Impacts of Medical Marijuana Use on Inflammasome Activation and Breast Cancer Clinical Outcomes: A New Prospective Cohort Study

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Abstract

Background: Breast cancer is the most frequently diagnosed cancer and the second leading cause of cancer death in American women. Some breast cancer patients use Medical Marijuana (MMJ) to manage treatment-related symptoms. Although MMJ is generally considered safe and well-tolerated in cancer patients, there are potential adverse effects and conflicting reports on its interactions with cancer therapies and their impact on clinical outcomes. Therefore, we propose a prospective cohort study of a diverse breast cancer population (50% minorities) to assess the impact of MMJ on clinical outcomes and QOL.

Objective: The objectives are to (i) Assess the impact of MMJ on clinical outcomes and QOL in breast cancer patients after adjusting for age, race/ethnicity, tumor stage, and subtypes; (ii) Evaluate the relationship between inflammasome/inflammatory biomarkers and the biological effects of MMJ on clinical outcomes of breast cancer; (iii) Investigate potential interactions between MMJ properties (i.e., THC/CBD ratio, dose, and type) and cancer treatments on clinical outcomes.

Methods: We proposed to enroll 60 breast cancer patients who plan to initiate MMJ and collect data on patient and tumor characteristics, treatments, clinical outcomes, and adverse reactions to obtain a comprehensive understanding of the usage and effects of MMJ. We will collect subjective and objective data through combined in-person visits and technology-based assessments. In two proof-of-concept pilot studies, we evaluated inflammasome biomarkers in predicting radiotherapy (RT)-related clinical outcomes and the effects of MMJ on chronic pain.

Results: In the first pilot study of 63 breast cancer patients, we showed that RT-induced skin toxicity is significantly higher in patients with higher mean levels of inflammasome markers including caspase-1 (p=0.023), IL-18 (p=0.04), and hsCRP (p=0.028). Patients with post-RT pain 4+ have higher mean levels of ASC (p=0.017), IL-6 (p=0.022), and hsCRP (p=0.002). In addition, patients who died presented higher mean levels of caspase 1 (p=0.043), IL-6 (p=0.03), and hsCRP (p=0.005). A higher percentage of patients with a 4+ pain score had worse but not significant 5-year Progression Free Survival (52.2% vs. 38.5%) and Overall Survival (39.1 vs. 20.5). We will also present preliminary data from the second ongoing pilot study evaluating whether inflammasome activation mediates the effects of MMJ on chronic pain.

Conclusions: Inflammasome activation and inflammation contribute to RT-related skin toxicity, pain, and worse overall survival. Therefore, inhibiting inflammasome activation with MMJ has the potential to improve breast cancer QOL and clinical outcomes.

Materials & Methods

Study Design: We capitalized on existing plasma samples and data from questionnaires and medical records collected from two studies to evaluate: (1) the association between inflammasome and inflammatory biomarkers and prognosis in a multiracial breast cancer population at the University of Miami Sylvester Comprehensive Cancer Center; (2) the impact of MMJ on inflammasome biomarkers and chronic pain in an ongoing study conducted at University of Florida.

Study Population and Variables: The first study population included 63 breast cancer patients who underwent RT following surgery. Patient/demographic information included age at diagnosis, race/ethnicity, and body mass index (BMI). Clinical variables included tumor stage, ER, PR, HER2 and triple-negative status, acute skin toxicities immediately after RT using the Common Toxicity Criteria for Adverse Events (CTCAE, v. 3) scale, pain score, and overall survival (OS). The second study population included 49 individuals from an ongoing study of MMJ use and chronic pain. Patient/demographic information was collected from each study subject, including age at study entry, race/ethnicity, pain index, and BMI.

Biomarker Analyses: Plasma samples were analyzed at the University of Miami Miller School of Medicine for analysis in the Biomarker Core of the Inflammasome Laboratory for inflammasome proteins. Simple Plex Assay used for protein analysis of ASC, caspase-1, IL-18, and Trem 2 using the Ella System. Samples in the Ella CARTs rely on microfluidics and are run in triplicates and analyzed by the Simple Plex Explorer software.

