there is no association found with caring versus curing.

The study results recommend further studies on wider sample in all the nursing' colleges in Kurdistan and to be compared with the viewpoints of medicine colleges' students in order to promote this vital issue between the students which affect the outcome of patient's care.

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Expression of PD-1 and PDL-1 on macrophages are stimulated by the conditioned media of pancreatic cancer cells and tumor associated macrophages.

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Abstract

Programmed cell death-1 (PD-1) and PD-1 ligand 1 (PD-L1) are target molecules for immunotherapy in many cancer types. PD-1/PDL-1 are expressed on many cells including cancer cells however promoted by the impact of several factors in the tumor microenvironment (TME). In this study, we used qPCR to investigate the role of conditioned media (CM) of Pancreatic cancer cells and tumor associated macrophages (TAM) on PD-1/PDL-1 expression on macrophages in vitro. Comparing the relative expression of treated macrophages at three different time points with the control group, both TAM and PDAC conditioned media significantly increased the expression of PD-1. Even though PDL-1 was also increased by both conditioned media at the 24 hrs. time point, however, a decreased expression was noticed particularly by the CM of pancreatic cancer cells. Generally, the study found that secretome of TAM and pancreatic cancer cells are closely related to PD-1/PDL-1 expression by macrophages, suggesting conditioned media a powerful inducer for immune checkpoint inhibition resistance.

Keywords: Tumor associated macrophages, pancreatic cancer, immune checkpoint,

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Introduction

The prevalence of cancer cases worldwide is increasing, accompanied by a significant mortality rate [1]. This in part is related to the fact that, tumors reduce immunogenicity as they divide and proliferate, resulting in immune escape which is one of the most important features of cancer [2]. One of the most deadly cancer types is pancreatic cancer which is the fourth leading cause of cancer-related deaths globally, with a 5-year overall survival rate of less than 8%. More than 90% of all pancreatic malignancies is pancreatic ductal adenocarcinoma (PDAC) [3] which is characterized by an immunosuppressive tumor microenvironment (TME) [4]. The tumor microenvironment of PDAC is a very complex and dynamic system consisting of various cell types including cancer associated fibroblast (CAF), immune cells such as macrophages, NK cells, T-cells, Dendritic cells (DCs), extracellular matrix, cytokines, and soluble factors. Collectively, these components contribute to the development of an immunosuppressive and hypoxic microenvironment that impacts the response to different therapeutic approaches, including chemotherapy and immunotherapy [5, 6].

Apart from tumor cells, immunosuppression in PDAC can be partially attributed to the presence of tumor-associated macrophages (TAMs) exhibiting an M2-like macrophage phenotype that are secreting anti-inflammatory or pro-tumoral factors, such as IL-4, 6, 10, 13, TGF-beta, and colony-stimulating factors [7, 8]. Furthermore, the expression of programmed cell death 1 (PD-1) and programmed cell death ligand 1 (PD-L1; also known as B7-H1) by TAMs contributes to the exhaustion of peripheral T effector cells, thereby diminishing the efficacy of checkpoint inhibitor immunotherapy [9, 10]. PD-1, is belonging to the CD28/Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) subfamily, specifically binds to PD-L1 on various cell types, including macrophages [11]. Studies have shown that the expression of PD-1/PD-L1 by macrophages inhibits the recruitment and function of T-cells, negatively correlating with the phagocytic potency of TAMs [12-14]. Tumors can evade the immune system by expressing a high level of PDL-1, which triggers the secretion of interferon-gamma upon T-cell activation [15] which leads to the overexpression of PDL-1 on both invading immune cells and cancer cells [16].

While the role of genomic aberrations, inflammatory signaling, and post-translational modification in determining PD-L1 levels has been extensively studied [17, 18], the understanding of the impact of stromal cells and their secretome (exosomes and vesicles) on PD-1 or PD-L1 expression by macrophages remains limited. In this study, we aimed to investigate whether human macrophages express PD-1/PD-L1 when cultured with conditioned media (CM) derived from PDAC cells and TAMs in vitro. Upon treating macrophages with the conditioned media from pancreatic cancer cells and TAMs, we observed a significant upregulation of both PD-1 and PD-L1 on the macrophages, albeit at different time points.

Materials and methods

Cell culture and conditioned medium preparation

The pancreatic cancer cell lines and primary PDAC cell lines which were used in the study are enlisted in (Table-1). All PDAC cells were cultured in Iscove's Modified Dulbecco's Medium (IMDM) (Invitrogen, Darmstadt, Germany) containing 10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin at 37°C in a humidified atmosphere of 5% CO2 and 95% air until reached 80-85% confluence. Cells were then washed twice with PBS buffer and cells were subsequently cultured for further 48 hours in serum-free IMDM. For the TAM conditioned medium, THP-1 cells were cultured in RPMI1640 medium following the same protocol as mentioned above. The conditioned media (CM) were then collected, centrifuged at 3,000 g for 10 min at 4°C and filtered through 0.22μM

filter to remove cell debris.

Table 1: List of cell lines used in the study.

Cell name	Cell origin	Sources
A818-1	Ascites	ATCC, Manassas, USA
AsPC-1	Ascites	ATCC, Manassas, USA
BxPC-3	Primary tumor	ATCC, Manassas, USA
CFPAC-1	Liver metas-tasis	ATCC, Manassas, USA
Colo357	Lymph node metastasis	ATCC, Manassas, USA
MIA PaCa-2	Primary tumor	ATCC, Manassas, USA
THP-1	Cell line (Hu-man mono-(cyte	Division of Redox Regulation, DKFZ, Heidelberg, Germany
PaCa-44	Primary tumor	ATCC, Manassas, USA
PacaDD119	Primary cancer cell line	Dr. Pilarsky, technical university of Dresden, Germany
PacaDD135	Primary cancer cell line	Dr. Pilarsky, technical university of Dresden, Germany
PacaDD137	Primary cancer cell line	Dr. Pilarsky, technical university of Dresden, Germany
PacaDD159	Primary cancer cell line	Dr. Pilarsky, technical university of Dresden, Germany
PacaDD161	Primary cancer cell line	Dr. Pilarsky, technical university of Dresden, Germany
PacaDD183	Primary cancer cell line	Dr. Pilarsky, technical university of Dresden, Germany
Panc-1	Primary tumor	ATCC, Manassas, USA
Pt45P1	Primary tumor	ATCC, Manassas, USA
SK-PC-1	Primary tumor	ATCC, Manassas, USA

Monocyte differentiation to macrophages and TAM

The differentiation of THP-1 monocytic cell line is already established and described before [19]. Briefly, THP-1 monocytes at a concentration of 0.5 x 106 Cell/ml were differentiated to macrophages using 100 ng/ml PMA for 48 h followed by washing twice with RPMI and 24 h resting stage in PMA-free RPMI1640 medium. Thus, monocytes were differentiated to macrophages and hereafter, the macrophages were polarized with 20 ng/ml M-CSF and IL4/13 to alternatively activated (M2) macrophages which have the phenotype of TAM. For cellular interactions, the macrophages were treated with the conditioned media or secretome of PDAC cell lines and TAM in the later experiments to observe PD-1 and PDL-1 expression.

Treatment of macrophages with TAM and PDAC CM

The macrophages (1x106 cells/ml) were cultured overnight in RPMI1640 medium in six-well plates. The cells were

then washed twice with pre-warmed serum-free medium (RPMI-1640) and subsequently cultured with the CM of TAM and PDAC cells for three days with the interval changing of the medium every 24 hrs. Each 24 hrs, considered a time point for harvesting the cells and preparing the cells for RNA isolation. In total, samples from 3 time points were collected, i.e., 24, 48 and 72 hours including the control (non-treated) and treatment.

RNA isolation

Total RNA was extracted from the non-treated macrophages, M1 and M2 positive controls as well as from macrophages which were treated with the secretome of PSCs using TRIzol LS reagent according to the manufacturer's instructions. Briefly, 106 cells were resuspended in 1mL Trizol and vortexed. The lysate was mixed with 200 µl chloroform and was shaken vigorously then incubated at room temperature for 2-3 min. The tubes were afterwards centrifuged at 12,000g for 15 min at 4 °C, leading to phase separation. The aqueous phase (colorless) was carefully pipetted into a new tube containing 500 µl ice-cold isopropanol and 2µl glycogen. The tubes were carefully mixed and incubated at -20°C for 60 min followed by centrifugation at 12,000g for 10 min at 4°C. The RNA pellets were then washed with 1ml of 70 % (v/v) ethanol and dried at room temperature for 15 min. The pellets were finally resuspended in 50µl pre-warmed nuclease-free water and incubated at 60°C for 10 min and RNA concentration was measured with the NanoDrop ND-100 spectrophotometer.

cDNA synthesis and qPCR

cDNA was synthesized from 700ng of total RNA of the treated macrophages using ProtoScript First Strand cDNA Synthesis Kit according to the manufacturer's instructions. The subsequent qRT-PCR reaction (20µl) containing 2µl of each cDNA template, 2µl of both forward and reverse primers, 10µl fast Syber Green master mix and 6µl nuclease free water was performed on LightCycler 480 (Roche Diagnostics, USA). The reaction was performed as follows: enzyme activation at 95 °C for 1 min, followed by 40 cycles of amplification (95 °C for 10 s and 60 °C for 35 s).

Table 2: Sequence of primers used for the qPCR.

Gene	('Primer Sequence (5'- 3	Company
PD-1	F- TTTCAGGAATGGGTTC-CAAG R- ACATCCTACGGTCCCAAG-GT	Biomers.net, Ulm, Germany
GAPDH	F- GAAGATGGTGATGG-GATTTCCA R- GATTCCACCCATGGCAAATT	Biomers.net, Ulm, Germany
PDL-1	F- GGTGCCGACTACAAGC-GAAT R- AGCCCTCAGCCTGACAT-GTC	Biomers.net, Ulm, Germany

Statistical analysis

Relative expression (fold change) of PD-1 and PDL-1 were calculated using Microsoft Excel by ΔΔCt method

using GAPDH as an internal control reference and non-treated macrophages (Ctrl) as the experimental control. Statistical data analysis was performed using one-Way ANOVA multiple comparison in GraphPad prism 8.3.0 and the data were represented as mean values with the respective standard deviations of at least two independent experiments or biological replicates. Differences between treatments were calculated where P-Values <0.05 considered significant.

Results

PD-1 expression was induced in all cases by TAM and PDAC conditioned media.

We investigated the expression of PD-1 by macrophages under the effects of conditioned media of both TAM and PDAC cells at three different subsequent time points (24, 48 and 72 hours, Figure 1, A, B and C respectively). Each treatment was separately analyzed, normalized to its internal housekeeping gene (GAPDH) and compared to the control (non-treated). Of all time points, PD-1 expression is significantly upregulated both by TAM-CM (P-Value 24hr= 0.04, 48hr= 0.0004 and 72hr= 0.007) and PDAC (P-Value of 24hr=0.02, 48hr=0.017 and 72hr= 0.027). However, the impact of TAM conditioned medium is more influential than PDAC cells as the effect is clearly showing an increasing PD-1 expression pattern at each time-point successively (Figure 1).

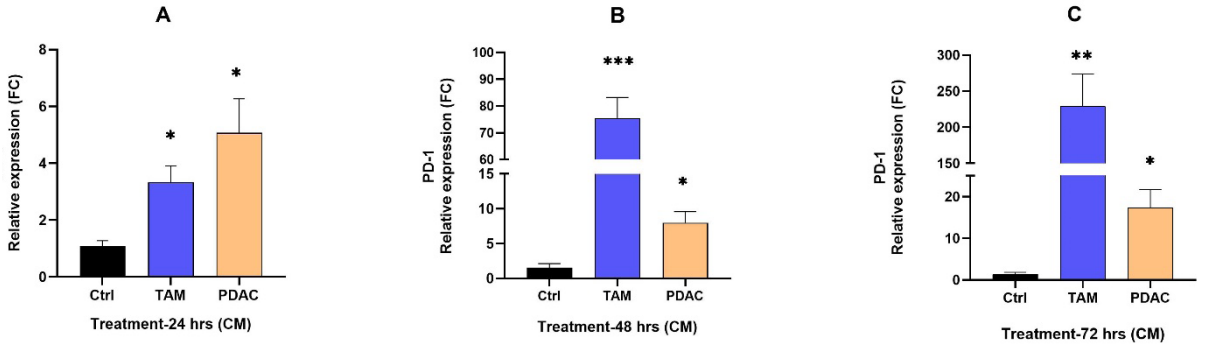

Figure 1: Relative expression (FC) of PD-1 gene by macrophages when incubated with conditioned media of TAM and PDAC cells, A= 24 hours treatment, B= 48 hours and C=72 hours. Ctrl= non-treated Macrophage, TAM= Treated with conditioned medium [CM] of tumor associated macrophages and PDAC= Treated with conditioned medium of pancreatic ductal adenocarcinoma cell lines. Error bars indicate standard deviation of the mean of three technical replicates. ANOVA results were reported comparing the treatments with the control based on the statistical significance * P<0.05, ** P<0.01, *** P<0.001.

Interestingly, the consecutive increase of PD-1 by TAM-CM is statistically significant when the result of each time point is compared to the other (Figure 2). The expression of PD-1 after 48 hours treatment with TAM-CM is statistically more significant (P-Value= 0.0002) than the 72 hours treatment (P-Value= 0.004) when both compared to the first time point (24 hrs.). Even, the difference between 48 hrs and 72 hrs treatment is significant (P-Value= 0.02) though it is not as significant as the difference between first and second time point treatments.

When the same comparison was made for the effectiveness of PDAC-CM at the three time point treatments, the influence is not similar to that of TAM-CM. Despite the upregulation of PD-1 under the effect of the PDAC conditioned medium, however, the increment of PD-1 at second time point is not statistically significant compared to first time point (P-Value= 0.18). The same pattern could be seen for the second and third time points as the increased PD-1 expression (P-Value= 0.08) is statistically non-significant. Despite that, the 72 hrs treatment with a (P-Value=

0.04) is considered a significant increase compared to 24hrs time point (Figure 2). Given the fact that, the increase is expected, however, it is not possible to compare the impact of both TAM and PDAC conditioned media as they contain different factors that may have roles in PD-1 regulation.

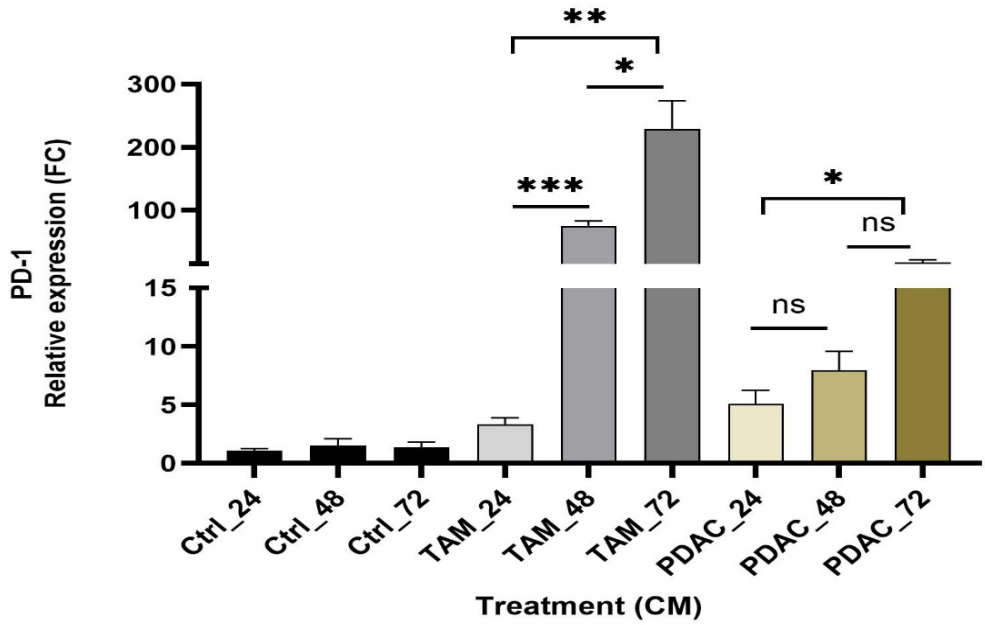

Figure 2: Collective data (three different time points) of PD-1 gene by macrophages when incubated with conditioned media of TAM and PDAC cells. Ctrl= non-treated Macrophage, TAM= Treated with conditioned medium [CM] of tumor associated macrophages and PDAC= Treated with CM of pancreatic ductal adenocarcinoma cell lines. ANOVA results were reported comparing the treatments with the control based on the statistical significance * P<0.05, ** P<0.01, *** P<0.001, ns= not significant

PDL-1 expression is highly induced at the beginning of treatment.

The measurement of PDL-1 gene expression is similarly performed as PD-1 gene. Also, the same treated cells were used in the analysis for both PD-1 and PDL-1. The relative expression (FC) of treated cells were compared to the non-treated ones (Ctrl) as a control. PDL-1 expression has upregulated significantly high after the 24 hours treatment by both TAM-CM (P-Value= < 0.0001) and PDAC-CM (P-Value= 0.03) as it has shown in (Figure 3/A). However, the results of 48 hours' time point (Figure 3/B), does only show a statistically significant expression by TAM-CM (P-Value= 0.001) but the PDAC-CM apparently had no effect on the PDL-1 expression as the analyzed value

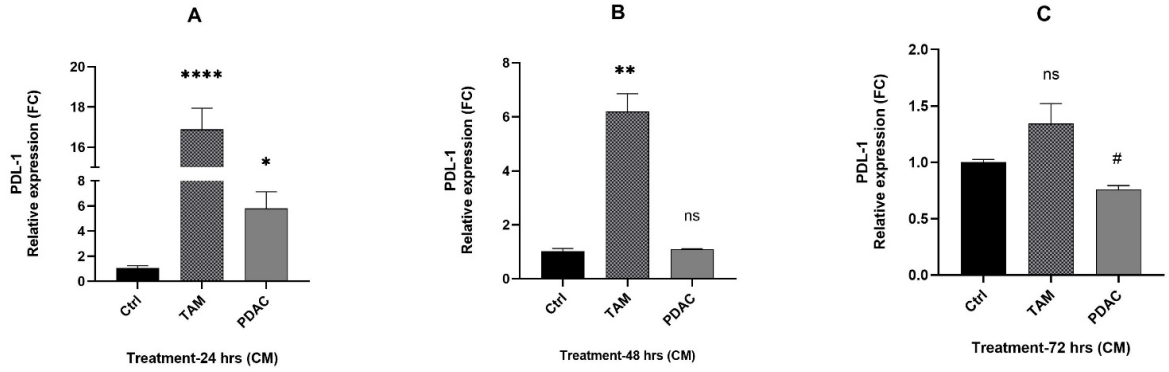

Figure 3: shows the relative expression (FC) of PDL-1 gene by macrophages when cultured with conditioned media of TAM and PDAC cells, A= 24 hours treatment, B= 48 hours, and C=72 hours. Ctrl= non-treated Macrophages, TAM= Treated with conditioned medium [CM] of tumor associated macrophages and PDAC= Treated with conditioned medium of PDAC. Error bars indicate standard deviation of the mean of three technical replicates. ANOVA results were reported comparing the treatments with the control based on the statistical significance * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001, ns= not significant

was not significant anyhow (P-Value= 0.8). What could be seen in the last time point (Figure 3/C), TAM-CM had shown no significant induction of PDL-1 expression (P-Value= 0.2) while PDAC-CM has significantly down-regulated the PDL-1 expression (P-Value= 0.0005). Accordingly, the different impacts of both conditioned media necessitate checking the treatments and graphing them together because a bearish divergence could be seen.By a close look at the combined results of TAM and PDAC effects on PDL-1, a declining pattern could be seen. Therefore, comparisons were performed among populations treated with TAM-CM, where clear statistically significant decrease can be seen among first, second and third time points (Figure 4). Comparing first time-point with both second and third ones has given a very significant decline (P-Value= <0.0001) likewise, comparing second and third time-point treatments resulted in a significant decrease in PDL-1 expression (P-Value = 0.0005). The result of PDAC comparison has given the same pattern where the second and third time-point treatments were significantly decreased compared to first one (P-Value= 0.01) but no significant difference could be seen between 48 and 72 hours treatment (P-Value= 0.07).

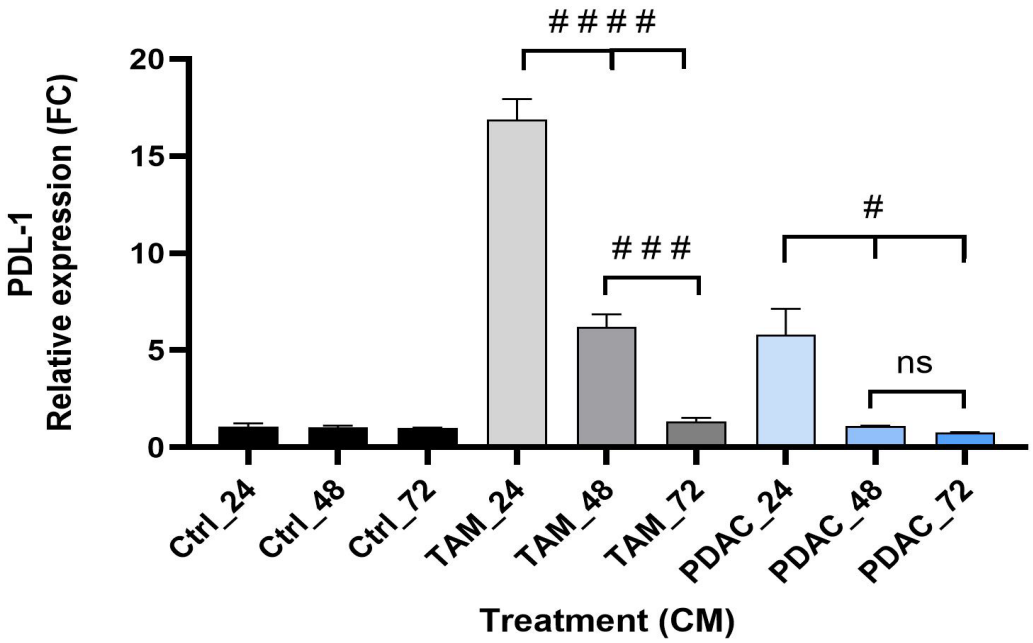

Figure 4: shows the collection of all PDL-1 data comparing the treatment time points to each other rather than to control. Effect of both TAM and PDAC conditioned media is significantly decreasing when compared alone. Ctrl= non-treated Macrophage, TAM= Treated with conditioned medium [CM] of tumor associated macrophages and PDAC= Treated with CM of pancreatic ductal adenocarcinoma cell lines. ANOVA results were reported comparing the treatments with the control based on the statistical significance # P<0.05, ## P<0.01, ### P<0.001, #### P<0.0001, ns= not significant.

Discussion

In recent years, cancer immunotherapy has been developed in an effort to improve the specificity and strength of the immune system against cancer. Programmed cell death protein 1 (PD-1) and its ligand, programmed cell death-ligand 1 (PD-L1), have been widely investigated in cancer [20] because they belong to the immune checkpoint proteins that are involved in the mechanism of immune escape by cancer cells [21]. Studies have shown that, PD-1 is expressed on T-cells, B-cells, Dendritic cells (DC), tumor associated macrophages (TAMs), Natural Killer (NK), and Treg and immune checkpoint blockade methods targeting cytotoxic-T-lymphocyte-antigen-4 (CTLA-4) or PD-1/PD-L1 are currently being pursued to treat cancer by restoring T-cell cytotoxicity against tumor cells [22, 23]. In this study we focused on the conditioned medium of TAMs in pancreatic cancer because they are the most potent stromal cells in the TME of PDAC that support tumor growth and have been associated with poor prognosis of PDAC and immunosuppression [7, 24]. In addition, our study aimed at evaluating the role of secretome or proteins available in the conditioned medium of TAMs and pancreatic cancer cells which have received less attention.

We have previously shown that PDAC secretome contains several important factors which regulate immune cells [25] and have investigated the role of pancreatic stellate cells on macrophage polarization (Mustafa S. A. et.al., Publication is process) therefore, hypothesized that TAMs may participate in PD-1/PDL-1 expression on the recruited monocytes. It is known that T-cells exposed to antigen constantly will harbor upregulated PD-1 on their surface which is one of the characteristics of exhausted T cells [26]. In the exhausted T cell, interferon α (IFNα) coupled with the IRF9 transcription factor bound to the promoter of the pdcd1 gene thus leading to the PD-1 expression [27]. What pathway is exactly involved in PD-1 expression by macrophages still is not well illustrated. However, in the current study, PD-1 was significantly up-regulated on macrophages treated by the CM of TAM and PDAC at all cases of treatment (Figure 1, A, B and C). In addition, the increase has an upward-rising pattern, such pattern suggests that the longer the cells incubated with the conditioned media, the higher the PD-1 expressed, it simply means that, at the third time point (72 hrs. treatment), the expression of PD-1 is the highest (Figure 2). Based on this result, it is highly necessary to profile the conditioned media of TAM and investigate their impact on PD-1 expression. PD-1 expression by TAMs inhibits phagocytosis and tumor immunity and is associated with increased Treg-cell proliferation and enhanced immunosuppressive function [20]. Therefore, in the higher grades of cancer, at the late stages, TAM may have more impact on treatment particularly because they are involved in resistance to immune checkpoint inhibitors.

On the other hand, our study has shown that PDL-1 is also significantly expressed on macrophages induced with the conditioned media of TAM and PDAC (Figure 3) still, TAMs CM is more potent than PDAC statistically (Figure 4). This is in accordance with the previous studies however on PDAC tissues which have shown that PDL-1 is highly expressed by the resident macrophages in the tumor microenvironment [22, 28]. In detail, high expression of PDL-1 on macrophages is induced by IL-27 that is related to STAT3 activation [29] and stimulated by the granulocyte–macrophage colony-stimulating factor (GM-CSF) in the conditioned media [30]. Meanwhile, GM-CSF, for example in the presence of CCL2, is involved in macrophage polarization towards M2-type which is the same phenotype of TAM [31]. Therefore, it could be postulated that more TAM will be available in the TME, as a consequence, a higher amount of TAM secretome in the TME will induce PDL-1 expression in turn. Our results (figure 4) clearly show the highly significant expression of PDL-1 at both 24- and 48-hours' time point, considering that the pattern is declining as the effect at the last time point (72 hrs) is significantly diminished (figure 3/C). This pattern is also pointing to the assumption that PDAC secretome/ conditioned medium will induce more TAM in the tumor microenvironment, therefore, more TAM will promote higher PDL-1 expression than PDAC alone.

This could be supported by the results previous studied which have shown that MMP-1, IL-6, FGF-2, VEGF-A, MIP-3α, and GRO-α concentrations were significantly increased in TAMs and released into the TME [32]. Such factors have been investigated, interleukn-6 (IL-6) for example, is necessary to induce the maximal expression of PDL-1 but not PD-1 on CNS-infiltrating macrophages [33]. PDAC-CM was only significantly induced PDL-1 at the first time point, still it is in agreement with other studies done on other cancer types such as cervical cancer [34] in which the authors found that both PD-L1 and PD-L2 strongly correlated with interferon gamma (IFNG) expression. IFN-gamma is considered as a key mediator of the anti-tumor immune response which is mainly produced by immune cells. Also, elevated PD-L1 in macrophages was correlated with high PD-L1 level in tumor and is the predominant immune cell type that expresses PD-L1 is CD68+ macrophages [28]. Therefore, the possibility of having more TAM in TME of PDAC is one of the most rational hypotheses which is already investigated [7]. In the long run, because of the variabilities of factors released into the TME by TAMs, some factors might be involved in the induction of PDL-1 on macrophages which is generally investigated in the current study. Remarkably, SIGLEC15 was identified as a novel TAM-related immune-checkpoint in PDAC and some other cancer types, which is correlated with poor prognosis and immunosuppressive microenvironment [35]. Accordingly, even though some studies have found that microbiome-derived metabolite trimethylamine N-oxide (TMAO) is reducing PDAC tumor growth via TAM activation [36], still, more investigations are needed on TAM to elucidate their role in immune checkpoint blockade.

Conclusion

In conclusion, PD-1 and PDL-1 are highly expressed on macrophages under the influence of conditioned media of pancreatic cancer cells as well as TAMs. The study suggests that secretome of pancreatic cancer cells and TAMs are closely related to PD-1/PDL-1 expression however, specific factors thmust be determined. Future studies could explore how macrophages contribute to the resistance to immune checkpoint inhibitors and immunosuppression, using both conditioned media and tumor tissues to gain a better understanding of the secretome (CM) role.

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Effect of Ramadan Fasting on Bone Profile in Healthy Female Adult

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Abstract

There is one month in a year which is called Ramadan in the Hijri calendar, Muslims fast during this month from dawn to dusk. In Ramadan, sleep and awake cycles are disturbed with fasting impacts the secretion of a key hormone in the human body which is parathyroid hormone (PTH) and hence bone metabolism at the same time. The study objective was to find the effect of Ramadan fasting on bone profile in female adults in Sulaymaniyah city in Iraq. Moreover, different parameters were taken with parathyroid hormone (PTH) to investigate their impact on the bone profile such as calcium, albumin, magnesium, inorganic phosphorus, total alkaline phosphate, and 25-OH vitamin D. The number of participants was only 30 healthy females from different ages. The result of this study depends on the double time of taking blood samples from each participant. The result of this study showed that there is no significant difference in each parameter before and after 28 days of Ramadan. However, serum parathyroid hormone showed a significant increase at the end of Ramadan (55.5±15.09 pg/ml, p=0.03) compared to the value of pre-Ramadan of fasting subjects (45.7±15.33 pg/ml). Overall, alterations in dietary habits throughout Ramadan influenced the secretion of PTH in a manner that could potentially have a positive impact on bone health.

Keywords: **Ramadan fasting, bone metabolism, parathyroid hormone, Kurdish**

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Introduction

Ramadan is the holiest month among Muslims and every healthy Muslim is obligated to fast. During this month, all healthy adult Muslims will abstain from food and drink between sunrise and sunset. The length of this period varies depending on the season, geographical location, and the precise timing of sunrise and sunset in each particular country or region, ranging from 12 to 18 hours. During fasting, two meals are taken per day - "Iftar" as the dinner & "Sahur" at the end of the night just before dawn. Daily physical activities and sleep duration will be affected due to changes in the frequency and quantity of foods and drinks. Therefore, Ramadan fasting may cause changes in body measurements, physiology aspects, hormonal metabolism, and haematological and biochemical compositions of the blood in the human body (Hosseini et al., 2013, Sayedda et al., 2013).

Numerous studies have been undertaken to investigate how Ramadan fasting influences the metabolic and physiological aspects of human health both during and after the Ramadan period. (Elnasri and Ahmed, 2006, Bahijri et al., 2013, Khoshdel et al., 2013, Bahijri et al., 2015).. In reality, there is a shortage of studies examining the impact of Ramadan fasting on bone biomarkers, and the outcomes reported in these studies are conflicting.

Modern-era Ramadan fasting is often linked to disrupted sleep patterns. Many individuals stay awake until dawn, sleep for a few hours before heading to work, and then take another nap after work or when they have the opportunity. Prior research has indicated that these sleep disturbances during Ramadan fasting are associated with alterations in circadian rhythms and cortisol hormone levels. These hormones can change the expression of other hormones such as parathyroid hormone (PTH). As a result, it has detrimental effects on many physiological conditions (Bahijri et al., 2013). Parathyroid hormone (PTH) is a hormone that is essential for bone metabolism (Kroll, 2000). It has been found that this hormone shows circadian rhythmicity and its circulating level is affected by sleeping disturbance (Nielsen et al., 1991, Logue et al., 1992).

Several studies have been conducted on the distinct effects of Ramadan fasting on those parameters that have an essential role in bone metabolism. For example, a study showed a slight change serum in albumin, and calcium levels after Ramadan fasting (Azizi and Rasouli, 1987). However, other studies reported unchanged levels of serum constituents such as alkaline phosphatase, calcium, phosphorous, albumin, vitamin D, and magnesium. (Furuncuoglu et al., 2007, Ibrahim et al., 2008, Mohammed, 2011, Sayedda et al., 2013, Bahijri et al., 2015). Although various studies have been conducted in order to find the effect of fasting Ramadan on various physiological conditions, their results are controversial. Therefore, this study aimed to find the effect of Ramadan fasting and the disturbance of sleep patterns on parameters of bone metabolism in healthy Kurdish women.

Patient and methods

Human subjects

This study was performed during Ramadan May-June 2019 in the city of Sulaymaniyah, Iraq. The study was conducted on 30 healthy women adults who were fasting during Ramadan. The present study was approved by the scientific advisory and the ethics committee of the Kurdistan Institution for Strategic Studies and Scientific Research. Subjects were examined twice, during their regular life (Pre-Ramadan) before, and again 28 days into the fasting period (End-Ramadan). Blood samples were collected from all subjects twice: first, one week before Ramadan and then on the 28th day of Ramadan. Serum was obtained by low-speed centrifugation at 1000g for 15 minutes, and samples were immediately separated into aliquots and samples were stored at -20C until measurements were conducted. To avoid day-to-day laboratory variation, all biological and endocrine parameters were analysed in a single batch.

Biochemical and endocrine assays

Serum biochemical and endocrine parameters of 30 serum samples were measured twice. Calcium, albumin, magnesium, inorganic phosphorus, and total alkaline phosphatase were assayed by a spectrophotometric method using cobas c111 auto-analyser (Roche-Germany). Intact parathyroid hormone and 25-OH vitamin D were measured by chemiluminescent immunoassay technique using cobas e411 auto-analyser (Roche-Germany).

Statistical analysis

The data have been recorded and tabulated using Microsoft Excel and analysed with the statistical software package SPSS 26 (SPSS Inc., Chicago, IL, USA). Results were presented as mean standard deviation. Data from before and at the end of Ramadan was compared using paired two-tailed student's T-test. The p-values less than 0.05 were considered statistically significant.

Results

A total of 30 healthy female volunteers were included in the study. The mean (±SD) age was 41.1 ± 14.3 years (range 18–71 years). The results of biochemical, and endocrine parameters are presented in (Table 1).

Table 1: This table shows the Serum biochemical parameters during Pre-Ramadan and End-Ramadan.

Parameters	Pre-Ramadan	End-Ramadan	Pre-Ramadan *versus* End-Ramadan
	Mean±SD	Mean±SD	P- value
(Magnesium (mg/dl	3.09±0.19	3.14±0.11	0.21
(Parathyroid hormone (pg/ml	55.7±15.33	65.5±15.09	**0.03**
(OH) vitamin D (ng/dl) 25	11.9±6.43	12.25±4.76	0.74
(Calcium (mg/dl	10.41±0.35	10.52±0.23	0.28
(Inorganic phosphorus (mg/dl	2.71±0.52	2.68±0.29	0.78
(Albumin (gm/dl	5.02±0.27	5.6±0.23	3.25
(Alkaline phosphatase (U/L	74.8±22.65	66.7±21.14	6.4

According to the most recent classification of vitamin D status proposed by (Thacher and Clarke, 2011), all subjects exhibited 25-OH vitamin D levels below the established deficiency threshold of ⩽20 ng/ml before Ramadan (Figure 1 A). Additionally, for all subjects at the end of Ramadan, their vitamin D levels remained within the insufficiency range, and none of them reached serum concentrations ⩾30 ng/ml, which have been suggested as the cutoff values to define an optimal vitamin D status by (Kennel et al. 2010). Notably, there was no statistically significant difference observed in the mean concentration of 25-OH vitamin D between the values measured before and after Ramadan fasting.

In this study, the effect of Ramadan fasting on electrolyte parameters showed no significant change. As shown in (Figure 1, B-D), there was no significant change in the concentration of magnesium, calcium, and phosphorous before Ramadan compared to the end of Ramadan. The serum values of electrolytes were within normal reference ranges. Additionally, it has been noted that there were no significant changes in serum albumin concentration compared to pre- and end-of-Ramadan fasting (Figure 2, A). Its value remained within the normal reference range. Although a reduction in the serum alkaline phosphatase concentration was observed after Ramadan, the level re-

mained around 76.7±21.14 U/L, indicating adequate enzyme production by the liver. No significant changes were noted in the means of this enzyme at the end of Ramadan fasting (Figure 2, B).

As shown in (Figure 2, C), serum parathyroid hormone showed a significant increase at the end of Ramadan (55.5±15.09 pg/ml, p=0.03) compared to the value of pre-Ramadan of fasting subjects (45.7±15.33 pg/ml), and fewer subjects having higher than normal reference levels (15-65 pg/ml).

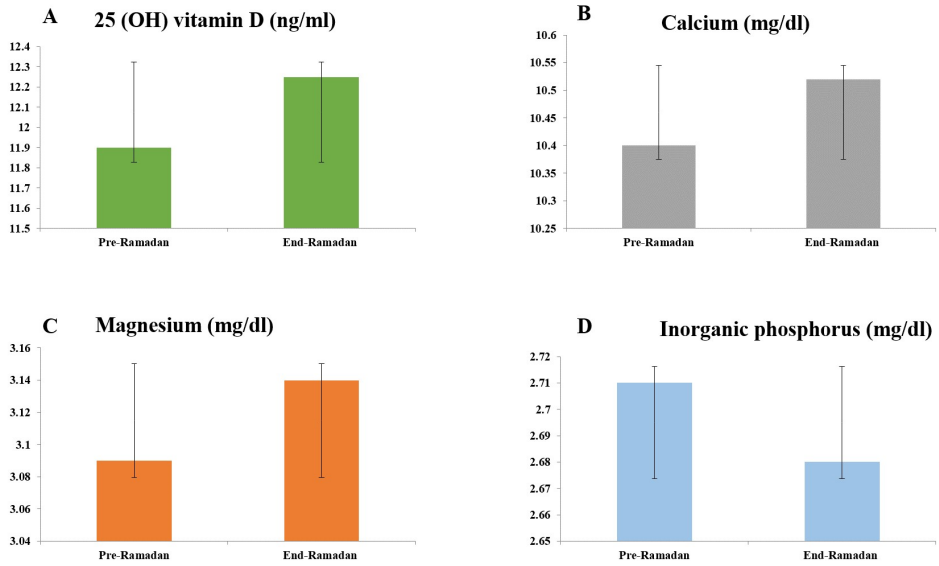

Figure 1: Serum biochemical parameters during Pre-Ramadan and End-Ramadan. The data is presented as number mean ± SD. The p-values are the resultant of paired two-tailed student's T-test. Significant p-values are indicated in bold.

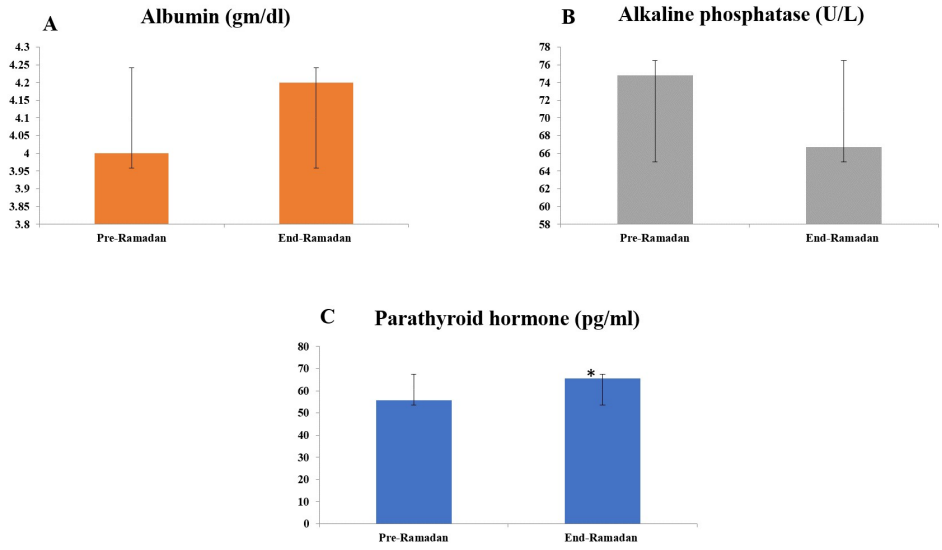

Figure 2: Serum biochemical and endocrine parameters during Pre-Ramadan and End-Ramadan. The data is presented as number mean ± SD. The p-values are resultant of paired two-tailed student's T-test. (asterisk represents a significant P value)

Discussion

During Ramadan, for most Muslims, fasting is a religious duty merely for adult Muslims, eept at same cases such as illness, travelling..etc, from dawn to sunset based on their religious belief they abstain from food and drink. As it was reported during this holy month, sleeping and daily life is changed which is influenced by calorie intake and different biochemical parameters in the body this might impact the life span with the improvement of health (Goodrick et al. 1990 and Anson et al. 2003). On the other hand, it has been suggested that fasting increases metabolic and endocrine dysregulation. This study was conducted to find out the impact of Ramadan fasting on bone metabolism and other different parameters including parathyroid hormone (PTH), calcium, albumin, magnesium, inorganic phosphorus, total alkaline phosphatase and 25-OH vitamin D.

This finding showed that the serum parathyroid hormone increased significantly at the end of Ramadan compared to pre-Ramadan time. The overall increase of PTH was observed between pre-Ramadan and post-Ramadan (45.7±15.33 pg/ml),), 55.5±15.09 pg/ml, p=0.03) subsequently. Similarly, Bahijri et al., 2015 reported that during Ramadan due to changes in dietary practices, the secretion of PTH modulated to a pattern that might be beneficial to bone health. PTH plays a key role in bone turnover, with studies indicating that continuous hypersecretion of PTH is associated with bone resorption, (Kroll, 2000). Thus, manipulation of PTH secretion has been suggested as a way of increasing bone strength and treating osteoporosis (Fraser et al. 2004, Silver and Bushinsky, 2004).

As mentioned in the result different parameters were observed to find out their impact on the bone profile. For example, total vitamin D levels were under the reference range pre and post of Ramadan which means the vitamin D levels were not affected by fasting. Comparably, this is confirmed by Bahijri et al., 2015. In addition, in this study, the effect of Ramadan fasting on electrolyte parameters showed no significant changes such as magnesium, calcium, and phosphorous. The serum values of electrolytes were within normal reference ranges. Calcium serum level was not changed before and after Ramadan. It was reported that when the serum calcium level falls to a critical low, the parathyroid glands are immediately activated to boost the production of PTH (parathyroid hormone). This finding was observed at the end of the Ramadan fasting period, where a substantial increase in serum PTH was observed in response to the decrease in serum calcium values. At the end of Ramadan, the serum calcium values returned to approximately normal values and slightly above the pre-Ramadan levels (Al-Kotobe, et al., 2006).

This study is also in line with finding reported by (Khoshdel et al., 2012) detected that there was no change observed in calcium and serum phosphorous at the end of Ramadan. In addition, alkaline phosphatase concentration was not changed after Ramadan in pregnant women. It is important to increase the rate of bone metabolism. Ramadan fasting, in general, did not show any adverse effects on the concentration of albumin, the results are in line with the earlier findings either reported a decrease (Mohtasham et al., 2001) or no changes in the serum albumin levels as a result of Ramadan fasting however there is a study confirmed that the serum albumin concentration was changed significantly increased in male and female during Ramadan (Nagra et al. 2011). Albumin makes up approximately 60% of the total serum protein and its major function in the blood is to maintain the colloidal osmotic pressure (Nagra et al. 2011). As albumin is synthesized within the liver, it is an important measure of hepatic function. Medically a generous concentration of albumin in the bloodstream is regarded as a measure of quality of life (Nagra et al. 2011).

Conclusion

Overall, the results suggest that Ramadan fasting has no adverse effects in normal healthy women on biochemical substances relating to bone function in healthy women.

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The Implications of Immunotherapy in Tumor through Targeting the Immune Checkpoints Inhibitors PD-1 /PD-L1.

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Abstract

In the last several years, immune checkpoint inhibitors (PD-1/PDL1) are currently looked at more positively to be a potential targeted treatment for cancer therapy for addressing tumor immune evasion. Specifically, checkpoint molecules such as programmed cell death protein 1 (PD-1) exhibit potent immunomodulatory properties by functioning as inhibitors of T-cell activation. Various forms of human cancer have been observed on the cell surface displaying the immune checkpoint known as programmed death-ligand 1 (PD-L1) (He et al., 2018), several kinds of cancer include kidney (Curran and Kopp, 2021), breast cancer (Erber and Hartmann, 2020), bladder (Eckstein et al., 2019), and ureter (Stenehjem et al., 2018), in addition to non-small cell lung cancer (NSCLC), (Pawelczyk et al., 2019), cancer of the pancreas (Liu et al., 2022b), esophageal cancer (Whooley et al., 2022), squamous cell carcinoma of the head and neck carcinomas (Crosta et al., 2021), and renal cell cancer (RCC) (Munhoz and Postow, 2018). Furthermore, Activated T cells are less effective at combating tumors when PD-L1 binds to its corresponding receptor, PD-1, blocking the transmission of signals designed to activate these cells. Following treatment with PD-1-PD-L1 inhibitor antibodies, there have been documented cases of tumor regression in patients with a variety of advanced cancer types. The findings of this study suggest that the presence of PD-L1 on tumor cells and other cellular elements in the tumor microenvironment has therapeutic implications. This review aims to investigate the structure and functions of the PD-1-PD-L1 axis within the framework of cancer. This study will primarily examine crucial elements including the therapies for PD-1 and PD-L1, the corresponding antibodies, and prospective innovative therapeutic strategies for future investigations. Extensive research is necessary in the coming years to determine the efficacy of immunotherapy, particularly PD-1 with PD-L1 immune checkpoint blockade, as a potential beneficial application in cancer treatment for specific cancer subtypes.

Keywords: Tumor; immunomodulatory treatment; the programmed cell death protein 1 (PD-1) and its ligand PD-L1.

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Introduction

Cancer is the uncontrollable development of cells in humans, and many forms of cancer currently lack treatment options. Delayed diagnosis of cancer performs a crucial role during the process of resistance to therapy. Nowadays, immunotherapy is recognized as can be a part of the most effective methods for cancer treatment, considered effective for tumors resistant to chemotherapy and/or radiotherapy, with fewer side effects compared to conventional therapies (Abdullah, 2019) (Wiersma et al., 2015).

In the past decades, surgical cancer treatment is generally followed by chemotherapy and/or radiation therapy(Abdullah et al., 2022). However, these therapies are unable to differentiate between healthy cells and malignant cells, posing an excessive danger. The immune system, on the other hand, can distinguish between regular cells in the body and foreign entities. Immunotherapy utilizes this ability by targeting foreign cells while sparing native cells (Nicholson, 2016). Consequently, many scientists have focused on identifying specific cancer antigens for the development of effective cancer vaccines and antigen-specific T lymphocytes, which form the basis of immunotherapy (Farkona et al., 2016) (Pandya et al., 2019). Immunotherapy works by inducing an immune response to eliminate cancer cells (Ventola, 2017).