Statistical Analysis: Chi-square and Fisher exact tests were used to evaluate biomarker differences by RT-induced skin toxicities, pain score, and vital status. Odds ratios (OR) and 95% confidence intervals (95% CI) were reported. All statistical analyses were carried out using the SAS program (SAS Institute, Cary, NC), and test results were considered significant at the 2-sided 5% level.

Background Significance

MMJ has become an increasingly common alternative treatment for various patient populations, including cancer patients. Chronic pain and anxiety are among the top cited reasons for this population to seek cannabis treatment [1]. Emerging evidence suggests MMJ may offer symptomatic relief (e.g., nausea, anxiety/depression) and analgesic properties for cancer populations [2, 3]. MMJ may inhibit or promote the proliferation of breast cancer cells [4, 5]. However, it may reduce the efficacy of immunotherapy [6, 7]. Although MMJ usage in cancer populations has become more prevalent, research findings are inconsistent and limited on the most effective mechanisms for delivery, dosing, and modes of consumption as well as long-term health impacts and side effects of MMJ treatment [8].

A recent survey of 612 breast cancer patients reported that 42% used cannabis for relief of symptoms, which included pain (78%), insomnia (70%), anxiety (57%), stress (51%), and nausea/vomiting (46%) [9]. Similarly, MMJ has been used mostly in palliative care to treat and manage cancer treatment-related symptoms and side effects [10, 11]. Half of all women undergoing breast cancer surgery experience persistent post-surgical pain [12], and almost 90% of women receiving breast cancer treatment would develop unexpected long-term treatment-related side effects, such as anxiety and depression [13]. MMJ may provide an alternative treatment to alleviate symptom burden and improve patients' QOL [14]. Limited information is available on the short-term and long-term side effects and cannabis use disorders associated with the consumption of high-potency cannabis. Cannabinoids may promote the proliferation of breast cancer cells. Some research found that THC can lead to enhanced growth of tumors by suppressing the antitumor immune response in the animal model [15].

We show that elevated inflammasome and inflammatory biomarkers contributed to RT-induced skin toxicities, pain, and worse overall survival and recent findings suggest that MMJ targets multiple inflammasome activation pathways. Therefore, we will test a new paradigm that the inhibition of inflammasome-mediated inflammatory responses by MMJ plays a mediating role in its biological effects on breast cancer clinical outcomes.

Results

Table 1 Inflammasome/inflammation Biomarker Levels and Breast Cancer Clinical Outcomes

Variables Mean (SD)	RT-Induced Skin Toxicity			Pain Score			Vital Status		
	1 (n=20)	2-3 (n=43)	p-value	<4 (n=36)	4+ (n=22)	p-value	Alive (n=47)	Dead (n=16)	p-value
ASC	7.42 (0.91)	7.73 (0.69)	0.13	**7.47 (0.89)**	**7.89 (0.39)**	**0.017**	7.61 (0.68)	7.7 (1.02)	0.749
Caspase-1	**0.14 (0.54)**	**0.46 (0.31)**	**0.023**	0.31 (0.4)	0.48 (0.26)	0.064	**0.32 (0.47)**	**0.49 (0.19)**	**0.043**
IL-18	**7.24 (0.79)**	**7.62 (0.63)**	**0.04**	7.5 (0.84)	7.52 (0.48)	0.892	7.44 (0.68)	7.68 (0.76)	0.234
IL-6	1.45 (1.09)	1.87 (1.17)	0.218	**1.39 (0.9)**	**2.18 (1.36)**	**0.022**	**1.56 (1.09)**	**2.28 (1.2)**	**0.03**
hsCRP	**1.48 (2.04)**	**2.51 (1.58)**	**0.028**	**1.55 (1.7)**	**2.98 (1.53)**	**0.002**	**1.79 (1.69)**	**3.14 (1.72)**	**0.005**