As immunological expertise expanded, T-cells have emerged as crucial players in the immune system response to malignancy, effectively eliminating cancerous cells and combating external threats (Raskov et al., 2021). Dendritic cells located within lymph nodes perform a crucial function by facilitating the stimulation and activation of T-lymphocyte cells. This process ultimately allows for the infiltration of T-cells into the cancer microenvironment, which includes tumour cells as well as other immune cells that have infiltrated the cancerous area (Marciscano and Anandasabapathy, 2021). The activation of T-cells recognises, bind to, with eliminate tumors cells. Additional T-cells engage in interactions with dendritic cells and other MHC class II antigen-presenting cells by recognising antigens presented on MHC class II molecules (Marciscano and Anandasabapathy, 2021). The T-cell receptor (TCR) is essential for T-cell activation and so plays a crucial role in controlling this interaction (Shah et al., 2021). In addition to their primary receptors, T-cells also express other receptors such as CD28, which serves as a co-stimulatory receptor and engages with CD80 and CD86 molecules found on dendritic cells (Kennedy et al., 2022). This interaction elicits the secondary activation signal for T-cells. Furthermore, the activation of T-cells is facilitated by cytokine signalling, specifically through the action of Il-2, which serves as a third signal. (Disis, 2010).

In the last decade, the utilization of "checkpoints" for instance, the programmed death ligand-1(PDL-1), has gained significant attention. PDL-1 is among the inhibitory ligands that contribute to sustaining in the homeostasis of immune regulation under normal conditions; nevertheless, it is found to be present in many malignancies (Gutic et al., 2023). Abundant expression of PDL-1 in tumors can assist them in evading the immune system, particularly interferon-gamma is secreted as a result of T-cell activation, which triggers this process (Qian et al., 2018). This leads to an overexpression of PDL-1 on both invading immune cells and cancer cells (Han et al., 2020). The interaction between PDL-1 and its receptor B7-1 (also known as CD80), follow by T-cell activation surface PD-1 expression inhibits cytotoxic T-cell function, as illustrated in Figure 1. PDL-1 and PD-1 are critical components that need to be activated for proper immune cell functioning. PDL-1 is commonly located on the outer layer of cancer cells that have been stimulated by interferon-gamma, PD-1 is commonly observed membrane-expressed antigens to be produced by the immune system (Ghosh et al., 2021).

Utilising the immune checkpoint molecules known as PD-1 with PDL-1has been recognised as a crucial aspect influencing the outcomes of immune checkpoint inhibitors (Figure 1). This approach can be implemented through various strategies, including: (i) understanding the mechanism of PD-1 and PDL-1; (ii) elucidating how PD-1 immunotherapy suppresses the anti-tumors immune response; and (iii) exploring treatment strategies to stimulate the immune response for combating cancer. This review is aimed at highlighting the essential function that immunotherapy plays in the treatment various cancers. and to explore novel prospects for developing effective combination therapies based on immuno-oncology that target the protein that inhibits cell death 1 (PD-1) and its ligand, PD-L1 signalling in high cases of cancer. Consequently, these advancements pave the way for potentially revolutionizing

cancer therapy, with promising clinical applications in the future.

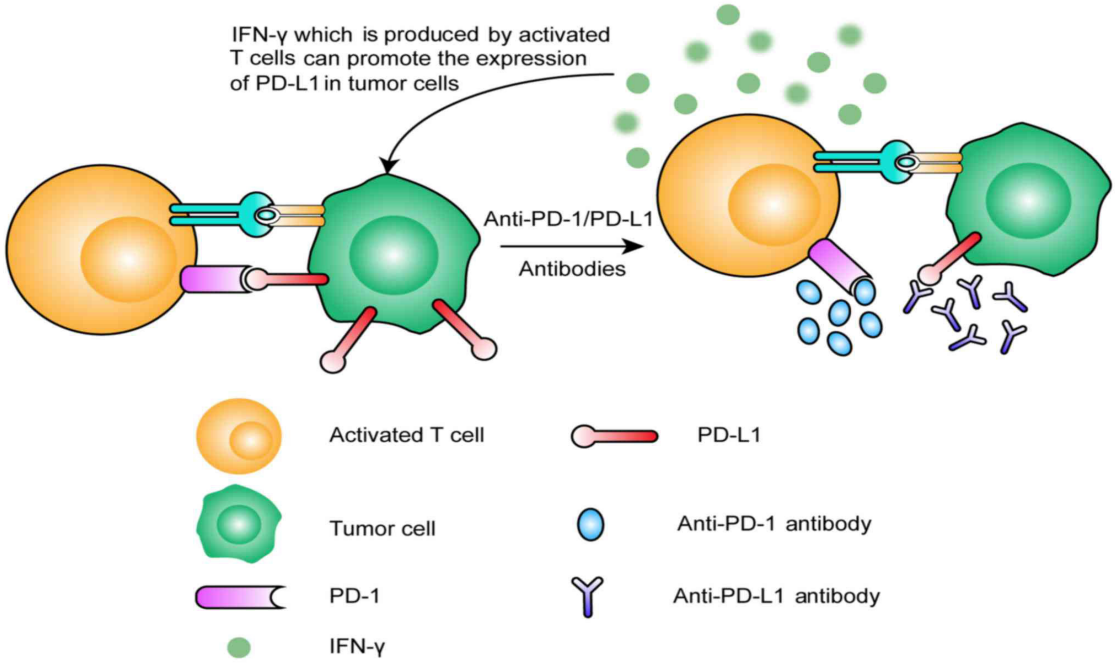

Figure 1. Outline of PDL-1 in the checkpoint immunity system. The immune checkpoints are regulators of immune activation (Fan et al., 2019). T-cells recognise tumour antigens presented by APCs to TCRs; however, the T-cell activation requires a second indication, known as the co-stimulatory signalling, following TCR binding; Co-stimulatory molecules bind to their ligands, such as B7 on APCs. Once these molecules bind to their ligands on the surface, T-cells stimulate immunological response. against cytotoxic antigens, simultaneously, when APCs come into contact with B7-1 or B7-2 (Abdullah, 2019), they also transmit suppressive messages. Furthermore, PDL-1 on activation of T-cells and tumour cells releases the cytokine interferon-gamma (IFN-), which may upregulate expression of programmed death ligand 1 (PD-L1) in malignant cells, allowing immunological escape (Fan et al., 2019).

The biological structure of PD-1 and its corresponding ligand.

PD-L1, also known as programmed cell death ligand 1 or CD274, is a protein that is produced by the PDCD1 gene. It is the protein that is responsible for the creation of CD274 (Kalim et al., 2020). The term CD274 is used to identify this molecule specifically (Wu et al., 2022). Its nomenclature as enhancement upregulation of PD-1 arises from its heightened presence in response to apoptotic stimuli observed in two distinct cell lines, namely 2B4.11 and LyD9t, which are involved in apoptosis (Fabrizio et al., 2018). In contrast to the T cell membrane, which expresses programmed cell death protein 1 (PD-1) at extremely high degrees, the B cell membrane only sometimes shows signs of expressing it (Han et al., 2020, Ishida et al., 1992). Structurally, PD-1 is characterised by its structural composition, which includes a type 1 transmembrane glycoprotein arrangement. This structure consists of a cytoplasmic tail and a single extracellular domain known as the immunoglobulin variable (IgV) domain. The IgV domain, containing a sequence of twenty amino acids, demonstrates a noteworthy characteristic of extending beyond the plasma membrane in the extracellular region (Patsoukis et al., 2020b). Additionally, the results show that 23 per cent of the sequence is identical to cytotoxic T lymphocyte antigen-4 (CTLA-4) (Chou et al., 2022). The PD-1 cytoplasmic domain indicates the existence of two tyrosine motifs. An acronym for tyrosine-based switch motif, ITSM stands for "immune receptor signalling inhibitor," and it is responsible for blocking immune receptor signalling (Qi et al., 2020). It represents an inhibitory motif that is frequently observed in immune receptors and is centred around the amino acid tyrosine

(Intlekofer and Thompson, 2013). Numerous Studies recently released data confirming the essential role that the intracellular tail of PD-1, also known as ITSM, plays in enhancing the immune-suppressive actions produced by PD-1 on activated T cells (Jiang et al., 2019b). Both programmed death ligand 2 (PD-L2 or B7-DC, also known as CD273) and programmed cell death protein l (PD-L1 or B7H1, commonly referred to as CD274) are members of the PD-1 ligand family and are categorised as class I glycoproteins (Jin et al., 2011). The ligands under consideration exhibit common structural features, such as The addition of immunoglobulin variable (IgV) and immunoglobulin constant (IgC) domains, as well as a transmembrane domain that is marked by hydrophobic characteristics, and a cytoplasmic tail (Li and Li, 2023). There is a significant amount of sequence conservation between the PD-L1 and PD-L2 molecules which are encoded by genes located on chromosome nine. Within the microenvironment of malignancy, an interaction between programmed cell death protein 1 (PD-1) and its ligand, programmed death-ligand 1 (PD-L1), is observed (Bolandi et al., 2021). The protein programmed death-ligand 1 (PD-L1) is expressed not only by antigen-presenting cells (APCs) but also by malignant cells. However, the appearance of PD-L1 is the major sign of T-cell activation (Liu et al., 2022a). Protein tyrosine phosphatase-2 (SHP-2) is attracted to phosphorylated tyrosine residues in the immunoreceptor tyrosine-based switch motif (ITSM) of PD-1 (Patsoukis et al., 2020a). The following signalling mechanisms are set off by this phosphorylation, which also inhibits the synthesis of cytokines and the cytotoxicity of T- cytotoxic lymphocytes (CTLs), among other biological activities of T-lymphocytes cells (Ross and Cantrell, 2018). It is possible that the interaction between the PD-1 receptor and its ligand, PD-L1, might result in a decline in the population of T-cells that are especially focused towards tumors. Eventually, more of these T-cells may die, which would allow tumor cells to avoid being attacked by T-lymphocyte cells. (Wang and Wu, 2020).

The role of PD-1/PDL-1 molecules and their respective functions in cancer cells.

Different types of immune cells express the PD-1 programmed cell death protein, including T-lymphocytes, B-lymphocytes, monocytes, dendritic cells, regulatory T-cells, and natural killer T cells (Zhang et al., 2020). The indicated evidence of a lack of T cells is notably observed in instances of protracted viral infections and malignancies (Wherry and Kurachi, 2015). A significant number of tumor-infiltrating lymphocytes (TILs) from a wide variety of tumors types have been shown to exhibit the protein PD-1 (Evangelou et al., 2020). Immunosuppression is present when tumor microenvironment (TME) contains activated Treg cells (Paluskievicz et al., 2019). Additionally, The activation status of CD4+ TILs can be detected by their increased PD-1 expression in Treg cells(Liu et al., 2021). Additionally, Increased PD-1 expression in CD8+ TILs may indicate cytotoxic T lymphocyte (CTL) exhaustion or dysfunction (Dolina et al., 2021). Recent studies have indicated that Macrophages that are associated with tumors (TAMs) express PD-1 in both mice and humans, impairing their ability to phagocytose malignancy cells (Peranzoni et al., 2018). However, the enhancement of phagocytosis can be achieved by blocking the interaction between PD-1 and PD-L1and reduces tumor growth. Programmed death-ligand 1 (PD-L1) exhibits a common occurrence of increased expression in neoplastic cells found in both solid tumors and hemangiomas (Jiang et al., 2019b). There exist various immune cell types, such as T cells, B cells, macrophages, dendritic cells (DCs), and mast cells, which originate from the bone marrow. However, it is worth noting that this specific characteristic is not limited solely to immune cell populations, but is also observed in certain non-immune cells (Patel et al., 2021). Cancer cells and antigen-presenting cells (APCs) can respond to type 1 and type 2 interferon stimulation by upregulating PD-L1. Activated macrophages and dendritic cells are where PD-L2 is most commonly found, despite the fact that PD-1 expression is ubiquitous (Dong et al., 2017). Furthermore, there is evidence that PD-L1 is expressed in the microenvironment of tumors, specifically in suppressor cells that are generated by myeloid cells, dendritic cells (DCs), and tumor cells (Peng et al., 2020). In colon cancer, myeloid-derived suppressor cells (MDSCs) with elevated levels of programmed death-ligand 1 (PD-L1) maintain an inhibitory role by dampening T cell activation (Weber et al., 2018). Additionally, Head and neck squamous cell cancers have been linked to high numbers of CD4+ and CD8+ tumor-infiltrating lymphocytes (TILs) and PD-L1 expression on tumor-associated macrophages (TAMs) (Li et al., 2021). The protein known as PD-L1 has been identified in plasma cells, as well as in specific sub-populations of dendritic cells, in instances of multiple myeloma (Ahn et al., 2021). Anti-PD-1/anti-PD-L1 antibody performance in stimulating anti-tumor T-cells can be compromised by the existence of PD-L1+ plasma cells and CD141+ mature

DCs (Costa et al., 2021). Consequently, the potential of this response as a therapy option that is both viable and effective for people suffering from multiple myeloma has become apparent (Kyle and Rajkumar, 2009). However, Autoimmune disorders, viruses, and their pathogenesis all depend heavily on the PD-1/PD-L1 pathway (Velu et al., 2015), organ transplantation immunology, and cancer immunity (Zhang et al., 2021). In healthy individuals, this pathway functions to prevent excessive tissue inflammation and autoimmune diseases by inducing and maintaining peripheral immunological tolerance(Qin et al., 2019). Tumor immune evasion takes place tumor-infiltrating lymphocytes (TILs) have their activation and programmed cell death inhibited when the programmed death 1 (PD-1) receptor interacts with PD-L1 (Vathiotis et al., 2021). Furthermore, this interaction has the effect of inhibiting the synthesis of cytotoxic T lymphocyte (CTL) granular enzymes and perforin, while also reducing the secretion of inflammatory mediators including interferon-gamma, interleukin-2, and tumor necrosis factor-gamma (Cunningham et al., 2021). Additionally, It promotes the synthesis of IL-10, an immunosuppressive cytokine that blocks the maturation of T-cell responses (Iyer and Cheng, 2012). In order to specifically target cancer cells, therapeutic approaches that make use of inhibitors of the PD-1/PD-L1 pathway have been used. It is essential to point out, however, that based on our present knowledge of the molecular mechanisms at play, it appears that only a small percentage of patients have experienced complete and long-lasting remission from their disease (Sun et al., 2020).

Checkpoint inhibitors that specifically target programmed cell death protein 1 (PD-1) and programmed death-ligand 1 (PD-L1) in the context of cancerous cells.

Evidence suggests that tumor cells can produce highly abundant levels of inhibitory signalling molecules (Jiang et al., 2019a). Tumor-induced immune suppression, which acts as an immunological gate, is a critical checkpoint mechanism in this network(Kim and Cho, 2022). The proteins PD-1 and PD-L1, also known as programmed cell death 1 and its corresponding ligand, respectively, are responsible for the regulation of this process. PD-1 is also known as programmed cell death 1, while PD-L1 is known as programmed cell death-ligand 1 (Salmaninejad et al., 2019).

Many different types of malignant tumor cells express PD-L1 (Akhtar et al., 2021), nonetheless, PD-1 is highly expressed in lymphocytes that are actively involved in immune system responses, including T cells, B cells, dendritic cells, and natural killer cells. PD-L1 is also present in many different kinds of healthy cells (Han et al., 2020). According to the findings of a number of studies, decreasing the amount of interaction that occurs between PD/1 and PD/L1 results in a better immune response from T cells and greater antitumor effectiveness (Seifert et al., 2017). However, The extracellular receptor known as programmed cell death protein 1 (PD-1) acts as a regulatory control for the action of effector cells that are involved in the process of cell depletion. When The suppression of T-cell activation is the end result of the contact between the programmed death-1 (PD-1) receptor and its ligand, programmed death-ligand 1 (PD-L1). This connection plays a critical role in mediating signal transduction, which ultimately leads to the inhibition of T-cell activation (Hudson et al., 2020) (Figure 2). Since solid tumors and antigen-presenting cells within tumor tissues can maintain the viability of cancerous cells by increasing their expression of PD-L1, there is significant interest in developing anti-PD/1;/PD/L1 antibodies as a potential cancer treatment (Zanello et al., 2022). Malignant cells can sustain their survival mostly under conditions where the levels of PD-L1 are elevated (Balar and Weber, 2017). There have been significant developments in immunotherapy, particularly in the clinical testing and application of checkpoint inhibitors. These inhibitors include antibodies that target PD1 and CTLA-4 as immunological checkpoints (Wojtukiewicz et al., 2021). By blocking the interactions between these checkpoints and their ligands, these inhibitors help to enhance the immunological response against tumors and restore the immune cell activity against cancer cells. In contrast to the mechanism of action exhibited by anti-CTLA-4 antibodies, which function by inhibiting the function of CTLA-4 on T cells, anti-PD-1 antibodies specifically target PD-1 receptors on T cells (Seidel et al., 2018). Both classes of inhibitors have demonstrated encouraging outcomes in clinical tests and have received regulatory approval for the treatment of a variety of cancers (Naimi et al., 2022). The demonstrated capacity to improve overall survival rates and achieve solid responses in patients establishes their significance as viable therapeutic options in the fight against cancer (Shiravand et al., 2022). Melanoma, a highly mutated form of human cancer, has exhibited a remarkably high rate of response to anti-PD-1 therapy, ranging from 30 to 40%.

(Gellrich et al., 2020). Immunotherapy with ICI has also exhibited promising results in germ cells testicular cancer that is resistant to platinum-based treatments with the remarkable outcomes observed, immunotherapy drugs have the potential to emerge as the fundamental treatment approach for several different kinds of cancer (Kalavska et al., 2020). In contrast, the effectiveness of PD-1 pathway-blocking antibodies in treating cancers, such as pancreatic and prostate cancer, which have lower median mutational loads, has been limited. The receptor known as programmed cell death protein 1 (PD-1), also referred to as CD279, exhibits co-inhibitory characteristics. Following antigen stimulation, the presence of T-cell activation is discernible on the outer membrane (Figure 2) (Xu et al., 2021). Additionally, mast cells, dendritic cells, and macrophages have all been found to express PD-L2 (Topalian et al., 2016). The protein PD-L1 has been identified in various categories of hematopoietic cells (Leon et al., 2020).

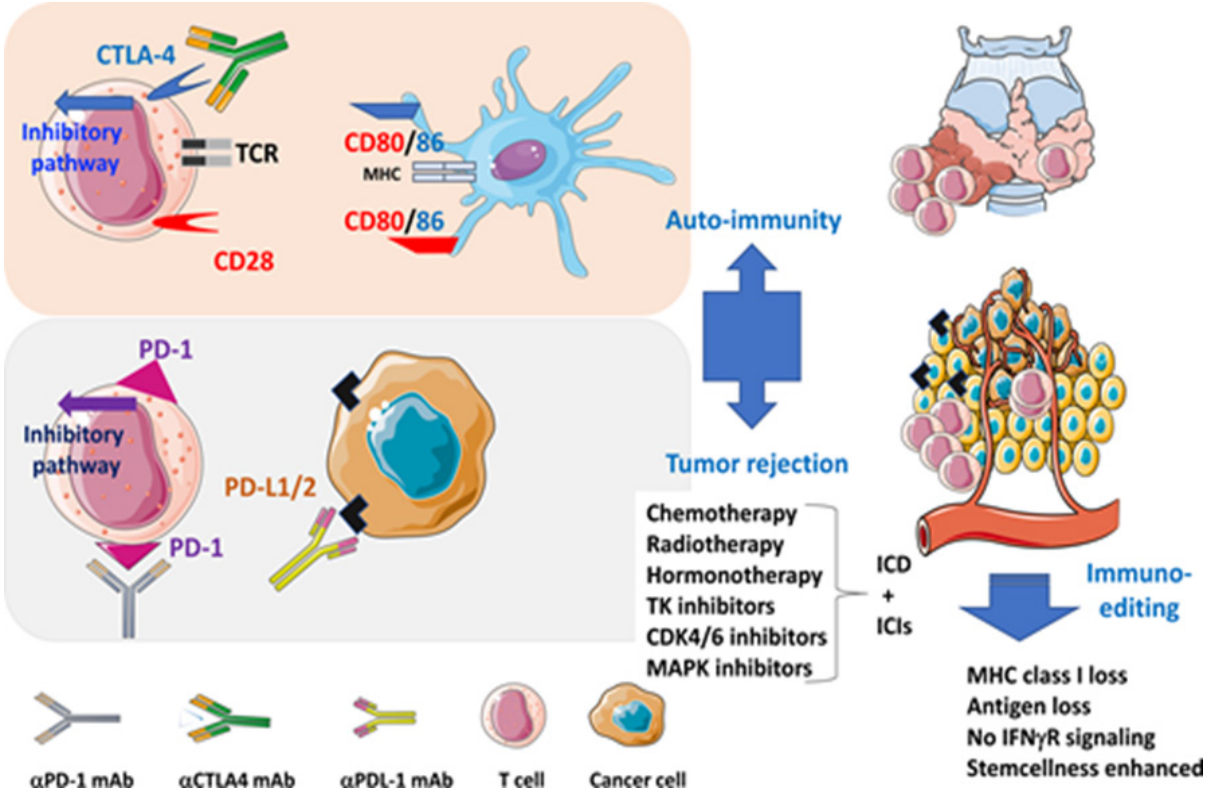

Figure 2. A diagrammatic image showing the primary tumour microenvironment, antigen-presenting cells known as dendritic cells are responsible for the processing of particular tumour peptides (TAA) and the subsequent association of those peptides with major histocompatibility complex (MHC) molecules. Dendritic cells are categorised as professional antigen-presenting cells (APCs). (Kroemer and Zitvogel, 2021).

Clinical applications of therapies targeting the blockade of PD-1 and PD-L1

PD-1 and PDL-1 blocking antibodies

Cancer immunotherapy induces the expansion of cancer-fighting T lymphocytes, which then eliminate malignant tumors by recognizing and targeting their presenting antigens (Abdullah et al., 2022). Monoclonal antibody molecules, commonly referred to as MAbs, have exhibited significant promise in the eradication of malignant tumors by selectively interacting with the programmed cell death protein 1 (PD-1) and its ligand, PD-L1 (Salmaninejad et al., 2019). Three anti-human PD-1 antibodies combine active immunization and nonspecific immune activation(Wang et al., 2018b). Nivolumab and pembrolizumab are representative examples of antibodies that have exhibited promising therapeutic capabilities about cancer therapy. The Food and Drug Administration (FDA) declined to issue their approval for the use of immunotherapy in the treatment of melanoma, non-small cell lung cancer, and other types of

malignancies until the year 2014 (Raedler, 2015b). In the year 2018, licensure was granted by the United States for the third-generation cemiplimab, which was designed to treat advanced or metastatic cutaneous squamous cell carcinoma (Migden et al., 2018). Pidilizumab, AMP-224, AMP-514, and PDR001 are not yet in clinical trials(Jiang et al., 2019b). Table 1 presents the indications of PD1/L1, antibody classes, and the side effects associated with FDA approval

Table-1

FDA-approved medications for immunotherapy of cancer

Target	Name of the medications	Route of administration	Purpose of using	Antibodies classes	Side effect
PDL-1	Avelumab (Bavencio)(Guo et (al., 2020	Intravenously (.(i.v	Malignant Merkel cell metastatic disease NSCLC cancer	IgG1	Body aches, swelling in the extremities, Bowel issues and a giant in weight
	Atezolizumab (Tecentriq)(Apo(lo et al., 2017	Intravenously (.(i.v	Urothelium, breast, bladder transitional cell cancers and carcinoma of the kidney	IgG1	Loss of appetite, dyspnea, dizziness .and coughing
	Duravulumab (Imfinzi)(Anto(nia et al., 2017	Intravenously (.(i.v	Cancer of the squamous cells of the head and .neck	IgG1κ	Bladder discomfort, oedema in the face, arms, hands, lower legs, or feet, tightness in the chest, shivers and sneeze
PD-1	Nivolumab(Overman et (al., 2017	Intravenously (.(i.v	NSCLC) cancer) .Liver cancer Lymphoma of the Hodgkin .type	IgG4	.Backache Skin blistering, peeling, or loos.ening Pain in the bones, joints, or .muscles Burning, numbness, tingling, or .unpleasant feelings are all possible .Taste changes or is lost Tightnetumour-associated
	Pembrolizumab(Robert et al., (2014	Intravenously (.(i.v	Metastatic melanoma	IgG4 kappa	They include a reduction in white blood cell count. and platelets in the bloodstream, diarrhoea and stomach ache
	Cemiplimab(Migden (et al., 2018	Intravenously (.(i.v	Cutaneous squamous cell carcinoma	IgG4	Hands or feet that are numb, muscle or bone pain; nausea, diar.rhoea, and lack of appetite

Nivolumab

The monoclonal antibody nivolumab is an IgG4 antibody that has been modified to have human-like properties and is targeted at the protein PD-1 (Opdivo, BMS-936558, MDX1106) inhibits the binding of PD/1 to its specific receptor, PD/L1 (Guo et al., 2017). The FDA granted Nivolumab approval in December 2014 for advanced cancer treatment or metastatic melanoma, marking an important milestone in its medical application(Robert et al., 2015a). Nivolumab was granted approval by the FDA in March 2015 for its use in the therapeutic management of metastatic squamous-cell non-small cell lung cancer. This term refers to the methods and treatments that are used to treat and control the spread of this type of cancer in addition to the lungs, and it provides patients who have this condition with an additional treatment option (Borghaei et al., 2015). Moreover, Nivolumab has exhibited notable effectiveness in combating a variety of cancers, including melanomas and non-small cell lung tumors (NSCLC), and hepatocellular cancer, indicating its broad potential in treating diverse malignancies (Lepik et al., 2020) (El-Khoueiry et al., 2017). The findings of the study indicate that Nivolumab exhibited promising outcomes in terms of cancer response and extended periods of disease remission in individuals diagnosed with non-small cell lung cancer (NSCLC) (Sundar et al., 2015). The overall response rate (ORR) was determined to be 23.7%, while the progression-free survival (PFS) was observed to be 91.1 days (Sato et al., 2018). Furthermore, it is important to note that around 80% of individuals diagnosed with Hodgkin's lymphoma experienced a minimum of three years of survival (Shanbhag and Ambinder, 2018). Moreover, the median duration of progression-free survival (PFS) fell within the range of 12 to 18 months (Goldkuhle et al., 2018) Adverse reactions such as pneumonia, colitis, and hepatitis, adrenal insufficiency, hypothyroidism, infusion reactions, cough, upper respiratory infection, peripheral edema, and fatigue have been reported (Ryder et al., 2014).

Pembrolizumab

Pembrolizumab (Keytruda, MK-3475) is The monoclonal antibody under consideration is designed to specifically target the programmed cell death protein 1 (PD-1) and has a significant binding affinity, as described by Longoria and Tewari (2016). Pembrolizumab is derived from lambrolizumab, which also targets PD-1. Pembrolizumab received FDA approval in 2014 (Keytruda, MK-3475, lambrolizumab) (Longoria and Tewari, 2016) specifically for addressing metastatic melanoma (Raedler, 2015a). The Phase I clinical trials conducted for melanoma provided evidence regarding the effectiveness and safety of pembrolizumab (Hamid et al., 2013), Additionally, the findings from Phase II clinical trials demonstrated a positive effect of pembrolizumab on metastatic melanoma, particularly when compared to ipilimumab, an antibody that specifically targets CTLA-4 (Robert et al., 2015a). The study findings revealed that Pembrolizumab was found to have a 33% objective response rate (ORR) in people with advanced melanoma (Robert et al., 2015b, Ribas et al., 2016). However, research on Pembrolizumab kinetics revealed that while its peak and trough levels remained essentially unchanged, over time, As doses were increased, the area under the plasma concentration-time curve increased (resulting in increased clearance and a wider AUC)(Longoria and Tewari, 2016).

Cemiplimab

The first checkpoint inhibitor that has received authorization from the FDA (Food and Drug Administration) was specifically developed for The primary therapeutic intervention employed for the management of advanced cutaneous squamous cell carcinoma (CSCC) involved the utilisation of cemiplimab (Libtayo), an anti-PD-1 antibody characterised by its high affinity (Fala, 2023). The results of a phase 1 clinical trial evaluating the efficacy of cemiplimab as a treatment for advanced cutaneous squamous cell carcinoma (CSCC) have demonstrated an extended response (Rischin et al., 2021), with no evidence of disease recurrence observed for a duration of up to 16 months following treatment (Naik, 2021) An expansion phase I study showed a 50% response rate and a durable impact, while a phase

II study revealed a 47% objective response, with adverse effects comparable to other PD-1 inhibitors (Migden et al., 2018). Common adverse effects of cemiplimab therapy include muscle or bone pain, rash, itching, nausea, and diarrhoea (LP, 2017). There are several anti-PD-L1 monoclonal antibodies available on the market. The FDA authorized atezolizumab, avelumab, and durvalumab in September 2014, May 2016, and May 2017, respectively. BMS-936559 and CK-301 are currently in the research and development stage (Akinleye and Rasool, 2019).

Atezolizumab

The monoclonal antibody atezolizumab (Tecentriq) is an Fc fragment of human IgG1 origin that was engineered using phage display technology (Alfaleh et al., 2020). It blocks tumor-surface PD-L1 and has shown potential for cancer treatment (Krishnamurthy and Jimeno, 2017). Since, the Fc portion of atezolizumab can undergo gene editing to reduce its antibody-dependent cell-mediated cytotoxicity (ADCC) effect (Powles et al., 2014). In May 2016, Atezolizumab is the first PD-L1 inhibitor to be approved by the Food and Drug Administration (FDA) for the therapeutic management of urothelial carcinoma. This authorization makes it possible for atezolizumab to take its place as the first PD-L1 inhibitor (Ning et al., 2017). Positive treatment responses with atezolizumab have been documented in various other malignancies, that includes renal cell carcinoma, transitional cell carcinoma of the urethra, and breast cancer (Balar et al., 2017). In a clinical trial, 62 people with renal cell carcinoma were given atezolizumab to test its efficacy and safety (Bosma et al., 2022). The results indicated an objective response rate (ORR) of 26% (Hodi et al., 2010). Additionally, The objective response rate (ORR) of treatment of advanced transitional cell bladder cancer with atezolizumab was found to be 26%, whereas for breast cancer treatment, the reported ORR was 10% (Bernard-Tessier et al., 2018) (Jiang et al., 2019b). Common adverse drug reactions associated with atezolizumab include fatigue, pneumonitis, colitis, inflammation of the thyroid, pituitary, and/or adrenal gland (0.4%), ocular inflammation, and infusion-related reactions (Corp., 2014).

Avelumab

Merck and Pfizer made an announcement in November 2014 regarding the development of avelumab, which is marketed as Bavencio and identified by its designation (MSB0010718C), is described as a monoclonal antibody of the IgG1 class that targets PD-L1 and is fully derived from human sources (gangolf.schrimpf, 2023). The intrinsic Fc region of Avelumab shows the ability to initiate antibody-dependent cell-mediated cytotoxicity (ADCC) by activating inactive T cells and inhibiting PD/L1. Furthermore, the administration of Avelumab demonstrated a notable objective response rate of 62.1% among patients diagnosed with metastatic Merkel cell carcinoma (D'Angelo et al., 2018). However, lung non-small cell carcinoma's overall response rate was only 12% (Gulley et al., 2017). Avelumab therapy is associated with the occurrence of exhaustion as a side effect, body aches, swelling in the extremities, bowel issues (both loose and hard stools), endocrinopathies, Crohn's disease, gastrointestinal and autoimmune disorders, as well as infectious diseases such as pneumonia and hepatic conditions. These results were published in 2018 (Bernard-Tessier et al., 2018).

Duravulumab

Imfinzi is a monoclonal antibody that targets immunoglobulin G1 kappa (also known as IgG1). Durvalumab (also known as Imfinzi) falls into this category that has undergone humanization to closely resemble endogenous human antibodies (Faiena et al., 2018). It inhibits the interaction between programmed death ligand 1 (PD-L1) and programmed death receptor 1 (PD-1), thereby preventing T-cell communication (Syed, 2017). Research investigating the administration of durvalumab therapy in individuals diagnosed with head and neck squamous cell carcinoma (HNSCC) resulted in an overall response rate (ORR) of 9.2% (Qiao et al., 2020) (BioIntron, 2021). Furthermore, it is currently being discussed what the progression-free survival rate is after six months for individuals who have been diagnosed and squamous cell carcinoma of the head and neck (HNSCC) was 20%, with a higher incidence of 25%

only in patients with a positive PD-L1 test result; progression-free survival rate (PFS) was not significantly different (Wise-Draper et al., 2022). However, In NSCLC patients, the ORR with durvalumab reached 66.3% (LP, 2017). Adverse reactions to durvalumab treatment include lethargy, urinary tract infections, muscle and joint discomfort, constipation, loss of appetite, peripheral oedema, infusion reactions, infections (such as pneumonia, hepatitis, colitis, hypothyroidism, and hyperthyroidism), and rashes(Lou et al., 2022).

Side effects of checkpoint inhibitors

antibodies of PD-1 generally exhibit reduced lethal and milder adverse effects in comparison to alternative therapeutic interventions (Wang et al., 2018a). However, they can still lead to significant adverse effects, including pneumonitis, which can occasionally result in death (Wu et al., 2017). Immune checkpoint inhibitors are commonly used for treating various types of cancer, but their potentially severe side effects pose a challenge (Ardolino and Joshua, 2019). Although combining two medications can enhance the effectiveness of cancer treatment such as melanoma, it is also associated with increased toxicity (Smalley et al., 2016). No evidence exists to indicate that managing toxicity through immune suppression compromises efficacy. Utilizing other checkpoint inhibitors can also be beneficial in boosting the patient's immune system, which performs a fundamental part in innate immunity (Achkar and Tarhini, 2017).

Promising advancements can be achieved through the utilisation of alternative checkpoint inhibitors that boost the immune system, a fundamental component of the body's natural immunological response within the patient (Darvin et al., 2018). The use of antibody-drug conjugates in combination with radiotherapy, either internal (via radioimmunotherapy) or external (through conventional beam radiotherapy), shows promise in cancer treatment (Dean et al., 2021). Prioritizing the management of suppurative symptoms is crucial, but it also carries risks for patients who choose this therapeutic approach. In conclusion, significant improvements can be achieved through the examination of multiple checkpoint inhibitors for enhancing the patient's immune response, with a simultaneous focus on wary toxicity monitoring and management, as well as the exploration of alternative therapeutic approaches.

Summary and future perspectives

The recent success of several immunotherapies, particularly checkpoint inhibitors, has brought immunotherapy to the forefront as a cancer treatment option. These therapeutic interventions have effectiveness in the treatment of a variety of different cancers based on clinical trials with demonstrate the capacity to contribute to the reduction of adverse effects associated with traditional chemotherapy. A number of recent researches have provided evidence supporting the effectiveness of cancer immune treatment that specifically targets the PD-1 as well as PDL-1 pathway can enhance sustained therapeutic immunity with reduced toxicity within multiple tumor types. While there is still much to learn about this signaling pathway, In the future years, the inhibition of the PD-1/PD-L1 signalling pathway represents a potentially important cancer immunotherapy strategy. The important questions remain unanswered in this field. Firstly, how do we select patients who are positive for PD-1and PD-L1? What traits are exhibited by individuals diagnosed with cancer? and which clinical detection method is the most effective? Secondly, what are the potential strategies that can be used to improve the infiltration of lymphocytes with CD8+ status into the tumor microenvironment (TME)? CD8+ T cells with tumor-reactive TCR repertoires have the potential to eliminate cancer cells, so understanding how to enhance their presence is crucial. Thirdly, how does PD-1 regulate CTLs and T-regulatory cells, and how does PD-L1 modulates the activity of cancer cells and antigen-presenting cells (APCs) within the tumor microenvironment (TME) is of interest? Can we develop more potent inhibitors based on these mechanisms? Fourthly, what is the effective treatment for PD-1and PD-L1-negative patients? Can these individuals take the advantage of alternative drugs or combination therapies that incorporate an anti-PD antibody strategy? Lastly, personalized indicators for guiding anti-PD therapy, alone or in combination with other targets, will be crucial for achieving clinical efficacy. Additional research is required to obtain a deeper comprehending of personal

genomic data to address these essential topics. Furthermore, the field of cancer treatment has recently undergone a transformative phase with the immunotherapy research and development, including PD-1 and PD-L1 pathway targeting. However, additional investigation is necessary to verify the efficacy and guarantee of using this method. It is important to note that immunotherapy takes time to establish itself as a key component of cancer treatment. In the past decade, it has been rapidly developed and authorized for various malignancies Although there have been notable advancements, the complications of cancer treatment have not been totally resolved by the use of immune checkpoint inhibitors (ICI). Although immunotherapy has presented promising opportunities, further research and development are still required. Our goals for the next decade include identifying biomarkers for predicting the efficacy and toxicity of ICIs, optimizing ICI regimens and exploring new combinations. Although cancer treatment has significantly improved patients' chances of survival and quality of life, response and toxicity predictions vary widely among different cancer types. Therefore, further investigation is needed in the area of cancer immunology in order to overcome these obstacles and drive the field forward.

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Quality Improvement of Cheese by Using Biological Methods in Sulaymaniyah Province

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Abstract

The use of biological approaches has been proposed as a way to improve the quality of Fresh cheese, which presents a significant issue for the dairy sector. The Aim of this research is to Improvement how using biological techniques can improve cheese quality in the province of Sulaymaniyah. Biological approaches can be used to enhance cheese quality in the Sulaymaniyah province. Producers can boost the value of their cheese by improving its flavor and texture with the help of starter cultures and ripening cultures. Chemical and microbial characteristics of Raw milk, curd, and finished cheese were compared between the two groups. Statistical packages SPSS can be utilized to examine information collected in Sulaymaniyah province related to the enhancement of cheese quality utilizing biological approaches. The data was summarized using descriptive statistics, t-test while the hypothesis can be tested using inferential statistics. The research found that by employing these techniques, cheese quality can be enhanced and its market value increased, benefiting both producers and consumers. The potential of these strategies in the context of Sulaymaniyah province has to be investigated further.

Keywords: Cheese, Quality, Biological Method, Microbial Characteristics, Milk

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Introduction

Cheese is a dairy product produced in wide ranges of flavors, textures, and forms by coagulation of the milk protein casein. It comprises proteins and fat from milk (usually the milk of cows, buffalo, goats, or sheep). During production, milk is usually acidified and either the enzymes of rennet or bacterial enzymes with similar activity are added to cause the casein to coagulate. The solid curds are then separated from the liquid whey and pressed into finished cheese.[1] Some cheeses have aromatic molds on the rind, the outer layer, or throughout. The governorate of Sulaymaniyah in Iraq's Kurdistan region is well known for its cheese. This study will examine the use of biological approaches in Sulaymaniyah province to enhance cheese quality. The utilization of microorganisms, including bacteria, fungus, and yeast, is at the heart of biological cheesemaking techniques. The fermentation process, in which these bacteria participate, is critical to the formation of cheese's flavor, texture, and scent. Shorter production times, higher quality and more consistent end products, and lower production costs are just a few of the benefits of using biological technologies in cheese production rather than more conventional approaches (Kandasamy et al., 2020).

Starter cultures are widely employed as a biological approach in cheese production. To begin the fermentation process, milk is typically mixed with a starter culture, which is a mixture of certain microorganisms Lactic acid bacteria. The acidic environment created by the bacteria's breakdown of lactose is essential for the coagulation of milk proteins. Cheese's flavor, texture, and scent can all benefit from the addition of starter cultures, and doing so also decreases the likelihood of spoiling (Alfaifi et al., 2020). Ripening cultures are another biological technique used in cheese producing. Cheese has finished maturing when a mixture of bacteria and fungi called "ripening cultures" is put on it. Cheese's proteins and lipids are degraded by these microbes, which leads to the creation of nuanced aromas and textures. Cheese's texture, flavor, and aroma can all benefit from the addition of ripening microorganisms (Jafarzadeh et al., 2021).

Biological approaches can be used to enhance cheese quality in the Sulaymaniyah province. Producers can boost the value of their cheese by improving its flavor and texture with the help of starter cultures and ripening cultures. The employment of biological approaches can also decrease the likelihood of rotting, which results in a longer shelf life and higher profits for farmers (Sharma et al., 2020). Because of its high nutrient content and distinct flavor, cheese is one of the most popular dairy products in the world. Cheese quality is affected by many variables, such as the milk used, the method of production, and the climate during ripening. In recent years, the quality of cheese has been enhanced by the use of biological approaches by increasing the microbial population during the cheese making process (Tang et al., 2020). The purpose of this research is to examine how using biological techniques can improve cheese quality in the province of Sulaymaniyah.

Literature Review

The quality of cheese, a widely consumed dairy product, can be affected by a number of factors, including the type of milk used, the temperature during processing, and the presence of microorganisms. The use of biological techniques to enhance cheese quality has gained popularity in recent years (Tilocca et al., 2020). The use of starter cultures is a typical biological strategy in the cheese producing process. When milk is fermented, the process is kicked off with the addition of starter cultures, which are made up of certain microbes. Cheese's flavor, texture, and scent can all benefit from the lactic acid produced by these microbes, which in turn lowers the milk's pH and facilitates the coagulation of milk proteins. The use of starter cultures has been proven in multiple studies to improve cheese quality. Bandyopadhyay et al. (2020), for instance, found that using specific starter cultures enhanced the cheese's texture and flavor.

Ripening cultures are another biological technique used to enhance cheese quality alongside starter cultures. Bacteria and fungus are introduced to cheese in the form of "ripening cultures" at a later stage in the production process. Cheese's proteins and lipids are degraded by these microbes, which leads to the creation of nuanced aromas and textures. Ripening cultures are used because they increase the cheese's quality and consistency. To give just one example, Zhou et al. (2019) found that using specific ripening cultures enhanced the cheese's sensory characteristics

and overall quality. There has been a rise in interest in the use of probiotics in cheesemaking as a biological technique. Probiotics are beneficial living microorganisms that are eaten by an individual. Research shows that adding probiotics to cheese boosts its nutrient content and health benefits. One study found that adding probiotics to cheese increased its antioxidant activity and lowered the risk of cardiovascular illnesses (Policastro et al., 2021).

Moreover, bacteriophages have been investigated for their potential use as a biological technique in cheese manufacturing. Bacteriophages, viruses that specifically target bacteria, can be employed to reduce the prevalence of harmful germs in cheese. Bacteriophages have been demonstrated to extend cheese's shelf life and decrease its susceptibility to deterioration. García-Cano al. (2020), for instance, found that the use of bacteriophages inhibited the development of Listeria monocytogenes in cheese, extending its shelf life. In conclusion, cheese's quality, nutritional value, spoilage risk, and shelf life can all be enhanced by the employment of biological approaches such as starter cultures, ripening cultures, probiotics, and bacteriophages. The impact of these biological approaches on cheese quality and their prospective uses in the food business require more study (Balkir et al., 2021).

It is common practice to employ starter cultures when making cheese because this boosts the product's overall quality. In order to kickstart the fermentation process, milk is typically inoculated with starter cultures, which are mixtures of carefully chosen microorganisms. These bacteria have the potential to enhance the flavor, texture, and aroma of cheese while also lowering the likelihood that the cheese will go bad. The utilization of starter cultures has been demonstrated in a number of studies to result in an improvement in the quality of cheese. For instance, a research project titled "Cheddar Cheese Flavor and Texture" carried out by Mohsin (2019) discovered that the utilization of a mixed starter culture greatly improved the flavor and consistency of Cheddar cheese in comparison to the group that served as the control.

One further strategy for improving the overall quality of cheese is the application of ripening cultures. When the cheese has been made, a mixture of bacteria and fungi called ripening cultures is added to it. Ripening cultures are also known as cheese cultures. Cheese's proteins and fats are broken down by these bacteria, which leads to the production of cheese's unique flavors and textures. The utilization of ripening cultures has been demonstrated in a number of studies to result in an improvement in the overall quality of cheese. For instance, the findings of a study carried out by Cai et al. (2021) indicated that the utilization of a particular combination of ripening cultures resulted in an improvement in the sensory quality of white cheese.

Another strategy for improving the cheese's overall quality is the use of probiotics. Live probiotic microorganisms are good for human health and are known as probiotics. Cheese's nutritional content can be improved with the addition of probiotics, which also have the potential to confer health benefits on those who consume them. Several studies have demonstrated that the addition of probiotics to cheese can result in an improvement in the product's quality. For instance, a research project that was carried out by Guerreiro et al. (2020) discovered that the addition of probiotic bacteria to cheese increased both its nutritional and sensory properties.

To summarize, the utilization of biological processes such as the addition of starter cultures, ripening cultures, and probiotics are all ways in which the quality of cheese can be enhanced. These techniques have been shown in a number of studies to enhance the flavor, texture, and scent of cheese as well as lower the danger of the cheese going bad and provide consumers with additional health advantages. By enhancing the product's overall quality and adding to its resale value, the implementation of these approaches has the potential to be beneficial for both customers and producers (Oh et al., 2019).

Materials and Methods

The cheese was made in the traditional fashion, beginning with raw cow's milk procured from a local dairy farm in the Sulaymaniyah region. Before the coagulation process began, the milk in the experimental group was treated with a microbial starter culture containing Lactobacillus bulgaricus and Streptococcus thermophilus. The standard pro-

cedure was used to create the control group, which lacked a starting culture. Chemical and microbial characteristics of milk, curd, and finished cheese were compared between the two groups. Statistical packages SPSS can be utilized to examine information collected in Sulaymaniyah province related to the enhancement of cheese quality utilizing biological approaches. The data can be summarized using descriptive statistics, while the hypothesis can be tested using inferential statistics.

Results

The results demonstrated that The cheese microbiota comprises a consortium of prokaryotic, eukaryotic and viral populations, among which lactic acid bacteria (LAB) are pH, moisture content, total solids, and protein content were dramatically raised after being fermented with the microbial starter culture. In addition, the population of beneficial bacteria grew, and the population of harmful microbes decreased, leading to improved cheese quality. According to the results of the tasting test, the cheese made with the microbial starter culture was superior to the control sample in both flavor and texture. It has been scientifically demonstrated that cheese quality can be elevated with the addition of microbial starter cultures. By supplementing the fermentation process with Lactobacillus bulgaricus and Streptococcus thermophilus, we were able to produce a cheese with enhanced flavor and texture. Starter cultures are used to regulate the microbial population and encourage the growth of favorable bacteria during the cheesemaking process. First, information about the chemical and microbiological characteristics of both groups at various points in the cheesemaking process must be entered into the program.

Table 1: chemical and microbiological characteristics of two different experimental groups at various points in the cheesemaking process.

Sample ID	Time point	pH level	Temperature ((°C	Moisture con- (%) tent	(%) Fat content	Bacterial count ((CFU/g
Group A_1	Pre-cul- ture	6.8	22	40	4.5	x 10^4 1.2
Group A_2	Pre-ren- net	6.4	28	38	4.3	x 10^4 3.5
Group A_3	Post-ren- net	6.1	33	37	3.9	x 10^6 6.8
Group A_4	Brining	5.9	20	35	3.5	x 10^5 8.1
Group B_1	Pre-cul- ture	6.9	22	40	4.6	x 10^4 1.5
Group B_2	Pre-ren- net	6.5	28	38	4.4	x 10^4 3.2
Group B_3	Post-ren- net	6.2	33	37	4.0	x 10^6 6.1
Group B_4	Brining	6.0	20	35	3.6	x 10^5 7.8

The pH, temperature, moisture, fat, and bacterial count of each sample are detailed in the table below at various

stages of the cheesemaking process. Each sample has a distinct ID, and the time stamp shows when the information was gathered. The two experimental groups being compared are Group A and Group B. All of the chemical and microbiological features of the cheese at various phases of the cheesemaking process can be compared between the two groups using this table.