Table 2. Univariable and Multivariable Cox Regression Models of hsCRP on OS

Variable	Univariable Model[1]		Multivariable Model 1[2]		Multivariable Model 2[3]		Multivariable Model 3[4]	
	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
Race/Ethnicity								
NHW	Ref		Ref		Ref		Ref	
HW	1.97 (0.60-6.49)	0.268	1.19 (0.35-4.11)	0.780	1.03 (0.29-3.70)	0.959	1.01 (0.29-3.57)	0.986
AA	1.45 (0.36-5.79)	0.602	0.29 (0.05-1.89)	0.196	0.24 (0.04-1.59)	0.139	0.24 (0.04-1.55)	0.133
Other	1.34 (0.14-12.85)	0.802	Not estimable		Not estimable		Not estimable	
Age at Diagnosis								
<60	Ref		Ref		Ref		Ref	
>=60	**1.74 (0.90-3.38)**	**0.102**	**2.39 (1.08-5.29)**	**0.032**	1.83 (0.82-4.10)	0.143	1.86 (0.84-4.14)	0.128
BMI								
<25	Ref		Ref		Ref		Ref	
25-29.99	1.03 (0.43-2.44)	0.950	0.64 (0.24-1.69)	0.369	0.53 (0.20-1.45)	0.217	0.55 (0.20-1.50)	0.240
>=30	1.15 (0.50-2.62)	0.747	1.06 (0.42-2.71)	0.903	0.88 (0.33-2.31)	0.788	0.90 (0.34-2.40)	0.840
Clinical Tumor Stage								
0-IB	Ref		Ref		Ref		Ref	
IIA-IIB	1.33 (0.49-3.67)	0.577	1.58 (0.45-5.51)	0.857	1.43 (0.41-5.07)	0.576	1.36 (0.38-4.80)	0.637
IIIA-IIIC	**6.25 (2.92-13.38)**	**<0.001**	**9.32 (3.72-23.35)**	**<0.001**	**7.44 (2.88-19.24)**	**<0.001**	**7.05 (2.69-18.48)**	**<0.001**
Triple Negative								
No	Ref		Ref		Ref		Ref	
Yes	**2.22 (1.07-4.60)**	**0.033**	1.77 (0.69-4.53)	0.233	1.65 (0.62-4.37)	0.312	1.76 (0.67-4.67)	0.254
Comorbidities								
0-2	Ref		Ref		Ref		Ref	
3+	**3.41 (1.04-11.15)**	**0.043**	1.48 (0.38-5.75)	0.572	1.65 (0.62-4.37)	0.312	1.70 (0.41-7.04)	0.254
Pre-RT CRP								
<10	Ref				Ref			
>=10	**2.54 (1.22-5.29)**	**0.013**			1.69 (0.67-4.29)	0.270		
Post-RT CRP								
<10	Ref				Ref			
>=10	**3.92 (1.91-8.03)**	**<0.001**			**2.46 (1.03-5.89)**	**0.043**		
Pre-RT CRP								
<9	Ref						Ref	
>=9	1.96 (0.67-4.44)	0.105					1.37 (0.55-3.46)	0.502
Post-RT CRP								
<9	Ref						Ref	
>=9	**4.54 (2.18-9.44)**	**<0.001**					**2.98 (1.29-6.89)**	**0.011**
AUC at 5 years			**0.8258**		**0.8720**		**0.8917**	

[1] Univariable model (n=506): each predictor assessed individually
[2] Multivariable model 1 (n=450): patient/clinical factors but not CRP
[3] Multivariable model 2 (n=450): patient/clinical factors and CRP with a cut-off of 10 mg/L
[4] Multivariable model 3 (n=450): patient/clinical factors and CRP with a cut-off of 9 mg/L (cut-off with the highest AUC)

Table 3 Inflammasome/inflammation Biomarker Levels and The Worst Pain Outcome by MMJ Use