Table 2: Descriptive Analysis

Variable	Group	Time point	Mean	Standard Deviation	Range
pH	Experimental	Pre-culture	6.8	0.2	7.0 - 6.6
pH	Experimental	Post-rennet	6.2	0.3	6.5 - 5.9
pH	Control	Pre-culture	6.9	0.1	7.0 - 6.8
pH	Control	Post-rennet	6.3	0.2	6.5 - 6.1
Moisture content (%)	Experimental	Pre-culture	40.5	1.5	42.0 - 38.0
Moisture content (%)	Experimental	Post-rennet	35.2	2.1	38.0 - 32.0
Moisture content (%)	Control	Pre-culture	39.9	1.0	41.0 - 38.0
Moisture content (%)	Control	Post-rennet	34.9	1.5	37.0 - 32.0
(%) Total solids	Experimental	Pre-culture	41.5	1.0	43.0 - 40.0
(%) Total solids	Experimental	Post-rennet	45.0	1.5	47.0 - 43.0
(%) Total solids	Control	Pre-culture	41.1	1.5	43.0 - 39.0
(%) Total solids	Control	Post-rennet	44.5	1.0	46.0 - 43.0
Protein content (%)	Experimental	Pre-culture	4.0	0.2	4.2 - 3.8
Protein content (%)	Experimental	Post-rennet	4.2	0.3	4.5 - 3.9
Protein content (%)	Control	Pre-culture	3.9	0.1	4.0 - 3.8
Protein content (%)	Control	Post-rennet	4.0	0.2	4.2 - 3.8

The table 2 above compares the experimental and control groups at various stages of the cheesemaking process in terms of several variables (pH, moisture content, total solids, and protein content). The descriptive statistics, including mean, standard deviation, and range, for each variable are shown in a table that is broken down by variable, group, and time point.

Data was summarized using descriptive statistics like mean, standard deviation, and range. pH, moisture, total solids, and protein content are only a few of the variables that may be measured and compared between the experimental and control groups throughout the cheesemaking process. This kind of examination is helpful for checking for any major changes in the cheese's chemical composition at various phases of the cheesemaking process and for comparing the differences between the experimental and control groups.

Table 3: t-test- comparison of cheese quality between experimental and control groups

Variable	Group	Mean	Standard Deviation	Sample Size	t-value	p-value
Flavor	Experimental	4.2	0.8	20	2.1	0.04
Flavor	Control	3.8	0.7	20	-	-
Texture	Experimental	4.5	0.6	20	3.6	0.001
Texture	Control	4.0	0.8	20	-	-
Appearance	Experimental	4.1	0.5	20	1.5	0.15
Appearance	Control	4.0	0.6	20	-	-

The above table 3 displays the outcomes of a t-test comparing the flavor, texture, and appearance of the experimental cheese to those of the control cheese. In addition to the sample size, t-value, and p-value, the table also displays the mean and standard deviation for each variable across all groups.

The experimental group's cheese is significantly better than the control group's, the p-value will be less than 0.05. With p-values of 0.04 and 0.001, respectively, the results suggest that there is a statistically significant difference in taste and texture between the experimental and control groups. Nonetheless, a p-value of 0.15 indicates that there is no discernible visual distinction between the two groups. This kind of study can help verify whether or not the application of a microbial starter culture results in superior cheese to the control group. When comparing the means of two groups, the t-test is a typical inferential statistical test used to assess if there is a statistically significant difference. The hypothesis that using the microbial starter culture significantly improves cheese quality relative to the control group can be tested using inferential statistics like the t-test or one-way analysis of variance. p 0.05 is a reasonable threshold for significance. If the p-value is less than 0.05, then there is a statistically significant difference in cheese quality between the experimental and control groups.

There is a connection between cheese's chemical and microbial features, and correlation analysis can help us figure it out. It is possible, for instance, to evaluate whether or not a substantial relationship exists between pH and moisture content by computing the correlation between these two factors. Lastly, descriptive statistics can be used to examine the outcomes of the sensory evaluation, summarizing the data and illuminating the favorite qualities of cheese among the participants. In conclusion, data analysis is a crucial stage in establishing the efficacy of biological techniques in enhancing cheese quality. Insights into the elements that affect cheese quality and help in building actionable plans to improve cheese quality can be gained through the application of appropriate statistical methodologies.

Conclusion

Finally, adding microbial starter cultures is one example of how biological approaches can boost cheese quality. The results of this study showed that the quality of cheese produced in the province of Sulaymaniyah was enhanced by the addition of Lactobacillus bulgaricus and Streptococcus thermophilus to the milk throughout the cheesemaking process. The findings of this study may be useful to the dairy industry because they suggest a simple and inexpensive way to improve cheese's quality. Research into the impact of different starting cultures on cheese quality in Sulaymaniyah province is encouraged.

In conclusion, the utilization of biological processes in the manufacture of cheese can improve the quality and consistency of the cheese, as well as lower the costs of production and raise the product's value on the market. In the province of Sulaymaniyah, the addition of starter cultures and ripening cultures to cheese has the potential to improve its flavor and texture while simultaneously lowering the possibility that the cheese will go bad. These methods have the potential to benefit both cheese farmers and consumers by boosting the cheese's profitability while also improving its overall quality.

Recommendations and Future Study

Several suggestions and areas for possible future research into enhancing cheese quality through the application of biological techniques in Sulaymaniyah Province are provided below on the basis of the mentioned literature review and results.

It is important to stimulate the dairy sector in Sulaymaniyah Province to use biological processes, such as starter cultures, ripening cultures, and probiotics, while making cheese. As a result, the cheese's quality and worth may rise.

Future research can examine the possibility that starter and ripening cultures isolated in Sulaymaniyah Province can be used to enhance the quality of cheese produced there. This has the potential to inspire the creation of innovative, high-quality cheeses that can be sold in the local market.

It is possible to study the impact of several climatic factors, including temperature, humidity, and altitude, on the development of starter and ripening cultures used in cheesemaking. As a result, the province of Sulaymaniyah may be able to optimize its cheese-making circumstances, leading to higher-quality cheese.

Examining the impact of probiotics on cheese quality is a promising area of research for the future, particularly in Sulaymaniyah Province. Because of this, probiotic cheese products that are good for people's health can be created.

Evaluation of the quality of cheese made utilizing biological processes can be accomplished by sensory analysis. This can be used to learn more about what customers want and improve production accordingly.

Limitations

There are few things that make it hard to study biological ways to improve the quality of cheese in Sulaymaniyah Province.The following are examples of such constraints:

Studies may have only used one type of cheese or one group of people, which limits how widely the results can be applied to other cheeses and groups.

Because some studies may not have had a control group, it may be hard to figure out how much of an improvement in quality can be attributed to the use of biological approaches.

Cheese quality may have been judged differently in different studies, which makes it hard to generalize the results.

Lack of the right tools, materials, and knowledge could make it hard for Sulaymaniyah Province to use biological methods to make cheese. Smaller businesses may find it hard to put these procedures into place because of this.

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Bisphenol A (BPA) Detection and Quantification in Plastic Bottles using Vertical Cultivation

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Abstract

Allium Cepa (Onion) is the most extensively produced vegetable in the Kurdistan region, with high production and resistance to environmental conditions. Considering its sensitivity to contaminants, it is commonly used for monitoring or testing environmental pollutants. Drinking water bottles made from polycarbonate plastics containing bisphenol A (BPA) are utilized. In the developing Kurdistan region of northern Iraq, there is an increasing issue of plastic bottle pollution. BPA traces have been found in bottled water samples. BPA release was assessed using HPLC in a vertical growing system with bulbs of the Allium Cepa plant placed in these plastic bottles and monitored growth. Vertical culture was discovered to have a low concentration of BPA in the plant cells than horizontal culture in soil, making it a safe growing method under certain climatic circumstances. The mean concentration of BPA in vertical cultivation is 0.19ug/ml (3.8ng for a 20uL injection), and the Limit of Quantification (LOQ) is 0.63ug/ml (12.7ng for a 20uL injection).

Keywords: HPLC, Bisphenol A, Vertical cultivation, Allium Cepa, plastic bottles.

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Introduction

Sulaimaniyah is one of the Kurdistan region's major cities that has developed significantly. The study area is classified as a separate Mediterranean-type continental interior and semi-arid climate; the summers are dry and extremely hot (average maximum temperature in July–August around 44 °C, and 5% humidity) with no rainfall, and the cool, rainy winters [1].

Allium Cepa (grain onion) is commonly cultivated in the Kurdistan Region considering the great production for obtaining plants with significant resistance to environmental conditions and impressive yield with diverse responses of plants to day length and temperature [2].

Allium Cepa has various advantages: its root growth dynamic is very sensitive to pollutants, it is commonly used for ecotoxicity testing, and it has been adopted for monitoring or testing environmental pollutants by the International Program on Plant Bioassays (IPPB) [2], [3].

Apart from the infrastructure revolution, the growing needs of the civilians cannot be met directly by the government. One of these needs is access to a clean water network. This shortage has forced people to drink water only bottled water. The demand is enormous as the area has more than 5 million inhabitants [4]. All plastic bottles ultimately find their way to landfills, causing environmental pollution. Waste recycling is currently unavailable in the research area that compiles with environmental regulations, and improper solid waste dumping has a negative impact on the environment. The local authorities incinerate the garbage, disregarding the fact that this process releases BPA into the air and soil, resulting in significant environmental pollution.

Solid waste is one of the significant environmental impact problems in developing countries. Approximately 3.5 million tons of MSW are generated daily globally [5]. Population growth, improving living standards after economic recovery, and industrial activities are all primary reasons for a significant increase in the quantity of solid waste [6]. The daily per capita waste generation in the Sulaimaniyah governorate was 1.32 kg in 2022, a cumulative solid waste of about 1,325,000 tons, and the plastic portion (bottles and bags) accounted for 13.30 % of the total waste [7]. Bisphenol A is a chemical compound widely utilized in several sectors, including synthetic polymers and specific applications in plastic containers, toys, and polycarbonate bottles. Microplastic ingestion and subsequent BPA are critical to the aquatic ecosystem's perceived harm and risk of pollution [8], [9]function and activity of endogenous hormones causing irregularity in the hypothalamus-pituitary-gonadal glands and also the pituitary-adrenal function. BPA has immuno-suppression activity and can downregulate T cells and antioxidant genes. The genotoxicity and cytotoxicity of BPA is paramount and therefore, there is an immediate need to properly detect and remediate its influence. In this review, we discuss the toxic effects of BPA on different metabolic systems in the human body, followed by its mechanism of action. Various novel detection techniques (LC-MS, GC-MS, capillary electrophoresis, immunoassay and sensors.

Among others, it releases BPA at room temperature. At higher temperatures, the rate of rise is faster. A considerable volume of bisphenol-containing items is already in the environment, making them significantly more hazardous [9] function and activity of endogenous hormones causing irregularity in the hypothalamus-pituitary-gonadal glands and also the pituitary-adrenal function. BPA has immuno-suppression activity and can downregulate T cells and antioxidant genes. The genotoxicity and cytotoxicity of BPA is paramount and therefore, there is an immediate need to properly detect and remediate its influence. In this review, we discuss the toxic effects of BPA on different metabolic systems in the human body, followed by its mechanism of action. Various novel detection techniques (LC-MS, GC-MS, capillary electrophoresis, immunoassay and sensors.

According to the United States Environmental Protection Agency (US EPA), the maximum amount of BPA permitted in the human body is 50 μg/kg BW (body weight)/day. However, after re-evaluation, a temporary TDI of 5 μg/kg BW/day was re-set by the European Food Safety Authority EFSA in 2014 [10], [11], [12].

Standard BPA detection techniques include gas chromatography - mass spectrometry (GC-MS) and high-pressure

liquid chromatography (HPLC) [13], [14], [15], [16].

vertical cultivation is an alternative for cultivation or gardens with limited space. This study aimed to establish a better approach to comparing BPA results in high-pressure liquid chromatography, particularly in mono compound manufactured materials. and to introduce the reuse of plastic bottles for vertical cultivation in private gardens and simultaneously explore whether this kind of cultivation would be safe for public health.

1.1 Bisphenol A (BPA)

Bisphenol A (BPA) C15-H16-O2 is the common name for 2,2-(4,4′-dihydroxy diphenyl) propane, 4,4′-isopropylidene diphenol, alternatively, 2,2′-bis (4-hydroxyphenyl) propane [17], an organic compound composed of two phenol rings connected by methyl bridge, with two methyl functional groups attached to the bridge [18]. It has been known since 1930 that BPA is an artificial estrogen, and its estrogen effect was used to promote industry profit [19]. Its essential properties include low vapor pressure, moderate water solubility, low volatility, and solid at room temperature [20], [21]. It is one of the highest-volume chemicals produced worldwide, with product estimations of more than 7 million tons in 2019 that were emitted into the ocean [22]either as macro- or microplastics. In this article, an overview is given of the presence of marine plastic debris globally and its potential to reach remote locations in combination with an analysis of the oceanic long-range transport potential of organic additives present in plastic debris. The information gathered shows that leaching of hydrophobic substances from plastic is slow in the ocean, whereas more polar substances leach faster but mostly from the surface layers of the particle. Their high content used in plastic of several percent by weight allows also these chemicals to be transported over long distances without being completely depleted along the way. It is therefore likely that various types of additives reach remote locations with plastic debris. As a consequence, birds or other wildlife that ingest plastic debris are exposed to these substances, as leaching is accelerated in warm-blooded organisms and in hydrophobic fluids such as stomach oil, compared to leaching in water. Our estimates show that approximately 8100–18,900 t of various organic additives are transported with buoyant plastic matrices globally with a significant portion also transported to the Arctic. For many of these chemicals, long-range transport (LRT. Being an important industrial chemical, Bisphenol A is used as a material for producing phenol resins, polyacrylates, and polyesters but is mainly used as an intermediate in producing polycarbonate (PC) plastics and epoxy resins. Polycarbonate plastics find extensive applications in various everyday items such as medical devices and food and beverage storage containers. This is due to their exceptional qualities including high impact strength, hardness, toughness, transparency, and resistance to temperature changes. Additionally, to safeguard food and beverages from direct contact with metal, epoxy resins are employed as inner coatings for food and beverage cans. BPA can also be present in children's toys as an additive, used in different types of plastics. [23] [24] [25], [26].

Although BPA is introduced extendedly in everyday life, supported by industry studies that showed various risks to human health [27], [28], studies funded by government agencies showed a wide range of effects on humans. BPA is an endocrine disruptor that can mimic the body's hormones [29], [30]. After entering the human body, BPA can disrupt normal cell function by acting as an estrogen agonist [29], [31], as well as an androgen antagonist [32], which may affect health. It has been suspected that BPA may be carcinogenic, potentially leading to the precursors of breast cancer [33]. In addition, exposure to BPA has been associated with chronic disease conditions in humans, such as cardiovascular disease and diabetes, and is a serum marker of liver disease [34], [35]. The ester bond linking BPA molecules in polycarbonate and resins is subject to hydrolysis, resulting in the leaching of BPA monomer even from new polycarbonate into the water at room temperature [25], [36], [37]. The heat and hydrolysis, such as that which occurs with the pasteurization and canning process, sterilizing, microwave heating, warming prior to serving, and washing of the containers, result in increased leaching of the BPA into the products that are consumed [38]. Now it is generally believed that consumer exposure occurs primarily via food in contact with BPA-containing materials, such as polycarbonate plastic baby bottles and table ware, plastic food containers and food and beverage cans lined with epoxy resins [27], [39]. It is also present in the air and drinking and other water sources. BPA leaches from the

soil into fresh water, and plastic and metal waste disposal are a major contaminant in landfills. High levels of BPA in the atmosphere that were measured in many regions in southern Asia are considered to be related to the burning of plastics for waste disposal, a treatment that takes place also at the Kurdistan region [26]. Measured concentrations of BPA in human blood, urine and other tissues confirm that exposure is widespread in the human population.

Materials and Methods

Small plastic bottles of 330ml were arranged in a vertical position. The narrow part with the cap was to wording the ground, a hole of 3cm in diameter was opened and 8cm diameter opening was created on the other side. The lower half part of the bottle was painted black from the outside and a small piece of sponge was placed inside the cap, as shown in Figure 1. A small leak was created on every cap and then the bottles were placed vertically with the top of the one into the bottom of the other. Another bottle was cut in the middle and placed on the top. A bigger one was placed at the bottom of the last bottle to collect the excess water. Apart from the first and the last bottles of each row, the rest were filled with soil. According to [40], The genus Allium is characterised by herbaceous geophyte perennials with true bulbs, some of which are borne on rhizomes, and an onion or garlic odor and flavor. Allium plants need light soil; thus, a mixture of sand-turf-soil (1-1-1) was used for plantation. The sponge at the bottom prevented the leak from being blocked by the mixture, and the black paint prevented the roots from coming in contact with the light, using the broader holes on the side the Allium bulbs were planted. Each vertical row consisted of one empty bottle on the top, five planted bottles in the middle and one empty bottle at the bottom. A total of 12 vertical rows were placed with 60 paired Allium bulbs in each bottle outdoors in the Autumn season, to ensure that a minimum of one of them will be successfully cultivated. The watering was done by hand-filling the top bottle. The water moved gradually from top to bottom, watering the plants and the excess water was collected and reused.

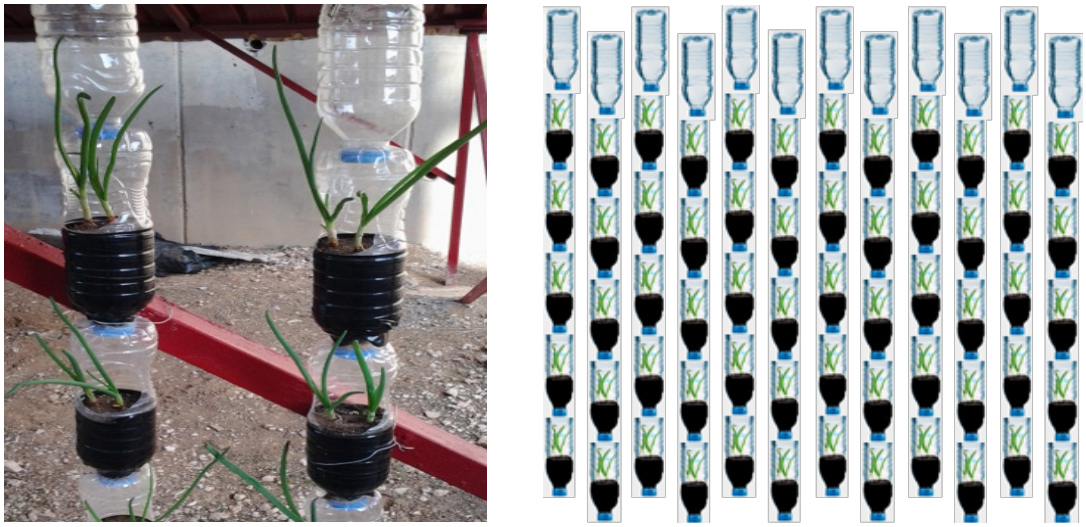
The same soil mixture was used for planting Alliums in the cultivation table, 60 paired bulbs were planted near the vertical arrangement to discover possible plant growth differences. For the laboratory data, BPA (>99%, CAS 80-05-7) was purchased from Sigma Aldrich (Germany). All other solvents used in extraction or chromatography were HPLC grade and purchased from Merck, Germany. Milli-Q water system was purchased from the USA (Millipore, Bedford, MA). In addition to standard laboratory materials, the following equipment is essential for the use of AFFINIMIP SPE cartridges (solid phase extraction):

(i) SPE vacuum manifold (Phenomenex, USA).

(ii) Mini vacuum pump.

The critical step is to follow the flow rate given in the protocol accompanied by AFFINIMIP SPE cartridges.

Fig.1 Vertical cultivation in plastic boatel.



HPLC Sample Preparation and Analysis

Samples were received in good condition and stored in glass containers in a refrigerator under -2 oC before analysis.

Cold spring onions are put in a desiccator to get to room temperature before extraction process. Then, 25g of spring onions, including the root system, and 50ml of water/acetonitrile are shaken for 30min in an orbital shaker at 150rpm. After that, solids are removed with filtration through a filter paper (4-7um), and the filtrates are centrifuged for 10min at 4000rpm. Supernatant solution is collected and filtered off through a filter paper as previously. Filtrates are diluted 1:1 with water to give the loading solution [41]. The clean-up method followed, as shown in the table below.

The solution is collected in an amber glass vial (4ml) and is evaporated until dry under nitrogen or in a speed vac concentrator. During analysis, the dilute factors should be considered in calculations. The obtained eluent was filtered through a 0.22 um filter before injection. To avoid cross-contamination, all vials were free from polycarbonate material or polymers[42].

Table 1. Sample Clean-up Method in HPLC

(Steps (Flow rate	(AFFINIMIP SPE BPA (100mg/6ml
Equilibration with 2drops/s .1	(3ml Methanol (2% Acetic acid 3ml Acetonitrile 3ml Water
Loading (ml)1drop/2s .2	20ml of diluted filtrate
(Washing of interferents (1 drop/s .3	9ml of ultrapure water (6ml of Water – Acetonitrile (60:40, v/v
Drying .4	Force the water down and out the bottom, apply vacu-.um for 30s
(Elution (1 drop/s .5	3ml of Methanol

HPLC Analysis Conditions

Chromatography analysis is performed using a Waters 2695 Alliance HPLC system with a 996-photodiode array detector (Waters, USA). BPA is monitored at 228nm. Chromatographic separation is carried out with a C18 reversed-phase column (Symmetry RP 18 150mm x 3.5mm, 5um) in gradient mode at a flow rate of 0.8ml/min, as shown in Table 2. HPLC data collection and manipulation were performed on a PC running Millenium (Waters, USA). The injected sample volume is 20 uL. The oven temperature was set at 25 oC.

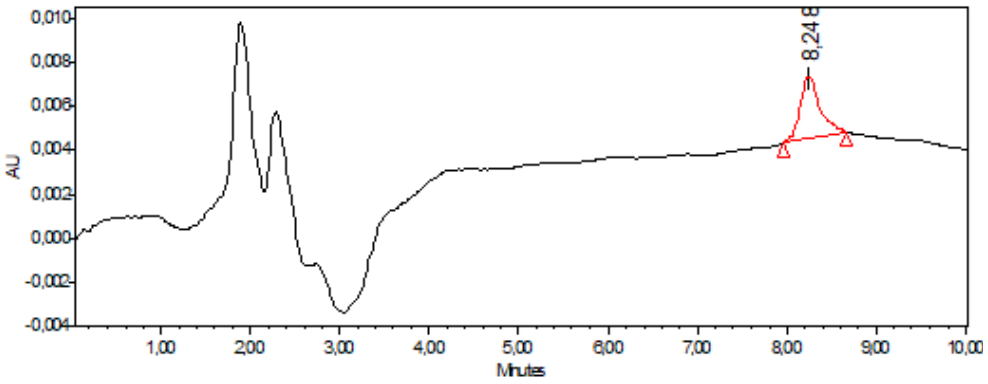
Table 2: HPLC Gradient system

(Time (min	(%) Acetonitrile
0	40
15	95
20	95
23	40

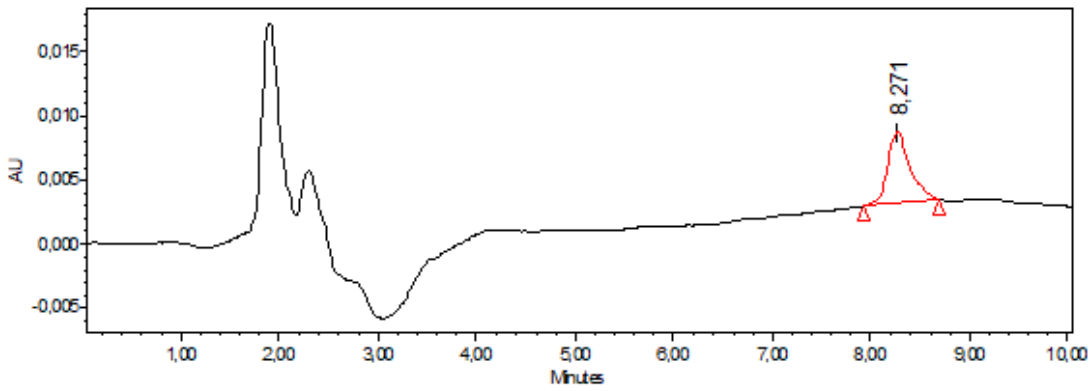
Results

Six standard solutions (250 - 10.000ng/ml) are tested to determine the linearity of BPA as shown in Figure 2.

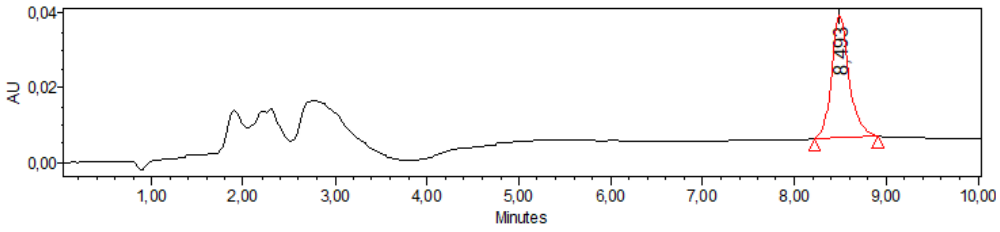
Std. of 250ng/ml



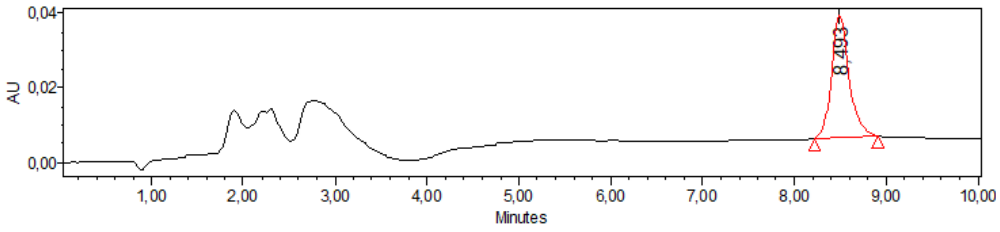
Std. of 500ng/ml



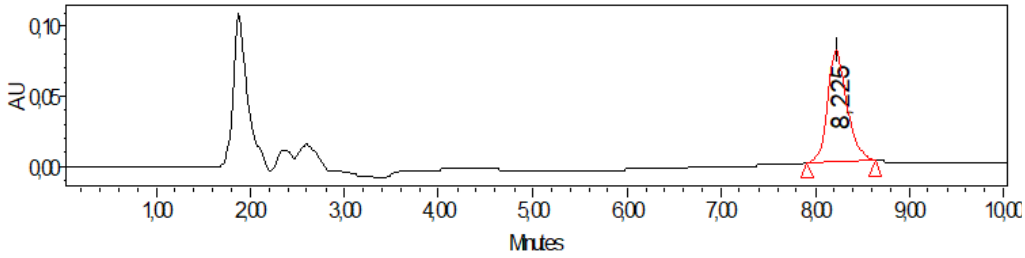
Std. of 1000ng/ml



Std. of 2000ng/ml



Std. of 5000ng/ml



Std. of 10000ng/ml

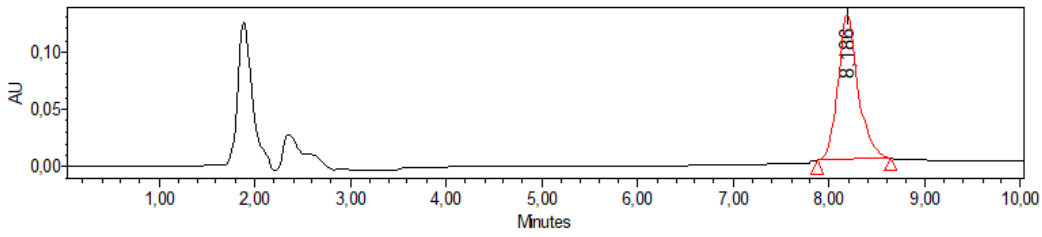


Fig 2. HPLC Chromatograms of calibration curve and samples.

The peak area and concentration of BPA are subjected to regression analysis to calculate the calibration equation and correlation coefficient. The regression equation of BPA is y= 0.0001 x+0.0805 (correlation coefficient 0.999), as shown in Figure 3. The limit of detection (LOD) of BPA is 0.19ug/ml (3.8ng for 20uL injection), and the limit of quantification (LOQ) is 0.63ug/ml (12.7ng for 20uL injection)

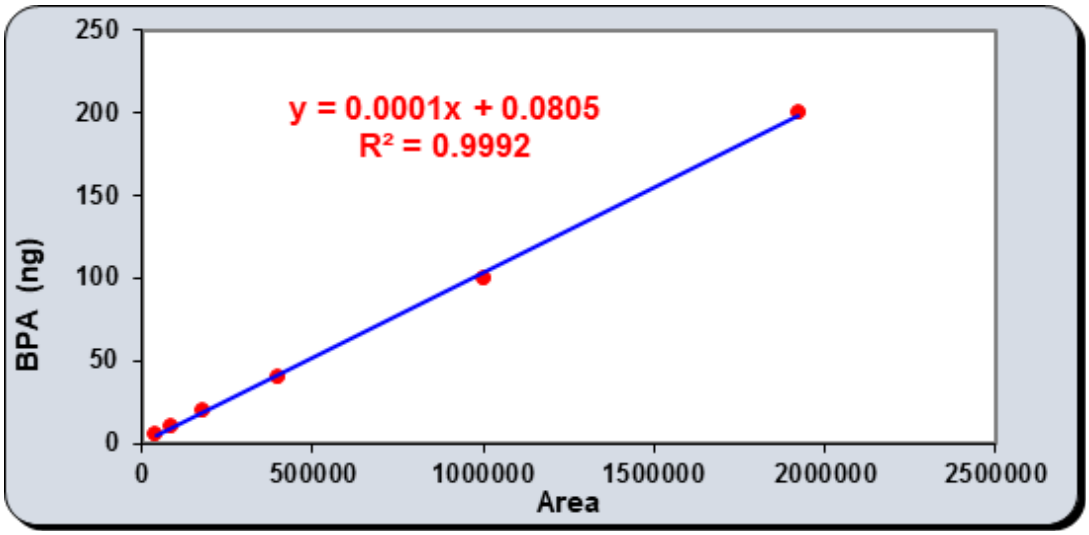
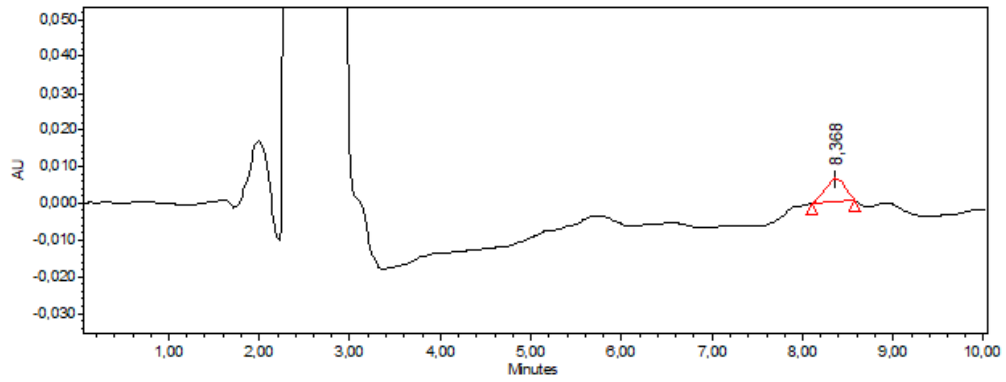


Fig. 3 correlation coefficient

Concentration of BPA in Planted Allium Cepa

AFFINIMIP SEP ® SPE cartridges are selected after testing other SPE methods (OASIS SPE, etc). MIPs are useful tools in sample preparation as analysis is entrapped in the polymer (BPA). Thus, MIPs offer high recoveries (>95%), repeatability and reproducibility. Method validation, robustness has to be examined by repeated experiments.

Figure 4. displays the chromatograms of the analyzed spring onions for BPA occurrence. BPA is detectable at an average level of 300 ng/g of Allium Cepa.



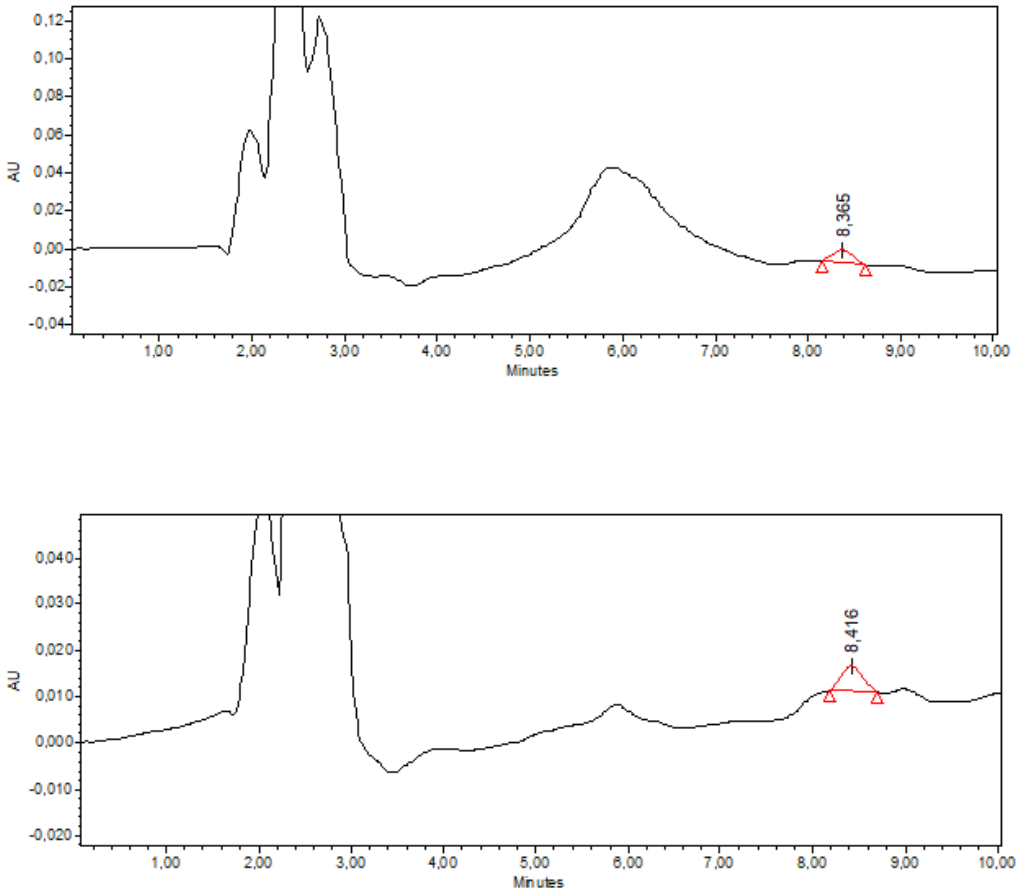


Fig. 4 HPLC chromatograms of the analyzed Allium Cepa for BPA

[43]2-bis (4-hydroxyphenyl set a total Tolerable Daily Intake (TDI) of BPA is 0.05 mg/kg body weight (bw)/day, which is gradually higher than the Allium concentrations that were found in current study The mean concentration of BPA in vertical cultivation is 0.19ug/ml (3.8ng for a 20uL injection), and the limit of quantification (LOQ) is 0.63ug/ml (12.7ng for a 20uL injection).. Even though the concentrations found are low, it is significant to consider that the daily intake refers to the total amount of BPA that an animal can intake, which includes many other sources of products that consist of BPA, like water, air and foods. Especially in the areas where the wastes are burned, the BPA concentrations in the air and underground water are high [44]. In the study area, the garbage is burned, as it is mentioned above; therefore, the habitants surcharged with BPA from the air. The low amount of BPA at the plant might be due to the climate conditions. It is reported that the higher rate of BPA migration from the plastic to the container occurs at high temperatures and sunlight exposure [45],[46]. During the experiment, the temperatures were low, from 2 oC to 16 oC, and the exposure to sunlight was less than 1 hour per day. Thus, the impact of these climatic conditions did not affect the migration of BPA to the soil. Furthermore, a very significant factor that affects the BPA concentration is that phytoremediation reduces the detected amount of it. The plants have the ability to transform the BPA into other forms that are harmless to human health [47], [48] and [49]. In addition, some microorganisms can metabolism the BPA concentration in the soil, causing a non-detected concentration in the plant cells [50].

Conclusion

In both cultivations, there was not a significant difference in plant growth. The only difference that was observed was at the time of bulb germination. The bottle cultivation occurred after four days, while the soil cultivation occurred after 13 days. This difference occurred because of the higher temperature in the root zone of bottles because of the sun heating. The plastic raised the bottles' soil temperature, germinating the bulbs faster than those in the ground soil. The rate of growth was the same after two months of cultivation. The weight of both cultivars was almost the same, with 25gr for each plant.

Although the concentrations of BPA at the cultivated Allium Cepa were significantly low,further investigation needs to be done under different climate conditions to ensure no health risk. The first results present a hopeful way of reusing plastic bottles, but at this point it is not safe to admit that there is no risk of BPA leach. Therefore, it is proposed to use this vertical arrangement of cultivation only for decorative purposes, planting small flower plants and cultivating low-growing vegetables during the winter time.

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Porosity and permeability distribution and their impaction on fluid production in carbonate reservoir rocks

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Abstract

Reservoir parameters including porosity and permeability are predominantly influence fluid flow movement and production rate in carbonate reservoir rocks. This impaction is corresponding to heterogenous distribution of these reservoir parameters which leading to anisotropic fluid production in the same reservoir intervals. In this work, the magnitude of matrix porosity and permeability were measured from core plug samples and available wireline log data was used for the porosity calculations in Baba Formation from Bai Hassan field. Besides of the petrophysical examinations, fluid loss data and formation test repeater results were used for evaluation of the impaction of porosity and permeability distribution on fluid flow and rate of hydrocarbon production. The rock types in the Baba Formation are characterized by a comprehensive distribution of measured matrix porosity and permeability. The magnitude of the effective porosity is started from 0.01 as the minimum value to 0.45 as the maximum with an average of 0.20 throughout the studied intervals which have been used for this study. The magnitude of measured matrix permeability is ranged from 0.01 mD to 827.04 mD with an average of 123.52 mD. The magnitude of average fracture porosity was 0.0036 and average fracture permeability was 0.394 mD. The rate of production is frequently controlled by the magnitude of matrix permeability throughout the studied wells. The highest rate of oil production is coincided with the highest magnitude of average matrix permeability and the lowest level of oil production was recorded was derived from the lowest magnitude of an average matrix permeability.

Keywords: Baba Formation; Fracture; Permeability; Reservoir; Carbonate.

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Introduction

The structure of the Bai Hassan field extends parallel to Kirkuk structure at its southwestern side within the Kirkuk embayment zone of Iraq Zagros basin. This anticline was identified by a stratigraphically complex area comprising multiple facies developments of a complicated diagenetic history. It is located within the Foothill Region of the northwest-southeast trending Zagros Fold and Thrust Belt, Figure (1). The field has been mapped previously as northwest-southeast trending doubly plunging anticline manifested as classic four-way structural closures (Dunnington, 1958).

The field consists of two domes with the SE – NW direction, Kithka Dome and Dauod Dome separated by a narrow saddle called Shahal saddle. It is mostly appeared that the Shahl Saddle is associated with a deep seated, axis-perpendicular, extension fault that was reactivated and influential in the structurally development of Bai Hassan during Miocene compression and folding. It is also likely that the structure is still in compression (NOC, 1992). Kithka dome is bigger in size and higher structurally by (335m) than Dauod dome (Buday, 1980). Dipmeter data attained on drilled wells show local dips in excess of 50 degrees that are most likely associated with faults. The top of the structure is relatively flat with dips less than 10 degrees. As a Tertiary reservoir Baba Formation has its importance in the process of petroleum exploration in Iraq and the region. Baba Formation was first defined by Bellen in 1956 from Kirkuk oil well-109, lithologically consists of porous dolomitized limestones, in surface outcrops the limestone has a chalky appearance, which is mostly massive, with some bedded parts (Bellen et al., 1959).

The Baba Formation in the Bai Hassan oil field is considered as the dominant reservoir rocks of the tertiary petroleum system. The reservoir productivity of this rock is predominantly varied throughout the field from the same interval. The initial rates of production obviously fluctuated from the drilled intervals in the same limb and between the two identified domes of the Bai Hassan structure. This phenomenon created a challenge during the production and development stage of the field for arranging and stabilizing rate of production and marketing from the Bai Hassan field.

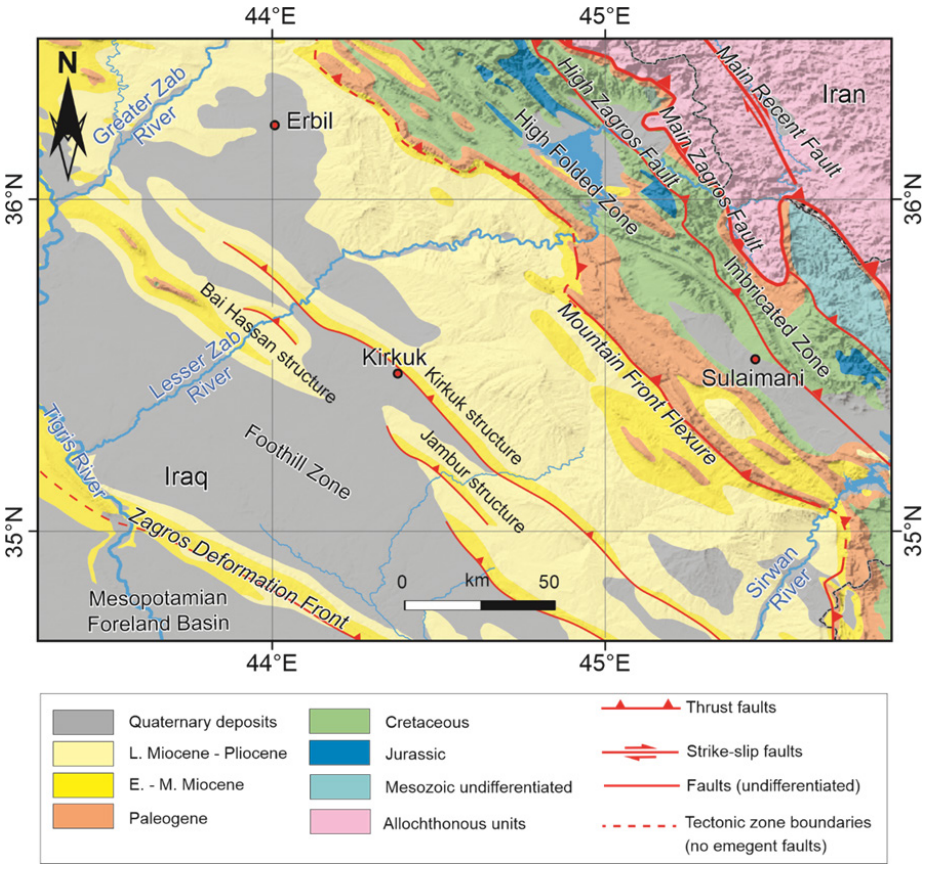

Figure 1. Geological map of the northeast of Iraq contains the dominant surface north-west and south-east trending structures. Bai Hassan locates in the Low Folded Zone (Foothill) of the Zagros basin which parallels to the Kirkuk, Jambur and other fields, modified from (Zebari et al., 2020).

This research works on the investigation of the quantitative distribution of porosity and its connection with the magnitude of permeability in the carbonate reservoir rocks of the Bai Hassan field in order to predict the lateral and vertical reservoir quality distribution throughout the studied field. It also examines fluid distribution and the level of the production based on these two parameters. The outcome of this research provides a clear vision for drilling new wells in development plan from the highest potential position in the field for producing the maximum rate of hydrocarbon. In addition, these results can be used as correlation data for supporting exploration strategy in the newly discovered field of the Kurdistan region licensed blocks.

Materials and Methodologies

The measured data for this study have been collected from five drilled wells of the Tertiary reservoir rock interval of Baba Formation in Bai Hassan oil field. The data cover core plug measurements of carbonate samples and composite wireline log raw data with in different intervals. The subsurface sections have been selected based on the location of the wells and position of the Bai Hassan structure with in the Kirkuk embayment zone. The preferred location of the wells started from the northwest to the southeast of the structure including well BH-20, BH-50, BH-78, BH-86, and BH-96, Figure (2). Well locations, elevations, the thickness of Baba Formation in the studied wells in addition to the depth intervals and type of data were used in this study are listed in Table (1).

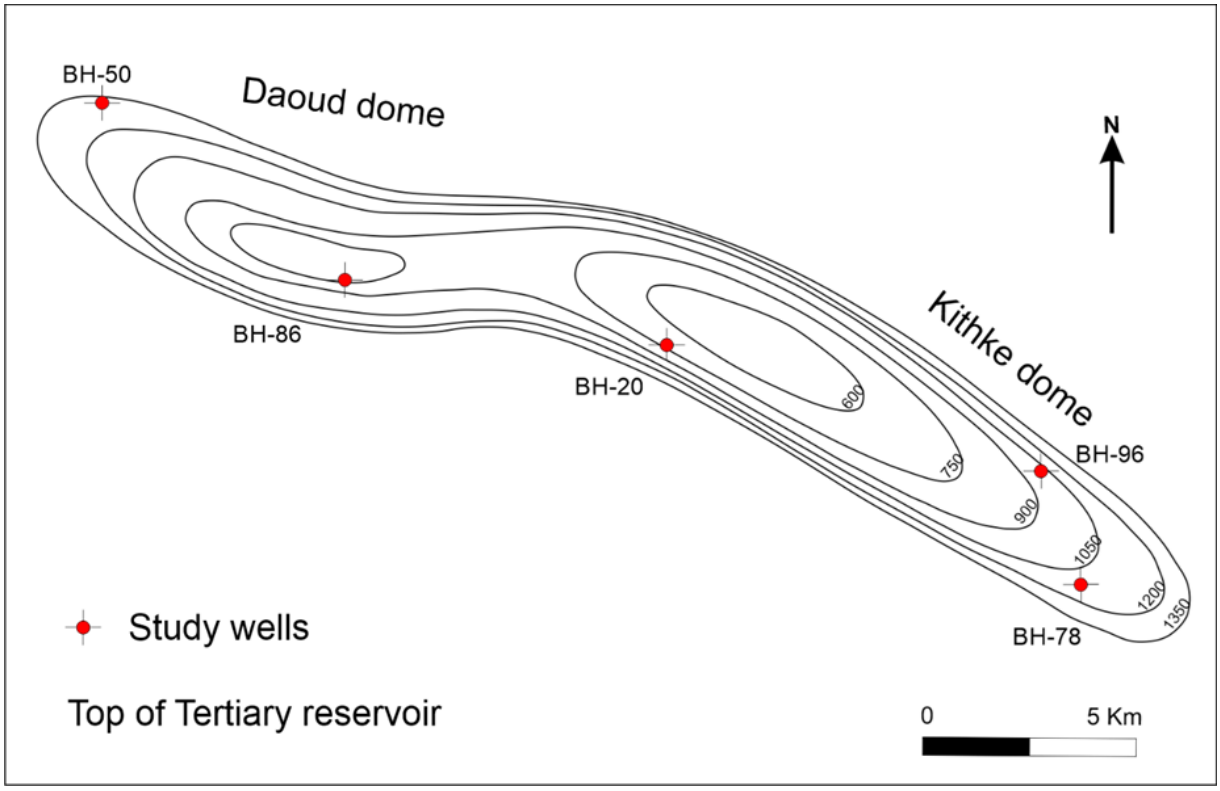

Figure 2. Contour map on the top of the Tertiary reservoir rocks of Bai Hassan field structure, modified from (Mustafa etal.,2020). The anticline composes of two dominant domes; Daoud, and Kithke. The studied wells including BH-20, BH-50, BH-78 BH-86 and BH-96 were drilled in different positions of the field.