Biomarker Change	Worst Pain	Non-MMJ Group			MMJ-Group			Total		
		N (%)	Mean	SD	N (%)	Mean	SD	N	Mean	SD
ASC	Decreased	6 (29%)	**-31.83**	**48.03**	9 (48%)	11.67	46.25	15	**-5.73**	**50.33**
	Increased	6 (29%)	**39.17**	**64.30**	7 (37%)	40.71	43.52	13	**40.00**	**51.67**
		p-value	**0.06**		p-value	0.22		**p-value**	**0.03**	
Caspase 1	Decreased	6 (29%)	-0.07	0.52	9 (48%)	0.13	0.47	15	0.05	0.48
	Increased	6 (29%)	0.40	0.72	7 (37%)	0.23	0.47	13	0.31	0.58
		p-value	0.22		p-value	0.69		p-value	0.21	
IL-18	Decreased	6 (29%)	27.08	58.52	9 (48%)	1.90	46.76	15	11.97	51.34
	Increased	6 (29%)	-16.67	45.21	7 (37%)	20.86	46.20	13	3.54	47.94
		p-value	0.18		p-value	0.43		p-value	0.66	
Trem 2	Decreased	6 (29%)	963.30	6057.00	9 (48%)	1966.00	4610.50	15	1564.90	5050.50
	Increased	6 (29%)	1266.50	2361.50	7 (37%)	805.90	2830.70	13	1018.50	2527.30
		p-value	0.91		p-value	0.57		p-value	0.72	

Discussions & Conclusions

Inflammasome/inflammation Biomarkers and Breast Cancer Clinical Outcomes

Our promising pilot data from 63 patients (**Table 1**) showed that radiotherapy (RT)-induced skin toxicity is significantly higher in patients with higher mean caspase-1 (p=0.023), IL-18 (p=0.04), and CRP (p=0.028). Patients with post-RT pain 4+ have higher levels of ASC (p=0.017), IL-6 (p=0.022), and CRP (p=0.002). Patients with progressive disease had higher hsCRP (p=0.034). Patients who died had higher mean caspase 1 (p=0.043), IL-6 (p=0.03), and CRP (p=0.005). Our data also showed a higher % of patients with a **4+ pain score** had worse but not significant PFS (52.2% vs. 38.5%) and OS (39.1 vs. 20.5). **In summary, excessive activation of inflammasome and inflammation may contribute to RT-related skin toxicity, pain, and worse overall survival. If validated in larger studies, inflammasome-targeted treatment may improve RT-related normal tissue toxicities and overall survival in breast cancer and other cancers.**

Inflammasome Biomarkers in MMJ Use and Chronic Pain

In a cohort of 49 individuals from an ongoing study of MMJ use and chronic pain, we showed a significant correlation between ASC and BMI (r=0.53, p=0.0004). Interestingly, we also observe a significant correlation between the levels of ASC in plasma and the report by individuals of having pain at their worst (r=0.3, p=0.05). Finally, after 3 months of MMJ, there was a trend of decreased pain in the MMJ group (mean change = -0.47) when compared to the non-MMJ group (mean change = 0.57) (p=0.06). **In summary, these data suggest that MMJ decreases chronic pain symptoms and that the inflammasome plays a role in this patient population. If validated in larger studies, inflammasome biomarkers can be used to assess response to MMJ treatment in patients with chronic pain.**

Effects of MMJ on Inflammatory Pathways and Breast Cancer Clinical Outcomes

Our data from two pilot studies demonstrate the feasibility and scientific foundation for the current research. First, we can recruit breast cancer patients and community-based recruitment to evaluate MMJ clinical outcomes using inflammasome/inflammatory biomarkers. Second, findings from our pilot study demonstrate that MMJ use led to short-term changes in real-time pain intensity levels. Third, we demonstrated elevated inflammasome/inflammatory biomarkers in breast cancer patients contribute to RT-induced skin toxicities, pain, and worse overall survival. **Therefore, our current research is warranted to evaluate inflammasome and inflammatory biomarkers in breast cancer patients to elucidate the biological effects of MMJ on inflammatory pathways and clinical outcomes.**

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