The core analysis encompassed 143 plug measurements for matrix porosity and permeability obtained from the cored interval, achieved from the North Oil Company-Kirkuk laboratory. The porosity was measured using Boyle's law of gas expansion technique (Spain,1992) and applying nitrogen gas for grain and pore volume calculation for the plug sample, equation (1). The permeability of the same sample was measured based on modified Darcy's law steady state equation for single point measurement (Ross,2011) and consequently klinkenberg-corrected permeability for each sample was calculated, equation (2). Besides of these measurement dynamic data which include test results were obtained from repeat formation tester tool for the studied intervals have been used for this study.

$$\phi = \frac{(VB - VG)\ 100}{VB}$$

ϕ : matrix porosity, fraction.

VB : sample bulk volume, cm3.

VG : rock grain bulk volume, cm3.

$$K = \frac{14700\mu QL}{A\Delta P}$$

K: Permeability, mD.

Q: Flow rate, cm3/sec.

A: Surface area of the sample cm2.

ΔP: Differential pressure, psi.

μ: Dynamic viscosity of the fluid, centipoises.

L: Length of the sample, cm.

Table 1. The studied wells with their elevations and collected data.

Studied Wells	Elevation from KB (m)	Samples		Composite log	Baba Formation		
		Core	Cutting		Top m	Bottom m	Thickness m
BH-20	254.22	8	-	Porosity logs	1380	1422	42
BH-50	260.5	16	-		1420	1501	81
BH-78	245	26	-		1678	1719	41
BH-86	271	-	10		1180	1273	93
BH-96	247	-	10		961	1032	71

The fracture porosity and permeability from the studied intervals was calculated from the tested intervals using following equations (3 and 4). The results of these equations are representing the tested intervals which are relevant with the producible zones within the drilled wells.

$$\emptyset_f = 0.00173 \left[\frac{JB_o\mu_o \log\frac{r_e}{r_w}}{h}\right]^{\frac{1}{3}} \qquad (3)$$

$$K_f = \frac{JB_o\mu_o \log\frac{r_e}{r_w}}{23.6h} \qquad (4)$$

$\emptyset_f$: fracture porosity, fraction.

Kf: fracture permeability, mD.

J : productivity index, m3/day/psi.

B_o : oil formation volume factor, 1.31.

μ_o : viscosity of oil, centipoise, 1.30.

h: production interval thickness, meter.

$\frac{r_e}{r_w}$: well radius affected by production / well radius, meter.

Results and discussion

The magnitude of the measured matrix porosity in well BH-50 which was drilled in the northern-east limb of the Daoud dome is characterised by wildly distribution throughout the selected samples. The minimum value of the matrix porosity is 0.05 and the maximum measured matrix porosity is 0.37 with 0.24 as average matrix porosity, as shown in table (2). These magnitudes of the measured matrix porosities are modified to 0.02 as the minimum value of the matrix porosity to 0.36 as the maximum values and 0.16 of average porosity in well BH-20 which was drilled in the southern-west limb of the Kithke dome. The magnitude of the measured effective porosity in well BH-78 which is located in the same side of well BH-20 in the Bai Hassan field starts from 0.01 to 0.35 and 0.19 is the average measured porosity in this well.

The matrix porosity values in well BH-50, BH-20 and BH-78 are measured from the core samples while in well BH-86 and BH-96 are calculated from the gamma ray logs.

Table 2: Static parameters of the measured and calculated effective matrix porosity in the Baba Formation throughout the selected wells in the Bai Hassan field.

Well	Location	Matrix porosity (-)			
		Minimum	Maximum	Mean	Standard deviation
BH-50	Northeastern limb of Daoud dome	0.05	0.37	0.24	0.07
BH-86	Southwestern limb of Daoud dome	0.01	0.27	0.17	0.04
BH-20	Southwestern limb of Kithke dome	0.02	0.36	0.16	0.10
BH-78	Southwestern limb of Kithke dome	0.01	0.35	0.19	0.06
BH-96	Northeastern limb of Kithke dome	0.06	0.45	0.21	0.06
Well	Location	Matrix porosity (-)			
		Minimum	Maximum	Mean	Standard deviation
BH-50	Northeastern limb of Daoud dome	0.05	0.37	0.24	0.07
BH-86	Southwestern limb of Daoud dome	0.01	0.27	0.17	0.04
BH-20	Southwestern limb of Kithke dome	0.02	0.36	0.16	0.10
BH-78	Southwestern limb of Kithke dome	0.01	0.35	0.19	0.06
BH-96	Northeastern limb of Kithke dome	0.06	0.45	0.21	0.06

The magnitude of the measured matrix permeability in the Baba Formation was achieved from the steady

state technique using nitrogen gas as an injected fluid. Similar to the matrix porosity the magnitude of the matrix permeability is characterised by an isotropic and wide range of distribution throughout the available core samples which starts from microdarcies to millidarcies. The highest magnitude of the measured matrix permeability was observed in well BH-50 which has five orders of the magnitude of the permeability, table (3). The magnitude of the matrix permeability in this well started from 0.090 mD to 827.04 mD with an average of 123.79 mD. The same magnitude of permeability has been recorded in well BH-78 which has the minimum matrix permeability 0.01 mD and maximum average permeability 721.71 mD with an average of 148.32 mD. The measured matrix permeability in well BH-50 started from 0.030 mD to 523.69 mD with an average of 118.40 mD.

The measured matrix effective porosity and Klinkenberg-corrected gas permeability from plug samples of carbonate rock intervals were achieved from the Baba Formation in Bai Hassan field are shown in a semi-log poroperm plot in the Figure (3). A non-linear trend of enhancing the magnitude of the corrected gas matrix permeability can be observed with increasing the amount of porosity throughout the measured samples. The plotted corrected gas permeability ($K: mD$) as a function of the gas porosity ($\emptyset : fraction$)has power law relationships with a coefficient of determination (R2=0.6845) and this correlation can be presented on the empirical module as shown in equation (5).

$$K = 11129 \times \emptyset^{3.52} \qquad (5)$$

Table 3. Static parameters of the measured matrix permeability in Baba Formation.

Well	Location	Matrix permeability (mD)			
		Minimum	Maximum	Mean	Standard deviation
BH-50	Northeastern limb of Daoud dome	0.09	827.04	123.79	176.23
BH-20	Southwestern limb of Kithke dome	0.03	523.69	46.39	109.33
BH-78	Southwestern limb of Kithke dome	0.01	721.71	148.32	174.87

This relationship is derived from a complex and heterogenous rock microstructure and pore system in the studied carbonate reservoir rocks (Rashid et al.,2015; Rashid etal.,2017; Hussein et al.,2018; Mustafa et al.,2020). This anisotropy created a wide range of porosity and permeability distributions throughout the reservoir rocks including 37 porosity units and five magnitude orders of permeabilities, Figure (3). The magnitude of measured matrix porosity and permeability is predominantly related to the reservoir rock fabrics (Zangana etal.,2022). The lithology of the Baba Formation in this field comprises of three distinctive rock types including dolomite(dolostone), dolomitic limestone and limestone. The dolomite rock unit contains macro-intercrystalline pores which provided the highest magnitude of matrix porosity and permeability. The magnitude of measured matrix porosity in this rock type ≥ 0.10 and the magnitude of measured matrix permeability ≥ 100 mD to 827.04 mD. The best reservoir quality and highest potential rock types corresponded to this rock type. This rock type is predominantly common in the north eastern limb (flank) of Daoud dome and south-western limb of the Kithke dome. However, once the dolomite rock pores are filled with anhydrite minerals the reservoir quality is totally destroyed as the pore space and throat occluded by anhydrite minerals. The hydraulic connectivity and permeability with occurrence of anhydrite filling rocks are reduced and the lowest permeability throughout the measured samples was observed in the anhydrite pore filling intervals.

The reservoir quality of limestone rock type is totally opposed to the dolomite rock type in term of pore system and magnitude of porosity and permeability. The pore system is dominantly consisting of micro-intercrystalline pores that were preserved between the calcite crystals. The magnitude of porosity in this rock type < 0.09 and value of permeability is located in tight reservoir rock as the measured permeability throughout the selected samples are lower than 1.0 mD. This reservoir rock type is observed in the north-eastern limb of the Kithke dome and limited extensions have been observed in the south western flank of the Daoud dome.

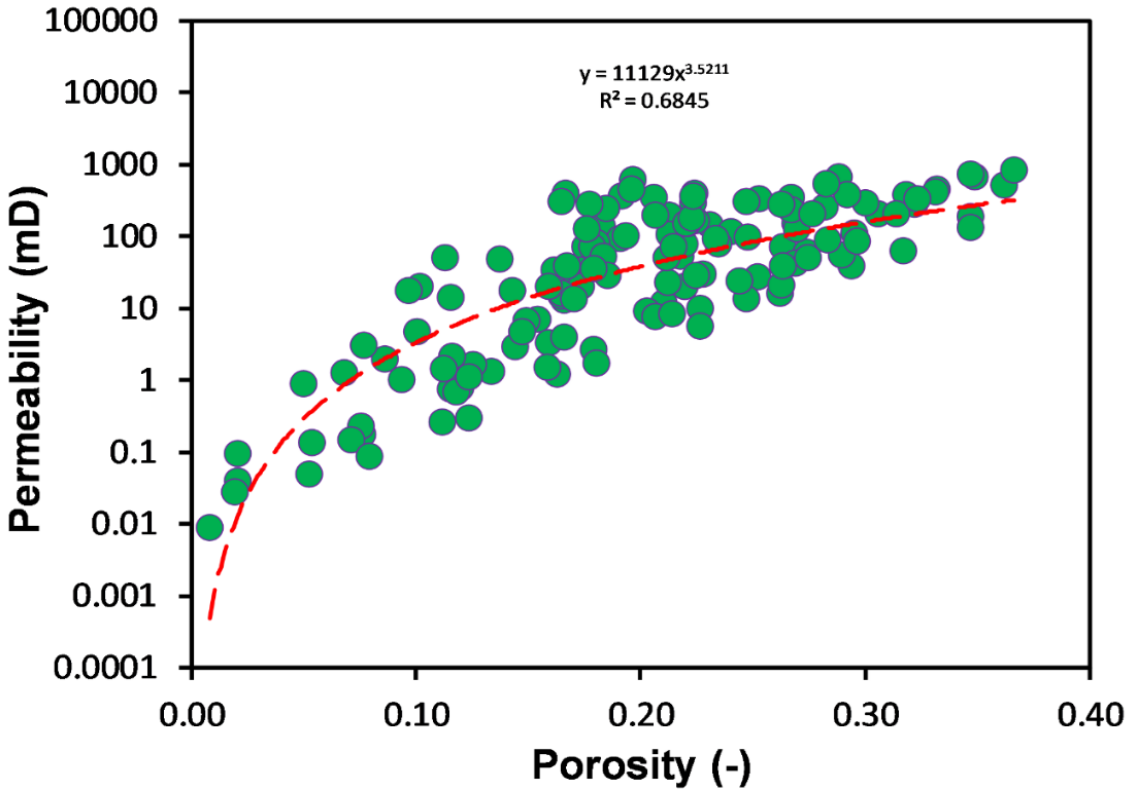

Figure 3. The measured Klinkenberg-corrected gas permeability as a function of the measured gas porosity for the achieved plug samples of Baba Formation in Bai Hassan field.

The most complex and complicated reservoir rock type is observed from the dolomitic limestone rock fabrics which are mineralogical composed of a mixture of calcite and dolomite minerals with some variable percentages of these two components. The pore system contains micro-intercrystalline pore between dolomite crystals, macro-intra-granular and macro-vuggy pores. The vugs are classified as isolated pores where they are interconnected through intercrystalline pore space with a small pore throat sizes. These pore throat sizes distribution gave a varied range magnitude of permeability which started from 1 mD to greater than 40 mD. The non-linear relationship between the matrix porosity and permeability in the poroperm plot, figure (3) is related to the ratio of pore size to pore throat size in the intragranular and vuggy pores. The pore size in this type of pores have a macro scale while the pore throat sizes have nano scales. This contrast in size distribution between pores and pore throats started from intragranular pore which pore size 6 time is larger than pore throat size in the same pore system to 14 times in vuggy pores. The dolomitic limestone reservoir type interval is frequently extended throughout the south-western limb of the Kithke dome.

The magnitude of average matrix porosity increases toward the north of the field including north-eastern limb of the Daoud dome and toward the southern part of the field which is covered by the south-western limb of the Kithke dome. The magnitude of porosity variations is influenced by pore size distribution in the Baba Formation. The highest values of the measured matrix porosities were observed in macro-intercrystalline pores between dolomite crystals and intragranular-vuggy pores of dolomitic limestone rock type. The magnitude of the matrix porosity in these two limbs are nearly similar and about 10 units of the matrix porosity is higher than the opposite limb of the same dome. The magnitude of matrix porosity was changed obviously in the dolomitic limestone rock type based on calcite and anhydrite filling pores extensions. The value of porosity increase in the dolomitic limestone once the pores were preserved and decreased when the rock fabrics contains fine crystalline of dolomite and the pores were filled with calcite and anhydrite cement. This rock type is available with high intensity of fractures in the northern east limb of the Kithke dome and southern west limb of the Daoud dome.

Similar to the matrix porosity, the magnitude of measured matrix permeability is increased towards north and northeast of the Daoud dome and towards west and southern west of the Kithke dome. The permeability enhancement is controlled by the rock microstructure and pore system as the largest pore throat size provided the highest magnitude of measured matrix permeability in the macro intercrystalline pores of dolomite rock fabrics. The average matrix permeability from core samples in well BH-50 which is located in the north-eastern limb of the Daoud dome was 123.79 mD, while this magnitude reduced to 87.20 mD in the same interval in well BH-59 which is drilled in the south western flank of the Daoud dome. The same variations have been observed in the Kithke dome where the magnitude of average measured matrix permeability in well BH-78 which is drilled in the south-western flank of the dome and it is 148.32. This value is reduced to 92.30 in well BH-32 which is suited in the north eastern flank of the dome.

Fracturing is an effective parameter which modifies the hydrocarbon storage ability and pathways of the reservoir rock. Natural fractures were observed throughout most of the core samples that were obtained from the Baba Formation. Some of the fracture surfaces are filled with calcite and anhydrite cements which totally or partially closed the interconnected fracture system and destroyed the fracture permeability. These fractures are characterised by inclined to sub-vertical fractures angles which are ranged between 40º-70º and have an average distance of 15-30 centimeters (NOC,2004). Fracture intensity is controlled by the tectonic position of the field and location of the drilled field in the Bai Hassan structure. The fracture distributions and fracture opening surfaces have been compared with the drilling mud losses (NOC,1989). The selected wells including BH-50, and BH-78 were drilled in different positions in the Bai Hassan field and they have no mud losses recording within the Baba Formation drilled intervals as all the wells located in the limbs of the domes, Figure (4). This result is related to low fracture intensity and fracture surface filling with calcite minerals. However, an extensive mud loss was recorded in the drilled intervals of the Baba Formation throughout the crestal part of the field including BH-70, BH-54.

The magnitude of the calculated fracture porosities is too low in comparison with the measured matrix porosity of the same intervals. Numerically, the matrix porosities are two units of magnitude greater than the fracture porosities. Consequently, available hydrocarbon in this reservoir rocks are dramatically stored in the matrix porosity in both domes and the matrix porosity has direct impaction on the reservoir storage potential. In addition, the measured matrix permeability is three orders of magnitude higher than the calculated average fracture permeability in the same reservoir intervals.

The average matrix permeability for the Baba interval in BH-50 which is drilled in the north eastern flank of the Daoud dome was 123.79 mD while the magnitude of the fracture permeability in the same intervals is 0.591 mD, table (4). The same results can be seen for the magnitude of permeabilities in the south western limb of the Daoud and Kithke domes. The magnitude of the fracture permeabilities in the listed wells is nearly similar while the flow rates are different in each well. This relationship between the magnitude of fracture permeability and flow rate proves that the production rate does not depend on the fracture distribution in the studied intervals.

The presented production data as shown in table (4) indicates that the potential fluid flow path ways in the Baba Formation are formed by the intercrystalline pore throats type and the magnitude of matrix permeability is controlled by the pore throat size distribution. The highest rate of production (12500 bbl/day) was recorded in the dolomite rock type intervals of the Baba Formation in well BH-50 which has an average matrix permeability of 123.79 mD. In well BH-20, the initial rate of production was 9300 (bb/day) which coincides with matrix permeability 118.40 mD. The flow rate in well BH-59 is reduced to 2875 bbl/day in the Baba formation which corresponds to an average of matrix permeability of 87.20 mD.

Well	Location	Net pay (m)	Flow rate bbl/day	re/rw	Fracture porosity (-)	Fracture permeability (mD)
BH-50	NE-Daoud dome	70	12500	3.118	0.0042	0.591
BH-59	SW-Daoud dome	49	2875	3.013	0.0030	0.2187
BH-31	NE-Kithke dome	46	1400	3.118	0.0037	0.4153
BH-20	SW-Kithke dome	66.5	9300	3.118	0.0035	0.3526

Table 4. Fracture porosity and permeability with production rate of the selected wells.

The lowest level of oil production was recorded in well BH-31 which is equal to 1400 bbl/day, and it derived from an average matrix permeability 34.55 mD. These numerical relationships between the magnitude of average matrix permeability and flow rate clearly give the result of the production rate corresponds to the value of matrix permeability.

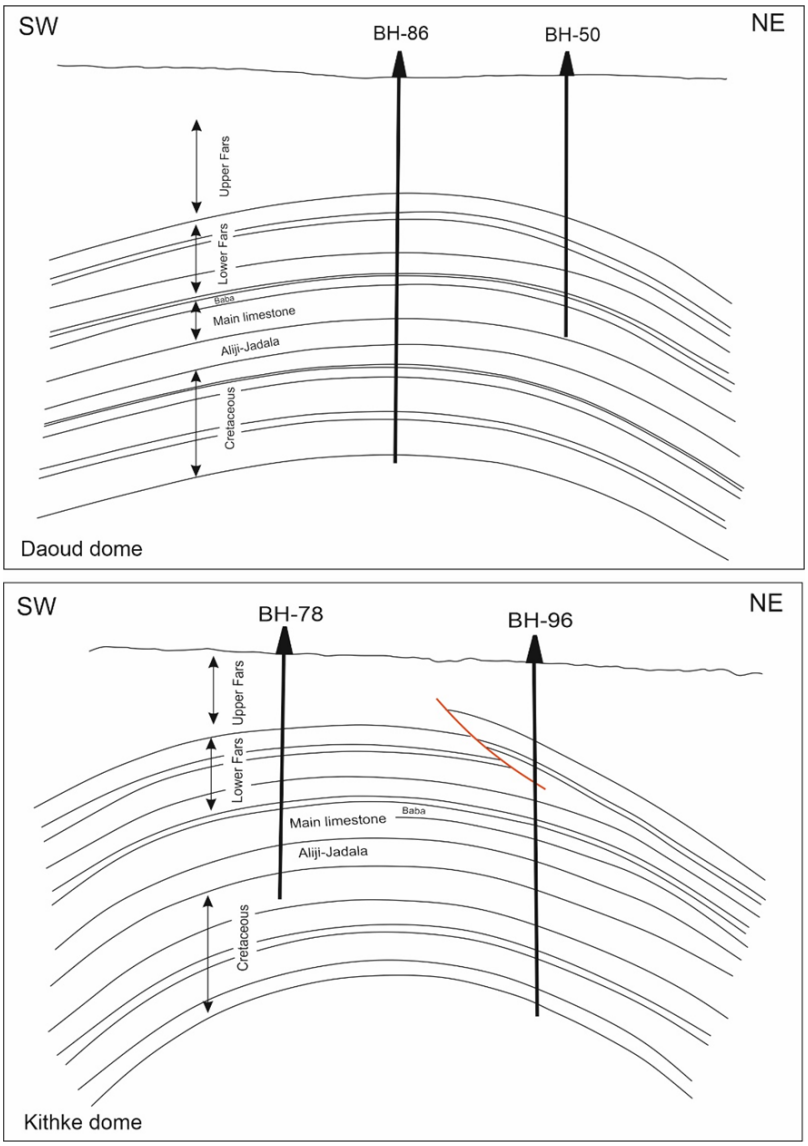

Figure 4. Diagram of Daoud and Kithke domes of Bai Hassan field. It shows the locations and paths of drilled well which are used in this study (NOC,1989).

4- Conclusions

This study analysed reservoir properties in heterogenous carbonate reservoir of the Baba Formation in the Bai Hassan field in Kirkuk embayment zone. The contribution of measured porosity and permeability in fluid flow and production rate has been examined using core samples, wireline logs, well tests and mud log data. The dominant outcomes of this work are summarised as follows:

- The rock types in the Baba Formation are characterized by an extensive distribution of matrix porosity and permeability in the Bai Hassan field. The magnitude of matrix porosity and permeability is predominantly influenced by pore structures and lithological components of the rock types of the Baba Formation.

- The value of matrix porosities are two units greater than the fracture porosities and available hydrocarbon in this reservoir rocks are dominantly stored in the matrix porosity throughout the field and the matrix porosity has effective role on the reservoir storage potential.

- The matrix permeability is three orders of magnitude higher than the calculated average fracture permeability in the same reservoir intervals as a result the production is mainly provided through matrix permeability which depends on the intercrystalline pore throat types in dolomite minerals of dolostone rock type.

- The matrix permeability value controlled the rate of production throughout the studied wells and the numerical relationships between the magnitude of average matrix permeability and flow rate clearly give the result of the production rate is corresponding to the value of matrix permeability.

Acknowledgement

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Classification And Noise Reduction of Electromyography Signals

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Abstract

This research aims to present a new method of noise reduction and feature extraction of Electromyography (EMG) signals.

Tow electromyography signals measured from the Right hand (RH) and Left hand (LH) of a person under test, spectrum analysis, and fuzzy modeling were applied to simplify the data classification and analysis of the measured EMG signals.

Keywords: Electromyography, Fuzzy modeling, Noise reduction, BrainBay software, OLIMEX 328, Spectrum analysis.

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1. Introduction:

The main purpose of this research is to implement fuzzy modeling, noise elimination, and classification of EMG signals.

Two EMG signals measured from the Right Hand (RH) and Left Hand (LH) of a person are used to simplify the classification and noise reduction of EMG signals. Human hand activities are explained by a large number of degrees of freedom (DOFs), (Zecca) [1]. For that, the classification and data analysis of the EMG signal is quite complicated (Rubana) [2]. The features of the EMG signal depend on the subject's internal structure, including individual blood flow velocity, skin formation, tissue structure (muscle, fat, etc.…), the measuring site, measured skin temperatures, and more, (Reaz) [3]. The attributes produce different types of noise signals that can be found within the EMG signals, (Ahsan) [4].

A fuzzy logic system gives advantages in biomedical signal processing and classification because the biomedical signals are not always strictly repeatable, and may sometimes even be contradictory, (Yang) [5], one of the most useful properties of the fuzzy logic system is that contradictions in the data can be tolerated, furthermore, the trainable fuzzy system can be used for efficient data analysis and decision making, it is possible to discover patterns in data which are not easily detected by other methods, (Rangaraj) [6].

On the other hand, one of the main difficulties in analyzing the EMG signal is due to its noisy characteristics, (Amrutha) [7].

If myoelectric control is selected as the primary control scheme, site identification must consider EMG signal level, EMG separation, and skin condition, marking the area on the skin surface that has acceptable EMG signal strength and separation and then donning the interface and transferring the site provides the best results, (Hatice) [8].

2. Experimental measurements:

With suitable laboratory conditions, EMG signals are measured from the left and right hands of a person (LH, RH), and the EMG records are different from one hand to another according to the reasons mentioned before.

Spectrum analysis was applied to the measured EMG signals, then converted to digital form using ADC, the similar components in both (LH) and (RH) signals will be considered as the noise, so the XOR process was applied to eliminate these components, while the other components will be added and used to reconstruct the EMG signals

Which must be converted to analog form using DAC.

EMG device measures the electrical currents that are generated in a muscle during its contraction and represents neuromuscular activities.

3. Fuzzy modeling of EMG signals:

Let the fuzzy implication R is of the format

R : IF (X1 is A1 ,…., Xk is Ak) then: y= g(X1,-----, Xk) (1)

Where:

y: Variable of the consequence whose value is inferred.

X1-----Xk: variable of the premises that appear also in the part of the consequences.

A1---Ak: fuzzy sets with linear membership functions representing a fuzzy subspace in which the implication R can be applied for reasoning.

f: Logical function connects the propositions in the premise.

g: Function that implies the value of y when x1----xk satisfies the premise, (Takagi) [9].

If we have Ri implications of the above format:

Ri (i =1,……., n)

(x1 =x10,--------,x1= xk0)

Where x10,--------xk0 are singletons, the value of y is inferred in the following steps

For each implication Ri, yi is calculated by the function gi in the consequence

yi = gi(x01,…. X0 k

= pi + pi 1 x01 ,……+ pi k x0k (2)

The truth value of the proposition y=yi is calculated by the equation

$I_{y=yi}I$ = (Ai1(x01) Λ…….Λ Aik(x0k)) Λ I Ri I (3)

I Ri I = 1

So, the truth value of the consequences obtained is

$I_{y=yi}I$ = Ai1(x01) Λ…….Λ Aik(x0k) (4)

The final output y inferred from n implications is given as the average of all yi wit the weights $I_{y=yi}I$: (Osman) [10].

y=[Σ $I_{y=yi}I$*yi] \ Σ $I_{y=yi}I$ (5)

Applying equation (5) for both EMG signals the LH and RH :

B1=[Σ $I_{RH=RHi}I$*yi] \ Σ $I_{RH=RHi}I$ (6)

B2=[Σ $I_{LH=LHi}I$*yi] \ Σ $I_{LH=LHi}I$ (7)

And by using Wilson amplitude (Wamp) which is the number of times that the difference between EMG signal amplitude among two adjacent segments exceeds a predefined threshold, (Gupta) [11].

Applying Wilson amplitude for B1 and B2 we get:

C1Wamp = ΣN k=1 f($I_{B1k= B1k+1}$ I) (8)

C2Wamp = ΣN k=1 f($I_{B2k= B2k+1}$ I) (9)

Converting C1Wamp and C2Wampto digital form (D1, D2) and XOR them the output y will be:

y=D1+D2 (10)

the block diagram of the fuzzy modeling used in this research is shown in fig. (1). Where the RH and LH EMG signals were fuzzified then after a suitable consequence has verified a defuzzification was applied to A1 and A2 to

get B1 and B2, then calculating Wamp for B1 and B2, as a result, we get C1 and C2, they both converted to digital form to have D1 and D2 as an input to the XOR gate in which the similar quantities were rejected(noise canceling), the output y will be in digital form if we need it as an analog signal a DAC can be used.

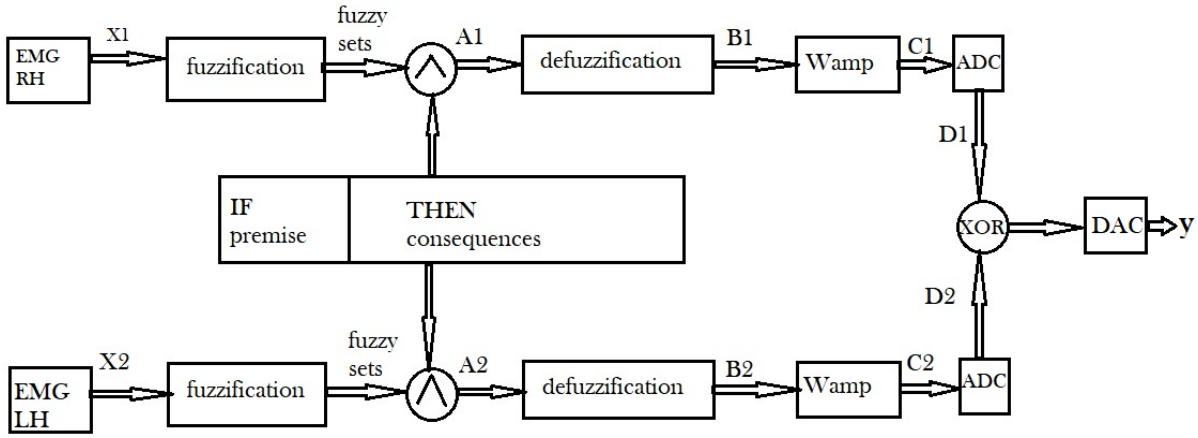

Fig. (1) Block diagram of measuring and fuzzy modeling of RH, LH EMG signals.

4. Experimental Results:

To measure the EMG signals of the right hand (RH) and the left hand (LH) of a volunteer in this research, a measuring system was built as shown in fig. (2).

Fig. (2) measuring system for EMG signals of the LH &RH.

An OLIMEX 328 microcontroller [12] used with two EMG shields was connected through a USB port of a computer and the measuring process was done by using BrainBay software [13], the complete design used in this research is shown in fig. (3).

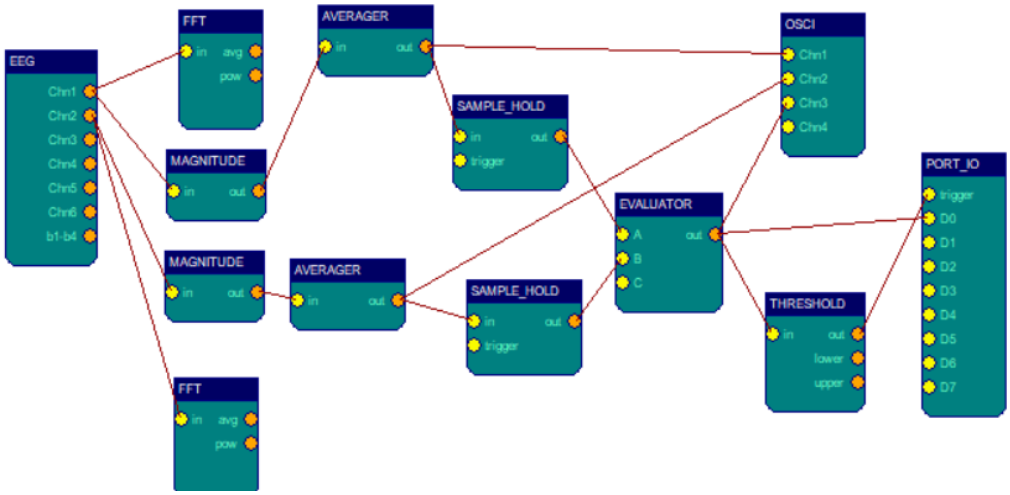

Fig. (3) Measuring system design using BrainBay software.

The raw measured and filtered EMG signals of the RH and LH are shown in Fig. (4).

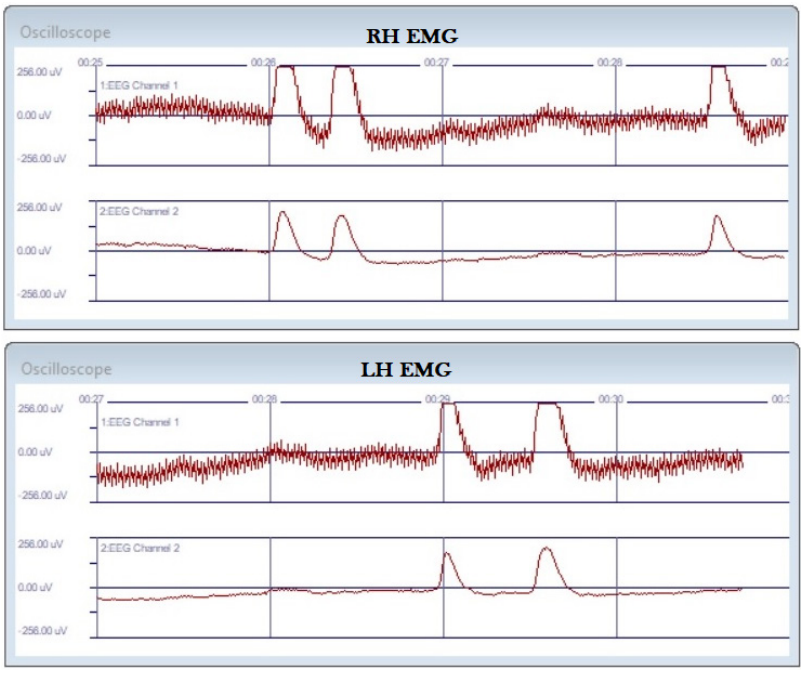

Fig. (4) Raw and filtered EMG signals.

To discriminate the noise of both EMG signals an FFT spectrum analyzer was used, fig. (5) shows the spectrum analyzer output.

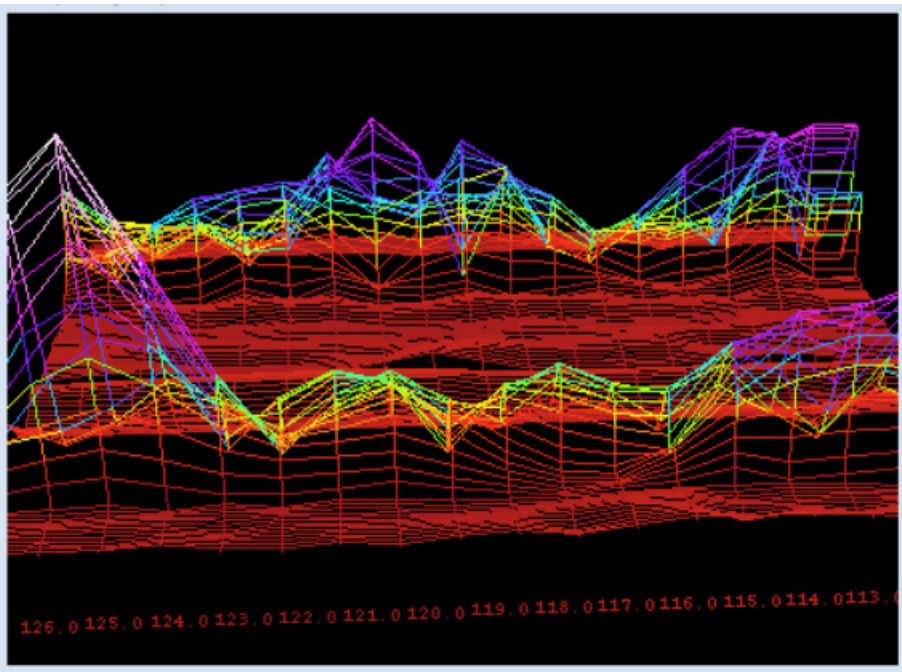

Fig (5) spectrum analyzer output of LH and RH EMG signals

The filtered signals were processed using the Wilson amplitude method [6], fig. (6) shows the resultant EMG signals, from this result the researcher decides the type of filter used and the frequencies that must be filtered.

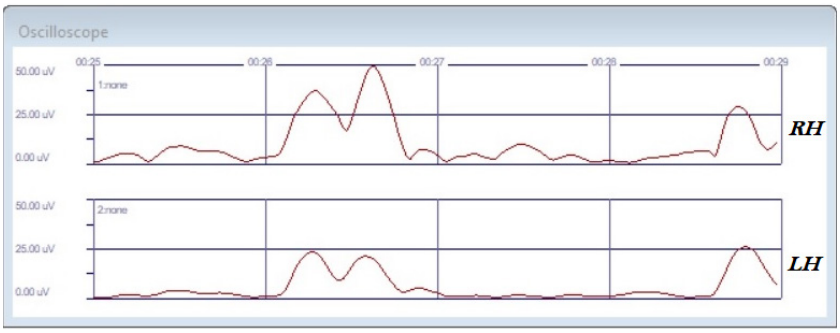

Fig. (6) EMG signals after applying the Wamp procedure.

The signals above in (Fig. 6) passed through a sample and hold process, the result of the sample and hold is shown in (Fig.7).

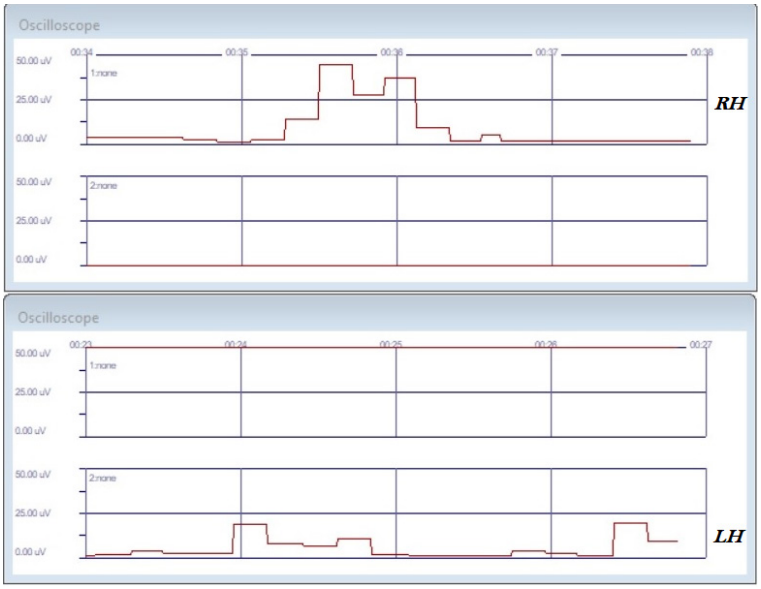

Fig. (7) EMG signals in digital form

The digital form of the two EMG signals (RH &LH) is used as an input to the XOR gate to element the similar values that represent the noise according to this research.

Finally, the output of the XOR gate is converted to an Analogue signal.

The resultant (one EMG signal) is shown in Fig. (8).

Fig. (8) The final EMG signal extracted from LH & RH EMG signals.

5. Conclusion:

The most difficult problem in using measured EMG signals in control applications is the EMG features because of their variety which depend on the natural properties of human skin, and it is hardly affected by environmental factors like humidity, skin temperature, skin conductivity, and noise, besides its poor repetition and similarity during the measurement for the same muscle action.

This research presents an easy way to noise canceling and overcome the issues mentioned above by using fuzzy logic.

The spectrum analysis of the raw EMG signals will help to choose a proper filtering process.

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Introduction to the Journal

The Kurdistani Journal of Natural Science and Biomedicine (JKSNB) is a quarterly journal published in the English language. It publishes original, important, effective and influential research and strategic studies across various fields of study. We promote academic communicationstrive to achive excellence, and stick to deadlines. The JKSNB keeps pace with current developments and keeps up with the aspirations of researchers and scholars inside and outside of the Kurdistan Region, in terms of scientific analyses and interpretations.

The journal aims at promoting strategic scientific research in Kurdistan, creating vast areas of scientific analysis to academics and researchers in Bio-Medical and Natural Sciences, and supporting the public and private sectors to conduct scientific research.

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Section One: Writing, Submiting and Publishing Guidelines

The article is to be submitted through the Kurdistan Institute for Strategic Studies and Scientific Research (KISSR) website, and researchers must bear in mind the following instructions and guidelines regarding writing and submitting their research.The number of pages should not exceed twenty-five pages of (A4) sized paper.

1. The article must contain the following.
- Title
- Researcher name(s), Department, College, University, Country, and an official e-mail address
- Abstract in English, and two other languages, which should be placed before the bibliography (references, works cited)
- Keywords
- Article content
- Results, findings, and conclusions
- -Sources and references.

2. A cover letter must be included with each manuscript submission. It should be concise and explain why the content of the paper is significant, placing the findings in the context of existing work. It should explain why the manuscript fits the scope of the journal.

All cover letters are required to include the statements:

- We confirm that neither the manuscript nor any parts of its content are currently under consideration or published in another journal.
- All authors have approved the manuscript and agree with its submission to JKSNB .

.3 Article Writing Template

A. Mechanical Instructions

a. The title of the article should be placed at the top of the first page, be centered, and an 18-point font size with bold formatting should be used (A 16-point font size with bold formatting should be used for subheadings, a 14 -point font size for the

body, and a 10-point font size for sources, annotations, and explanations.

b. Researcher name(s), Department, College, University, Country, and an official email address should be written in size (12Indent the first line of the first paragraph by putting your cursor at the beginning of the paragraph and press the tab key once.
c. For articles written in English, font type Times New Roman should be used for the body, a 16-point font size with bold formatting for the main title, a 14-point font size for the subheadings, a 12-point font size for the body, and a 10-point font size for the sources and margins.
d. Use single line spacing between paragraphs.
e. Leave a 2 cm margin on the sides of each page.

B. Follow the Harvard Referencing System for citing and referencing sources or references,
C. The abstract should be in no more than 250 words and should be in one paragraph only. Leave a single line spacing
D. Keywords. Remember that, the keywords should be between 5-7 single words.
E. The article is presented in two different modes: Personal details are to be deleted before uploading the article to the electronic platform, and this is for the review process to run anonymouslyand for a fair review to be conducted, way from favouritism and biases.
F. When the review process is complete and the article is conditionally accepted by the reviewer(s),the editorial board contacts the researcher via their e-mail address and asks them to make the corrections suggested by the reviewer(s).Once the corrections are made, the researcher should resend the article to the journal to be checked by the reviewer(s).
G. Upon completion of the review of the article by the reviewers, the editorial board of the journal shall informs the researcher whether their research was accepted or rejected.
H. When submitting the article via electronic upload on the the journal's website, publishing costs amounting to 250,000 IQD) must be paid, and this is according to the the Ministry of Finance's letter no. on/ / 2020If this payment is not made, the article will not be forwarded to a reviewer.
I. Submission is made electronically via the journal's website or via journals@kissr.edu.iq.

4. Full experimental details must be provided so that the results can be reproduced. *Biomedicines* requires that authors publish all experimental controls and make full datasets available where possible.

Section Two: Article Review

The review process is in three stages:

The first stage: Initial (Preliminary) review:

The article is reviewed by the journal's management body in a preliminary assessment to see if the article meets the journal's requirements and that it is eligible for a revie, Here is what the process is like.

- If the language used is not proficient enough, the article will be sent to a linguist for proofreading.

The writing style is benchmarked with the one approved of by the journal to see if it is in alignment - .with the publishing instructions of the journal

-The journal staff offer formatting changes to the author if required. And, if the work is returned by the author and is still unorganized, it will rejected and will not be forwarded for a review.

- Every article or written piece will be subjected to a plagiarism detection tool or software to identify any instances of plagiarism. Authors are held responsible for the originality of their article content and the information it contains.

The Second Stage: Scientific Review

After the article passes the preliminary review stage, it is sent to two specialist reviewers in the field. If the article is rejected by one of them, the article is sent to a third reviewer, and this is because an article is only accepted with the approval of two reviewers.

The Third Stage: Approval of the Evaluation

The reviewer chooses one of three options:

1 -Rejection: a complete and final rejection of the article.

2 -Conditional Acceptance: It is accepted under the condition of making the required amendments and changes.

3- Accepting the article as it is: The journal staff might still make some minor formatting changes, but the article is not subject to a second review and further scrutiny.

i: Evaluation Guidelines for Reviewers

During evaluation, please consider the following questions

1) Does the research make a valuable contribution to current knowledge and literature, in terms of the development of a theory, generating new data, or developing new methodology?
(2 Is the research written and submitted in the light of the journal's instructions to the authors?
3) Does the research adhere to the criteria that follow?

i. Knowledge and depth of understanding of basic concepts and issues
ii. Argument and its relevance to the assignment title
iii. Analysis, including originality of examples provided
iv. Evidence and critical use of sources researched
v. Independent thought and personal evaluation of issues under discussion
vi. Accuracy and clarity of expression, grammar, and punctuation
vii. Logical organization and linking of ideas
viii. Systematic and standardized in-text citation and bibliographical references

4)The reviewer should either accept the research as submitted, demand a second evaluation after the suggested changes are made by the researcher, or, reject the research altogether.

ii.Classification Guidelines

1. An original scientific research demonstrates research results that have not previously been fully or partially published elsewhere.
2. The article under evaluation contains a comprehensive review of recent and current research in a particular area. Research in this category include questionnaires by their very nature and should contain references and critical assessments. References must be sufficient enough to allow for a good view of the subject.
3. Professional research should not be based on original research but must contribute to the application of known research results and the introduction of theoretical concepts.

iii: Final Evaluation:

Please make your final decision by ommenting A and B, and filling in Tables 1, 2 and 3.

A. **In the light of the determination of the evaluation and the final judgement of your choice, it is possible to put in place the justifications that led to that decision: (Your judgments to be written here)**

I accept the article because . . .

I reject the article because . . .

B. **In the light of the determination of the evaluation and the final judgment you have chosen, recommendations for amendments by the researcher are to be made:**

I accept the research only if the researcher makes the following changes:

1.

2.

3.

Table (1) Procedural aspects of the research

Aspects	V.Good	Good	Fair	Accepted	Weak
Title of the article					
Research address					☐
Research references					☐

The purpose of the research Argument/ Claim					☐
Its importance and merit the study					☐
Its scientific value					☐
(Findnings align with the resarch goal(s					☐
.Hypotheses				☐	
Research approach/ Methodology			☐		
Content					☐
Conclusion					☐

Table (2) Final assessment

Classification	Evaluation Notes	Tick the box
Title of the article		
Very good	does not need to be amended	☐
Good	needs minor adjustments before publication.	☐
Acceptable	is not suitable for publication until the recommended adjustments have been made.	☐
Weak	is not suitable for publication in a scientific journal that is strict at all	☐
Others	needs a second review and further scrutiny.	☐
The Reviewer	**Signature**	**Date:**

Table (3) Personal information of the reviewer

Reviewer Name			
Academic Title			
Organization			
Address			
Mobile No.		**e-mail (Official)**	
I hereby state that the review submitted is conducted objectively and is based on professional, scientific, and ethical standards.			**Signature**

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